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MESSAGE

Several attempts to come up with an issue of this Journal of the Philippine Pharmacists Association, Inc. have been tried and finally, the team has come up with this edition. Congratulations to the team and all the contributors of this important endeavor. Thank you too to Bryan for taking the lead!

A number of very good studies and researches are in this issue. This definitely will be another source of information for our pharmacists, students of pharmacy and everyone who may need some information about medicines and their use, patients and their practices and pharmacy operators and their services, including noteworthy researches in pharmaceutical science. I also hope this journal will serve an inspiration for those who would want to conduct studies and publish them, a venue for one’s work to be known and shared to others.

Again, my warmest congratulations to everyone. I am proud of you!
Gender sensitivity in clinical pharmacy: a descriptive study on pharmacy students’ perspectives and gender-based patient characteristics

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ABSTRACT

The interrelationship between sex and gender results to differences in needs and preferences, thereby requiring customized approach to health care provision. The objective of this paper is to determine the relevance of gender sensitivity in clinical pharmacy education. Forty-seven graduating pharmacy students were given questionnaires before and after a gender sensitivity lecture to assess ability to differentiate sex from gender, identify influences on gender perspective, and determine perception on gender-sensitive health care. Psychological profiling of patients, a total of three males and three females, in terms of gender-related attributes and preferences was also conducted after interviews.

Majority of students differentiated adequately sex from gender. Family ranked highest among the important influences on gender perspective. Concept of gender-sensitive health care considered gender differences in terms of lifestyle, drug effects, preferences and psychological attributes. Patient respondents alleged a lack of gender sensitivity in current health system, and indicated preference to health professionals of their corresponding sex. Gender sensitivity in clinical pharmacy education is important to overcome barriers brought about by gender differences, to ensure full cooperation and effective communication between patient and pharmacist.
Gender sensitivity in clinical pharmacy: a descriptive study on pharmacy students’ perspectives and gender-based patient characteristics*

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The interrelationship between sex and gender results to differences in needs and preferences, thereby requiring customized approach to health care provision. The objective of this paper is to determine the relevance of gender sensitivity in clinical pharmacy education. Forty-seven graduating pharmacy students were given questionnaires before and after a gender sensitivity lecture to assess ability to differentiate sex from gender, identify influences on gender perspective, and determine perception on gender-sensitive health care. Psychological profiling of patients, total of three males and three females, in terms of gender-related attributes and preferences was also conducted after interviews.

Majority of students differentiated adequately sex from gender. Family ranked highest among the important influences on gender perspective. Concept of gender-sensitive health care considered gender differences in terms of lifestyle, drug effects, preferences and psychological attributes. Patient respondents alleged a lack of gender sensitivity in current health system, and indicated preference to health professionals of their corresponding sex. Gender sensitivity in clinical pharmacy education is important to overcome barriers brought about by gender differences, to ensure full cooperation and effective communication between patient and pharmacist.
INTRODUCTION

The terms *sex* and *gender* have been used interchangeably, despite the differences in the scope of their definitions. This is due to the commonality in their basic element of distinguishing males from females (Berg, 2002). Sex is based on the fundamental differentiation of biology, whereas gender expands such distinction through a different perspective, that is, sociology (Zelek *et al.*, 1994).

For many years, biology has considered the anatomy and physiology of the males and females as the key points of differentiation (Westerstahl, 2003; Uncommon Sights, LLC, 2007; Zelek, 1994). In recent years, medical research has expanded its exploration into the genetic, hormonal, and metabolic influences that shape the distinctive attributes of males and females (Gopal 2001; Westerstahl, 2003). The interplay of these variables results to certain predisposition, not only in the physical level, but also in the intellectual, emotional, and psychological aspects (Guimond 2007 cited Keller 2005).

According to the World Health Organization (2008), gender refers to the socially constructed roles, behavior, activities, and attributes that a particular society considers appropriate for men and women. Although gender is rooted in biology, it is the environment and culture that give it its distinctive character (Berg, 2002). Gender perspective, however, is not culturally universal (Guimond 2007). It may vary among societies, cultures, and even evolves through time. The disparity of the roles assumed and activities engaged with by men and women, either within the family or the society in general, bypasses intercultural variations, and nevertheless, remains evident.

There are two common sets of traits that are consistently used to differentiate the two genders from each other. These are the dimensions of agency and communion (Guimond, 2007 cited Bakan, 1966). Agency comprises of masculine traits that project assertiveness, such as ambition, self-concern, and dominance, while communion is associated with the feminine traits like being caring, and concerned with others, both of which pertaining to tender-mindedness (Guimond, 2007). Research findings show that, unlike women, men are less willing to acknowledge anxiety, fear, shame, or other negatively toned emotions, as these are not within the confines of social expectations for manhood (Beck, 1994; Kruger, 2004). Moreover, men even feel compelled to engage in more high-risk activities, while maintaining constant denial of possible problems and concerns.

The roles and attributes associated with each gender have grave implications in their lifestyle and health practices. In the course that both genders seek professional help, certain biological and social predispositions often hinder effective communication. The activities and roles of a clinical pharmacist basically revolve around the patient. Effective communication skills are, therefore, important to successfully impart to and exhaust from the patient necessary information relevant to the case. And as patients were shown to have gender-based differences, it is of utmost importance that clinical pharmacists are equipped with the knowledge and skills to apply the most appropriate and effective approach to patients from either gender. Moreover,
these knowledge and skills may eliminate personal biases and gender-based differences which are present among clinical pharmacists themselves.

This study aims to determine the relevance of gender sensitivity in clinical pharmacy education. In line with this, the following are the specific objectives of the study: to determine the ability of pharmacy students in differentiating sex from gender; to identify the important influences on the gender perspective of students; to determine the perception of pharmacy students on gender-sensitive health care system, in relation to clinical pharmacy practice; to formulate a qualitative profile of patients in terms of gender-based differences in health practices and behavioral patterns; and, to determine patients’ perspective on the current health care system as far as gender-sensitivity is concerned.

METHODOLOGY

The first part of the study was an evaluation of the students’ perspectives regarding gender. The study covered a total of forty seven graduating pharmacy students from the College of Pharmacy, University of the Philippines Manila, enrolled in Pharmacy 56 Introduction to Clinical Pharmacy during the second semester of academic year 2008-2009.

At the start of the course, a gender sensitivity lecture was given. The session began with students answering a pre-lecture questionnaire. In the first segment, students were asked to give their own definitions of “gender” and “sex.” It was followed by a set of 10 statements which the students needed to classify as either pertaining to sex or gender. Then, the students were asked to cite agents or factors that they consider most influential to their gender perspective. The last part of the pre-lecture exercise was a question about the student’s notion of gender-sensitive health care. The questionnaires were then collected as the lecture began.

At the end of the lecture, a set of three more questions were asked. The students were asked to explain the importance of gender sensitivity in the context of clinical pharmacy. And lastly, the students were made to identify at least three areas covered by pharmacy practice in which they consider gender sensitivity to be of primary importance. Data gathered was then subjected to frequency determination and qualitative analysis.

The second part of the study aimed to provide concrete evidence on speculations about gender-dependent patient preferences, with the formulation of a psychological profile of patients from both genders. This was done to confirm or deny the applicability of gender differences in the Philippine setting. It involved the participation of a total of six adult Filipino respondents, equally distributed between the two sexes. Respondents were purposively selected from three categories defined by the researchers according to different age brackets. This was to take into account the age-dependent variables in the responses. These categories were college students, young professionals, and middle-age. College students should be at least 19 years old, taking up non-health science courses. Young professionals should have at least 2 years of working experience with a non-health science job description, and had completed non-health science tertiary education. Finally, middle-age respondents should be at least 40 years old, should be
married and with child, working with a non-health science job description, and attained at least tertiary level of education with a non-health science degree. Non-health science tertiary education and job description were added to the criteria for subject selection so as to eliminate biases in opinions. Respondents were also screened for past health experiences, and should have at least one personal encounter with a health care professional. Free consent was given by all respondents prior to the inquiry.

The inquiry was conducted through a pre-scheduled one-on-one casual interview. The same set of questions was asked to all categories, with additional questions for the middle-age Filipino female in reference to pediatric health care. The interview was conducted in either English or Filipino, whichever was preferred by the respondent. The interview was then recorded on an audio cassette tape, which was then reviewed for the results and findings. Data gathered from the inquiry were subjected to qualitative analysis.

RESULTS AND DISCUSSION

Part I: Pharmacy Students

In the pre-lecture assessment, 46 out of 47 students were able to differentiate sex from gender. Common terms used in the definition of sex referred it as a classification of males and females; as pertaining to biology; with a few who specifically defined it in terms of physiological and anatomical sense; or in reference to the reproductive system, particularly the sex organs. The terms morphological and physical attributes were also mentioned. Despite the variety of the words used in the definition of sex, all of which were somehow related to a more scientific and biologically defined classification of human beings. Gender, however, was perceived differently.

The students came up with a longer list of terms and phrases in their attempt to define gender. Some of the most pertinent terms were linked to society, roles and responsibilities, culture, norms, and traditions. Gender, like sex, was considered as a means of classification of individuals. However, such classification was no longer limited to the dichotomy based on natural attributes. Rather, more factors were considered in defining the different genders, with marked relevance to the personal preferences of the individual. Categories, which sprang from such classification, included masculine or men, feminine or women, homosexuals or transgender, with some specifying gays and lesbians. Despite the diversity in their definition of gender, all can be unified to the basic concept that gender is psychologically and socially defined and influenced.

The following table summarizes the terms and phrases used in sex and gender definition, as they appeared in the pre-lecture questionnaire responses of students, with their corresponding frequencies.
The inquiry was conducted through a pre-scheduled one-on-one casual interview. The respondents prior to the inquiry at least one personal encounter with a health care professional. Free consent was given by all biases in opinions. Respondents were also screened for past health experiences, and should have education and job description were added to the criteria for subject selection so as to eliminate tertiary level of education with a non-health science degree. Non-health science tertiary married and with child, working with a non-health science job description, and attained at least same set of questions was asked to all categories, with additional questions for the middle-age females; as pertaining to gender. Common terms used in the definition of sex referred it as a classification of males and anatomical sense. In the pre-lecture assessment, 46 out of 47 students were able to differentiate sex from femininity. The following table summarizes the terms and phrases used in sex and gender definition, as they appeared in the pre-lecture questionnaire responses of students, with their corresponding frequencies.

<table>
<thead>
<tr>
<th>SEX (n=47)</th>
<th>GENDER (n=47)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male or Female (23)</td>
<td>Society (16)</td>
</tr>
<tr>
<td>Biological (22)</td>
<td>Roles/responsibilities in society (13)</td>
</tr>
<tr>
<td>Physiological or anatomical (14)</td>
<td>Personal/sexual preference/behavior (13)</td>
</tr>
<tr>
<td>Reproductive system or sex organs (11)</td>
<td>Culture/practices/traditions/norms (12)</td>
</tr>
<tr>
<td>Morphological or physical attributes (11)</td>
<td>Male, Female, Homosexual, Bisexual (8)</td>
</tr>
<tr>
<td>Scientific (4)</td>
<td>Based on Sex (3)</td>
</tr>
<tr>
<td>* roles, intercourse, boy and girl biased, no emphasis on identity</td>
<td>Others: feminine/masculine, emotional, not physical nor biological, psychological, based on factors other than M/F, subjective/theoretical</td>
</tr>
<tr>
<td>* natural/physical attributes</td>
<td></td>
</tr>
</tbody>
</table>

* refers to the definition by the single outlier

In the identification of statements as whether pertaining to gender or sex, only six percent of the students scored below average. Sixty-six percent garnered a perfect score, while 23 percent missed only one question. It was also observed that all those who had missed at least one statement, had commonly wronged the 10th statement, which is as follows: “Filipino female infants have higher chances of survival than male infants.” It is hypothesized that the word “survival” must have been the source of confusion since such word has both biological and social implications.

When the students were asked to indicate the most influential factors in their gender perspective, 81% considered family, while 66% indicated the church or religion as another influence. Mass media, friends, and society were also indicated, with school ranking as the sixth most influential to gender perspective. Ranking of these factors were done based on inclusion frequency without regard to the actual ranking indicated by the students. However, it should be noted that the family consistently ranked as the top influence in the students’ gender perspective, in both frequency determination and individual ranking. Such findings may be explained by the Filipino culture of close family ties, hence the high regard for the opinions of family members. Just as Filipinos are attached to their families, so are they to their religion. The Church has been an essential part of the Filipino culture and history, with its doctrines and traditions integrated into the homes and educational institutions. Thus, it exerts a strong influence in the individual’s perception of society and self, gender included. The relatively high impact of the mass media was also expected as it is somehow considered as a constant variable in the urban setting as it is encountered on a daily basis. The influence of friends and social circles to an individual’s perception may be traced to the social nature of humans. As social beings, people want to belong and to maintain relationships, thereby making them prone to peer pressure. It is surprising, however, that despite them having been in school for around 17 years of their lives, only 17 out of 47 students considered school as a major influence in their gender perspective. Such may be due to the diffusion of emphasis in the educational system, where the focus is on the essentials of
the individual subjects, without much, if any, emphasis in discussing gender-related issues. However, the imperative nature and the time spent by students in school gives it a very big potential as a venue in shaping minds towards gender sensitivity.

The following table shows the important influences on gender perspective, their respective ranks, frequencies and percentage.

<table>
<thead>
<tr>
<th>Rank</th>
<th>Influences</th>
<th>Frequency (n=47)</th>
<th>Percentage (n=47)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Family</td>
<td>38</td>
<td>81%</td>
</tr>
<tr>
<td>2</td>
<td>Church/Religion/God</td>
<td>32</td>
<td>68%</td>
</tr>
<tr>
<td>3</td>
<td>Media</td>
<td>28</td>
<td>60%</td>
</tr>
<tr>
<td>4</td>
<td>Friends/Peers</td>
<td>27</td>
<td>57%</td>
</tr>
<tr>
<td>5</td>
<td>Society/Culture</td>
<td>21</td>
<td>45%</td>
</tr>
<tr>
<td>6</td>
<td>School/Education/Institution</td>
<td>17</td>
<td>36%</td>
</tr>
</tbody>
</table>

The last segment of the pre-lecture exercise aimed to exhaust the student’s ideas on gender-sensitive health care system, particularly focusing on its applications in clinical pharmacy practice. According to the pharmacy students, gender-sensitive health care should consider the differences between genders, in terms of their lifestyle, occupation, social status, familial and social roles, and patient preferences. There was also the mention of pharmacokinetic and pharmacodynamic gender differences, thus the proposition of gender-specific approaches in patient drug therapy. Moreover, attitude of health care professionals was also considered crucial in the attainment of gender sensitivity, mentioning qualities such as caution, empathy, respect and understanding of the patient, so as to gain patient cooperation and avoid possible offense.

Post-lecture assessment revealed that 46 out of 47 students claimed positive benefits from the gender sensitivity lecture. However, one student claimed that the ideas presented in the lecture were not novelty, although he admitted sharing the same gender perspectives. Among those who claimed change in their gender-perspective, clearer understanding in the definitions and scope of the terms sex and gender was considered as the most common change. Also, 24% claimed that the lecture motivated them to be more gender-sensitive individuals. Some cited the need for gender-fair language and gender-equality, with a few considering the session as an eye-opener to the gravity of gender issues in the society.

All of the students agreed on the importance of gender sensitivity in clinical pharmacy practice, citing the differences in the backgrounds, needs, and preferences of the patients, and the necessary attitude of the health care professionals in dealing with the patient, as primary reasons. It was also pointed out that clinical pharmacy involves direct and personal contact to the patients; hence gender sensitivity should be practiced to increase patient comfort in discussion and patient compliance, ensuring optimum health care. Other reasons mentioned were equal rights between genders, formulation of gender-specific approaches in treatment, counseling and drug development, and also the need for the use of gender-fair language.
However, pharmacy practice has a very broad scope. In order to evaluate the students’ perceptions, they were asked to identify at least three specific areas in which gender sensitivity is deemed to be most needed. Frequency test results showed that patient counseling is the specific area perceived to require gender sensitivity the most. Such may be due to the fact that it is this particular area of practice that has the greatest personal contact with the patient. Also, success of patient counseling greatly depends on effective communication between the patient and the pharmacist. And a gender-sensitive approach would play a key role in gaining the patient’s trust and cooperation. Tying in second is dispensing and drug therapy. In dispensing, the issues raised were more on dissemination of correct information about drug products, while in drug therapy, variations in patient dosage requirements, gender-related risk factors and adverse drug responses, as well as gender-specific non-pharmacologic treatment, were considered. Dealing with sensitive issues, particularly sexually-transmitted diseases and other reproductive health concerns were pointed out as requiring gender-sensitivity. The field of drug research and development were also cited as areas where such approach should be applied.

**Part II: Patients**

Patients were asked to independently describe generalized traits for men and women. Both genders described men as straightforward, less articulate in explaining themselves, more reserved and secretive, and have the tendency to take things lightly. Moreover, men were also perceived as possessing a generalized reluctance to admit weaknesses. Such was unanimously admitted by the male respondents. According to them, reasons for such behavior included their desire to solve things on their own, not wanting to cause any trouble to family and friends. Moreover, constant admission to weaknesses or problems was considered as a feminine trait, and therefore, if engaged with would be harmful to their “macho” image.

Women, on the other hand, were perceived as meticulous with details, more responsible, caring, emotional, sensitive to others’ and one’s feelings, and more articulate than men. However, despite their superiority in communication, it was also cited by both genders that women also tend to euphemize some of their personal details, so as to protect their feminine image which is supposedly conservative. Therefore one can say that as much as males are hesitant to admit weaknesses or toned-down emotions, women are embarrassed to admit socially deviant behaviors. And while men resort to secrecy, women sugarcoat the truth. Overall, these findings are consistent with the biological and psychological evidences found in literature.

Behavioral patterns among men and women may have serious implications in their health care practices. In this section, the respondents were asked to compare and differentiate men from women, in terms of their behavior as patients. Male respondents described male patients as having stronger physique, thereby making them less susceptible to pain. However, female respondents also claimed high pain threshold as a female attribute, citing the pain associated in childbirth, and menstrual cramps. Unfortunately, no literature was found to support either claims, as pain is considered a subjective experience.
The attributes mentioned earlier to describe men, in general, also translated in their health practices. Both genders agreed that male patients would initially be reluctant to seek professional help, due to reasons already mentioned. Aside from that, the middle-age male respondent also added that older men tend to deny the fact that they are getting older, and are “deteriorating,” a perception seconded by the other respondents in reference to their own fathers. Furthermore, both of the young professional respondents found the idea of making appointments too much of a hassle. When asked which gender would most likely seek professional help, 4 out of 6 responded females, with the college student and middle-age female respondents indicating otherwise. This was because females were perceived to be more health conscious, and paranoid, hence the need for constant assurance. However, upon review of their personal health practices, both genders were following the same parameters before seeking professional help. These parameters included the length of time waited from onset of symptoms to actual medical checkup which was a week on the average, severity of the condition, and practice of self-medication upon onset. However, it was also observed that both college students were more cautious and would consult their own mothers when relief was not attained after a day or two. Also, a relatively unique response was given by the middle-aged male respondent, wherein he claimed to seek immediate help either from his wife or children, or directly calls the community doctor for assistance. However, professional help was sought more urgently for pediatric cases, as reported by the middle-age female respondent; although, self-medication is still initially practiced. This was because children were perceived to be more susceptible to illnesses.

In the case that both genders seek medical help, all agreed that male patients tend to be more straightforward in discussing their symptoms, and would only mention information which they deemed necessary. On the contrary, female patients were expected to be very elaborate on recounting details during consultation, and such disclosure would be voluntary. This difference in information disclosure was pointed out as a possible barrier in communication, such that it was suggested that more questions should be asked when dealing with male than female patients. As far as patient compliance is concerned, female patients were found to be more conscious in following the medical advice, while their male counterparts admitted noncompliance, though not deliberately, but due to forgetfulness.

All respondents claimed to have encounters with health care professionals from both genders. Their perceptions were also consistent such that female health care professionals were described as more nurturing, attentive, showing greater concern, and had a motherly approach regardless of the patients’ gender. On the contrary, male health care professionals were described as more professional in their approach, and were said to spend less time with their patients. However, the young male professional claimed that female health care professionals were bookish, while male health care professionals were more open to different approaches to a particular case. Despite these differences, all respondents admitted partiality to same gender health care professionals. Even with pediatric cases, same partiality was reported by the mother in her choice of female health care professionals. These preferences are for the reason that same genders would understand each other better, even beyond the technicalities of the case.
Moreover, better understanding would facilitate the communication process, and same gender health care professionals were expected to be able to give more relevant and realistic recommendations to the patient.

In line with preferences, respondents were asked to specify the preferred gender of the health care professionals who would handle them. Results revealed that gender of the health care professionals was not a consideration in neuter diseases. Neuter diseases are those which do not have a direct relation to the reproductive system. These include hypertension, mumps, measles, dengue, asthma, bronchitis, pneumonia, and skin problems. However, skin problems, though neuter, were considered as a female expertise, hence both genders preferred female to male health care professionals. Moreover, hypertension, being a lifestyle disease, was also indicated with same gender health care professional preference by the young professionals and college students. This was because of their belief that same gender health care professionals would be able to give them more relevant and realistic recommendations as it is affected by lifestyle. Non-gender specific cancers include those that affect organs common to both sexes. Although no gender preference was indicated by male respondents in this condition, female respondents unanimously specified female health care professionals. This was because of the possible terminal nature of the disease which they think would be handled well by the nurturance and genuine concern perceived to be common among female health care professionals. As expected, gender-specific diseases, considered as sensitive issues, which entail direct or indirect involvement of their sex organs were indicated with same gender health care professionals. Reasons cited were the discomfort and embarrassment in physical examination of the sex organs, disclosure of intimate details regarding the history of the disease, perceived awkwardness in both health care professional and patient during the medical session, and as mentioned earlier, the feeling of being understood better by the same gender health care professionals in discussion of the history, the actual experience, and other lifestyle-related factors which may be relevant to the case. Moreover, the decision on the gender of the health care professionals in pregnancy and family planning was considered by males as dependent on their partner, while females still indicated same gender preference. Respondents claimed that their indicated gender preferences and reasons were shared by the rest of the population of their gender in their respective age groups.

However, the patients indicated the familiarity, reputation and experience of the health care professional as the primary criteria for seeking medical help. Other factors considered were accessibility, location of the health care facility, and the type of approach in dealing with patients. Generally, a friendly approach was chosen over the conventional type. Privacy was also highly regarded in settings of medical sessions.

Before concluding the interview, the concept of gender-sensitivity was introduced to the respondents. All were not familiar with the concept and had no idea what it meant. After it was briefly explained, all respondents claimed that the current health care system is not practicing gender-sensitivity, and that it is important that it would make efforts towards becoming more gender-sensitive.
CONCLUSION

Gender differences have been confirmed by anecdotal and scientific evidences presented in this paper. These differences have seriously affected the health practices and preferences of patients. Furthermore, these have implications to the behavioral patterns of health care providers. Therefore, it is of great importance that the recognition and understanding of gender psychology be incorporated into the health care system. The length of time spent by individuals in formal education makes the school a high-potential venue in effecting major changes in the future. As it is the training ground for members of the health care system, pharmacists included, it could equip the future health care professionals with the necessary values, knowledge and skills to make them gender-sensitive.

In clinical pharmacy, aside from the staple criteria of superior knowledge and skills in pharmacotherapeutics, effective communication skills are also important so as to successfully convey the necessary information to the patients. Knowledge and understanding of gender differences will help health care providers to become more sensitive to the unique needs of their patients. This will allow them to apply the most appropriate approach in order to ensure optimum health care provision. The current health care system, however, is perceived to be lacking gender sensitivity, hence more efforts are needed. As members of the health care team, clinical pharmacists should contribute to the attainment of a more gender-sensitive health care system.

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Special thanks to Dr. Anthony Cordero, Director of UP Manila Center for Gender and Women Studies.
Medication knowledge and attitudes towards professional collaboration among pharmacy assistants in Manila: a descriptive study

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ABSTRACT

One of the challenges in the health care delivery is the lack of human resource. The scarcity of pharmacists in the community setting, and the increasing demand for pharmaceuticals have led to acquiring pharmacy assistants. In many occasions, the pharmacy assistants are the ones dispensing the drugs without the supervision of the pharmacist. This study aimed to determine the pharmacy assistants’ extent of knowledge on selected items of safe medication use, and their attitudes towards professional collaboration with the pharmacist. With the use of a pre-tested survey questionnaire, the study involved 100 pharmacy assistants based in the City of Manila.

Pharmacy assistants have positive attitudes and practices toward professional collaboration, but lack essential knowledge about proper use of medications and drug safety. While almost all the drugstores implemented formal training, their methods vary and do not generally provide the necessary and adequate skills for pharmacy assistants. Interventions to improve drug knowledge and safe medication practices should be given continuously to pharmacy assistants to ensure safe use of medications by the public.
INTRODUCTION

Drugs are important in the management of diseases as they are used to mitigate, prevent, diagnose, or treat medical conditions. However, if drugs are erroneously prescribed by physicians or improperly dispensed by pharmacists, the condition of the patient may worsen. Thus, the ability of those who are responsible in prescribing and dispensing is critical in ensuring the safety and welfare of the patient. In the Philippines, pharmacy assistants play a crucial role in the medical or non-medical use of drugs and herbal products, as the traditional workload of pharmacists is increasingly handled by them. This means that the competency of pharmacy assistants is paramount and should be measured objectively through their ability to perform and possess significant job-related knowledge, skills, attitude and practices in a community drug store.

In countries like Canada, competencies of pharmacy assistants include: making legal, ethical and professional judgment, ensuring good quality of the medications, promoting safe and effective drug distribution, participating in the management of operations and financial activities associated with the processing of prescriptions, communicating effectively with patients and other health care team members, educating if appropriate in order to improve and promote patient well being, and working in collaborative relationships within health care teams to optimize patient safety and improve health outcomes (NAPRA, 2007).

Filipino pharmacy assistants perform various roles in a community drug store. The common tasks of a pharmacy assistant are: drug preparation (counting, weighing, measuring and labeling); clerical and computer duties; inventory of prescription and OTC medication; maintenance of pharmacy equipment and supplies; managing the cash register; and, stocking of shelves (Camu, 2004).

At present, the concern about the pharmacy assistants lies on their capabilities and qualifications. Most likely, the pharmacy assistants employed do not have sufficient knowledge on drugs and adequate skills in patient counselling upon entry to practice. Most of them receive training only after being hired even if most employers prefer those who already have formal training, certification or previous experience. While some drugstores have training program for pharmacy assistants before employment, it may not be sufficient. The absence of a standard training program and assessment system has a tremendous effect on the health care system since pharmacy assistants are directly interacting with the public in selling drugs, with or without supervision of the pharmacist.

Formal pharmacy assistant education programs, like in the United States, include the study of medical and pharmaceutical terminology, pharmaceutical calculations, pharmacy recordkeeping, pharmaceutical techniques, and pharmacy law and ethics. They are also required to learn medication names, actions, uses, and doses. Many training programs include internships. After completion, the students receive a diploma, a certificate, or an associate’s degree, depending on the program. A national certification examination is also given to certify the pharmacy assistant’s formal education and training. The certification is voluntary in most States although some may
require it. In addition to this, pharmacy assistants must be recertified every 2 years, after undergoing 20 hours of continuing education within the 2-year certification period (Bureau of Labor Statistics, U.S. Department of Labor, 2007). In the study by Camu et al (2004), the major training process for pharmacy assistants in the country is on-the-job training. Examinations and seminars are generally not provided by community drugstores. For chain drugstores, aside from their all-college-graduate policy, they also have their own system of training, although often not standardized.

Pharmacy assistants, apart from fulfilling their routine duties, must also work in collaboration with health professionals, particularly the pharmacist, to ensure a patient-oriented care. This collaborative relationship will promote the safe use of medications, wellness, disease prevention and management of the disease. They must understand their roles as members of the health care team, even if they are usually not recognized as such. They must actively participate and contribute as a team to promote patient care (NAPRA, 2007).

The study attempts to provide answers to the following questions: 1) What is the extent of knowledge of pharmacy assistants on selected items of safe medication use?; 2) What are the prevalent attitudes of pharmacy assistants towards professional collaboration with the pharmacist?; and, 3) What are the means by which pharmacy assistants maintain and enhance their own competence?

METHODOLOGY

A descriptive, cross-sectional study was used in order to determine the perceptions of pharmacy assistants on their role in the community drugstore, their medication knowledge and their attitudes towards professional collaboration with the pharmacist. The choice of the participants was done on the basis of the location of the community drugstore. The drugstores to which they were affiliated are located nearby major hospitals in Manila (Philippine General Hospital, Ermita-Malate; Chinese General Hospital, Blumentritt; Manila Doctors Hospital, Ermita; University of Santo Tomas Hospital, Sampaloc; Jose Reyes Memorial Hospital, Tayuman; San Lazaro Hospital, Sta. Cruz; and, Mary Johnston Hospital, Tondo).

Responses were gathered with the use of a self-administered survey questionnaire, developed and pretested for this particular study. Pharmacists-in-charge or the managers were oriented with the purpose of the study and its methodology. The researchers distributed survey questionnaires to 120 pharmacy assistants from February 2009 to March 2009. A total of one hundred (83.33% participation rate) completed and returned the questionnaire. After the collection of survey forms, the data set was organized and summarized using Microsoft Excel. Further interpretation and analysis of the results had been done.
RESULTS

Demographic data of the participants

Table 1 shows the summary of the demographic characteristics of the participants, categorized according to size of the establishment. “Small” drugstores are those which are manned by not more than one pharmacy assistant; “medium” drugstores are those that are manned by two or more pharmacy assistants, but do not have satellite branches. “Large” drugstores are those that have at least one satellite branch.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Small sized drugstore</th>
<th>Medium sized drugstore</th>
<th>Large sized drugstore</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Male</td>
<td>6</td>
<td>18</td>
<td>9</td>
<td>17</td>
</tr>
<tr>
<td>Female</td>
<td>28</td>
<td>82</td>
<td>45</td>
<td>83</td>
</tr>
<tr>
<td>Age</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>15-20</td>
<td>9</td>
<td>26</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>21-30</td>
<td>20</td>
<td>59</td>
<td>39</td>
<td>72</td>
</tr>
<tr>
<td>31-40</td>
<td>2</td>
<td>6</td>
<td>9</td>
<td>17</td>
</tr>
<tr>
<td>&gt; 40</td>
<td>3</td>
<td>9</td>
<td>4</td>
<td>7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Educational attainment</th>
<th>No.</th>
<th>%</th>
<th>No.</th>
<th>%</th>
<th>No.</th>
<th>%</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elementary</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Secondary</td>
<td>17</td>
<td>50</td>
<td>15</td>
<td>28</td>
<td>0</td>
<td>0</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td>Vocational</td>
<td>4</td>
<td>12</td>
<td>4</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Undergraduate</td>
<td>4</td>
<td>12</td>
<td>3</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>College graduate</td>
<td>8</td>
<td>24</td>
<td>30</td>
<td>55</td>
<td>12</td>
<td>100</td>
<td>50</td>
<td>50</td>
</tr>
</tbody>
</table>

Pharmacy assistants’ roles and duties in a community pharmacy

Almost all (97%) of the respondents consider their work as an important part of community pharmacy practice. Majority (95%) also find their job interesting, enjoyable and worth doing. However, only slightly more than half (56%) of the respondents see themselves working full-time pharmacy assistants on a long-term basis.

Aside from their usual involvement in pharmacy operations, a considerable fraction (73%) of the study participants claimed that they engage in patient interview and patient needs assessment. Of these, majority said that they ask the patient’s primary complaints (75%) and collect other relevant information from the patients (62%). On their end, the information they provide to the clients includes the following: generic name (91%), brand name (88%), dose of
Pharmacy assistants’ medication knowledge

Twenty true-false questions were used to evaluate pharmacy assistants’ knowledge on drug use and safety. With a perfect score of twenty points, the mean score of the respondents is 10.43. Areas/topics covered in the tool include: anti-hypertensive medication use; antibiotic use; indications for antacids; medication storage for ointments, gels, suspensions, and syrups; proper use of NSAIDs and analgesics; and cough preparations. Individual scores were extremely diverse, ranging from 17% to 81%. Below is the list of knowledge items included in the tool with their corresponding percentage of respondents who provided correct response.

Table 2. Summary of percentages of correct responses per knowledge item

<table>
<thead>
<tr>
<th>Knowledge items</th>
<th>% Correct Responses</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Antihypertensive drugs can be discontinued when blood pressure returns to</td>
<td>41</td>
</tr>
<tr>
<td>normal range.</td>
<td></td>
</tr>
<tr>
<td>2. Use of antibiotics can be stopped by the patient when the symptoms of fever</td>
<td>59</td>
</tr>
<tr>
<td>and sore throat are relieved.</td>
<td></td>
</tr>
<tr>
<td>3. Paracetamol can be used liberally without expected side effects.</td>
<td>37</td>
</tr>
<tr>
<td>4. Some tablets, like antacids, are to be chewed before swallowing.</td>
<td>79</td>
</tr>
<tr>
<td>5. Antacids should be added into all prescriptions to avoid gastrointestinal</td>
<td>41</td>
</tr>
<tr>
<td>upset.</td>
<td></td>
</tr>
<tr>
<td>6. Vitamins are health food so they do not cause negative effects to human body.</td>
<td>21</td>
</tr>
<tr>
<td>7. Storing ointment/gel in the refrigerator could extend the expiration date.</td>
<td>62</td>
</tr>
<tr>
<td>8. Shelf life of suspension can be extended if kept inside the refrigerator.</td>
<td>39</td>
</tr>
<tr>
<td>9. Dosage of cough syrup is one bottle per use.</td>
<td>69</td>
</tr>
<tr>
<td>10. Food/drinks/tea/alcohol may interfere with the effects of medicine.</td>
<td>46</td>
</tr>
<tr>
<td>11. Pregnant women are not allowed to take antibiotics.</td>
<td>45</td>
</tr>
<tr>
<td>12. Flu-like symptoms must be treated with antibiotics readily.</td>
<td>56</td>
</tr>
<tr>
<td>13. Enteric coated tablets can be crushed.</td>
<td>38</td>
</tr>
<tr>
<td>14. Tetracyclines can be taken with milk and other dairy products.</td>
<td>49</td>
</tr>
<tr>
<td>15. Patients may swallow chewable tablets even without chewing them.</td>
<td>28</td>
</tr>
<tr>
<td>16. Antibiotics can be used for fever.</td>
<td>77</td>
</tr>
<tr>
<td>17. Patients can take NSAIDS such as mefenamic acid or ibuprofen on an empty</td>
<td>72</td>
</tr>
<tr>
<td>stomach.</td>
<td></td>
</tr>
<tr>
<td>18. Mucolytics can be used for dry cough.</td>
<td>15</td>
</tr>
</tbody>
</table>
19. One can double the next dose to compensate for the missed dose. 64
20. Effervescent tablets can be taken directly without dissolving in water. 63

Professional collaboration between pharmacy assistants and pharmacists

Items on professional collaboration utilized a 5-point Likert Scale (5= always, 4= usually, 3= sometimes, 2= seldom, 1=never). The scale was reversed for negative statements. Table 3 presents the respondents’ mean rating to each of the 12 collaboration statements. Higher scores represents more positive attitude towards professional collaboration, except for statements number 8 and 11, where the scale was reversed.

Table 3. Rating for professional collaboration activities by average

<table>
<thead>
<tr>
<th>Professional Collaboration Activities</th>
<th>Mean Rating</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Participation in activities of drugstore to promote patient health</td>
<td>4.09</td>
</tr>
<tr>
<td>2 Sharing knowledge on relevant information to clients</td>
<td>4.02</td>
</tr>
<tr>
<td>3 Sharing knowledge on relevant information to co-workers</td>
<td>4.22</td>
</tr>
<tr>
<td>4 Accepting help and guidance from co-workers to optimize health outcomes</td>
<td>4.35</td>
</tr>
<tr>
<td>5 Contributing for the achievement of determined goals and objectives</td>
<td>4.13</td>
</tr>
<tr>
<td>6 Recognition and referral of matters to pharmacists requiring knowledge, skills, and abilities of a pharmacists</td>
<td>4.43</td>
</tr>
<tr>
<td>7 Understand and participate in, and promote patient safety initiatives</td>
<td>4.16</td>
</tr>
<tr>
<td>8 Giving medicines to consumer without a doctor’s prescription</td>
<td>2.59*</td>
</tr>
<tr>
<td>9 Asking the pharmacist when unable to read the prescription</td>
<td>4.53</td>
</tr>
<tr>
<td>10 Consultation with the pharmacist before giving medicines without prescription to consumers</td>
<td>4.56</td>
</tr>
<tr>
<td>11 Giving advice to the consumer to try other medicines without the consent of the pharmacist</td>
<td>2.16*</td>
</tr>
<tr>
<td>12 Consultation with the pharmacist when they receive a special and uncommon drug</td>
<td>4.67</td>
</tr>
</tbody>
</table>

*Scale was reversed for negative statements.

Pharmacy assistants’ education and training

Majority (80%) claimed to have had formal training related to their work as pharmacy assistants before or during their employment. Of this majority, 59 (74%) had on-the-job training; 27 (34%) attended seminars; and, 19 (24%) had examination. From those who had examinations, 9 had oral exam, 17 had written exam and 10 claimed to have undergone practical exam. For the majority (63%) of trained pharmacy assistants, the licensed pharmacists of the drugstore were the ones who conducted the training program. The drugstore owner also served as the trainor for 31 (39 %) respondents.
There are also pharmacy assistants that claimed to have been trained by medical representatives, senior dispensers, representatives from organizations (e.g. Drugstore Association of the Philippines), and main office personnel for “large” drugstore. The length of training period of pharmacy assistants was extremely diverse, ranging from 1 day to 24 months. The training period for most respondents is 14 days.

Eighty one percent (81%) of the respondents believed that they have acquired sufficient knowledge and training to perform their duties as pharmacy assistants. However, 39% said that they do not have monthly or yearly examinations given by the establishment that may motivate them to update their knowledge and skills. Reading materials, such as the MIMS (81%) and product leaflets (69%), serve as the main self-learning activities of pharmacy assistants in augmenting their knowledge on drugs. When asked to rate their overall knowledge on drugs with 5 being the highest score, the respondents’ mean rating is only 3.05.

Seventy percent (70%) of the respondents thought there is a need to improve the training programs for pharmacy assistants to maximize their potential and improve their competence. The respondents suggested that there should be more seminars and trainings on medication use.

DISCUSSION

The present set of data strongly indicates that pharmacy assistants’ knowledge is less than satisfactory, although majority of them thought that they have acquired sufficient knowledge and training to perform their duties. This can possibly be attributed to many factors. One, the emphasis of pharmacy assistant training may be heavily tilted on the non-technical, non-cognitive aspects of the practice, such as order taking, actual dispensing of items and item stocking. There is the absence of formal training that truly equips pharmacy assistants when it comes to the more technical roles that involve patients’ items of interest, such as in counseling and in educating them about indications of medicines and description of drug action. Almost all the drugstores, regardless of size, implemented training for their pharmacy assistants. However, their method varies and may not generally cover all the necessary and appropriate items, as suggested by the knowledge scores and as indicated by the respondents themselves.

In this study, it was also found out that pharmacy assistants have positive attitudes and practices toward professional collaboration. Almost all respondents in this study recognized the practice situations within the collaborative relationship that require pharmacist intervention and those that are team based in nature. However, it is important to mention that this study only utilized self-administered questionnaires. Biases are to be expected to have influenced the trends in responses, most especially that the collaboration item responses were not validated by actual observation due to limited time and resources.

Moreover, although pharmacy assistants showed positive attitudes toward the necessity of medication consultation, more than half still dispenses prescription medicines to consumers without a doctor’s prescription and almost half of them advise their consumer to try out medicines and other products without the consent of a pharmacist. Efforts to improve pharmacy assistants’ medication dispensing practices must be implemented.
CONCLUSION AND RECOMMENDATIONS

There are various methods that could be implemented to address the problems identified in this study. First and foremost, in this era of patient-centered pharmaceutical care, pharmacy assistants need to be formally acknowledged as members of the health care system. They should be properly integrated into the health care team to maximize their potential as partners of other professionals in providing quality care for better health outcomes. With this comes the notion that they must take more responsibility for proper medication use, still under the supervision of a competent pharmacist. Pharmacists then need not only to teach pharmacy assistants how to dispense drugs but also to teach about the proper use of medications and other information that are necessary to benefit the consumers. Pharmacy assistants must work hand in hand with the pharmacists to promote and protect public health. Interventions to improve drug knowledge and safe medication practices, such as providing medication information, behavioral simulation, or even cognitive intervention, should be made continuously by pharmacists to improve the dispensing practice.

There is certainly a room for the establishment of education framework and standard training protocol for pharmacy assistants in the country in order to ensure competence and continued professional growth. Certification that verifies competence of a pharmacy assistant at entry level can also be considered, using existing international models. Moreover, to increase the quality of work of pharmacy assistants, it is essential to give focus on maintaining and upgrading their skills by providing the pharmacy assistants continuing education opportunities.

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National Association of Pharmacy Regulatory Authorities. 2007. Professional Competencies for Canadian Pharmacy Technicians at Entry to Practice. Ottawa, Canada, NAPRA.
Need for uniform national standards for the education and training of pharmacy technicians.


Retrospective Drug Utilization Study on NSAIDs in Osteoarthritic Elderly Patients in a Tertiary Government Hospital

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*This paper was presented at the 12th Asian Conference on Clinical Pharmacy, Hong Kong, SAR China in 2012.

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ABSTRACT

Management of chronic pain in the elderly should be carefully tailored. This study evaluated the utilization patterns of non-steroidal anti-inflammatory drugs (NSAIDs) in a government tertiary hospital for geriatric patients with osteoarthritis. A retrospective descriptive research was carried out by reviewing medical records of osteoarthritic patients aged 60 years or older who received NSAID therapy during hospital stay between 1 September 2009 and 30 September 2011. Patients who had undergone joint replacement surgery were excluded. Medical charts of twenty-four (24) patients were reviewed for compliance with standard clinical practice guidelines by identifying drug therapy problems. The identified problems were over-utilization of celecoxib, etoricoxib, and naproxen (66.7%), administration of NSAIDs in patients for whom NSAID therapy is contraindicated (58.3%), possible adverse NSAID-related events (25%), and administration of NSAIDs not recommended for the elderly (4.2%).

Keywords
Non-steroidal anti-inflammatory drugs, osteoarthritis, drug utilization, elderly
INTRODUCTION

Osteoarthritis (OA) is a degenerative joint disease mainly affecting the articular cartilage. It is the most prevalent joint disease worldwide and the leading cause of pain and disability in the elderly. The condition is characterized clinically by joint pain on loading, tenderness, crepitation, limited movement, occasional effusion, and variable degrees of inflammation (Woolf, 2003). Risk factors for developing the disease include non-modifiable factors such as age, gender, genetics, and race; and modifiable factors such as trauma, vocational factors, congenital musculoskeletal abnormalities, and obesity.

Non-steroidal anti-inflammatory drugs (NSAIDs) are one of the drug classes prescribed for managing pain related to OA due to their analgesic, anti-inflammatory, and antipyretic properties. Their mechanism of action involves blocking prostaglandin synthesis through the inhibition of cyclooxygenase (COX) enzymes. Prostaglandins are responsible for mediating vasodilation, which leads to inflammation and pain. A specific NSAID subclass known as COX-2 inhibitors (COXIBs) functions by inhibiting only the COX-2 isoform, which is specific for inflammation. The COXIB class was developed to provide benefits equal to traditional NSAIDs with decreased GI distress. NSAIDs should be carefully prescribed for populations who are at greater risk of experiencing the adverse effects associated with these drugs. For the elderly, NSAIDs must be given with caution because they are at greater risk of experiencing serious GI bleeding and perforation, which may even become fatal.

This study aimed to evaluate the NSAIDs utilization pattern in osteoarthritic geriatric patients admitted in a tertiary government hospital. Specifically, the study aims to: compare the patterns of NSAID utilization in OA with local and international standard clinical practice guidelines; identify drug therapy problems associated with NSAID use by examining presence of adverse drug events, over-utilization, therapeutic duplication, contraindications, drug-drug interactions; and, determine the number of patients who are and are not receiving appropriate NSAID therapy with respect to their age and co-morbidities.

METHODOLOGY

The study employed a retrospective descriptive research design and was conducted in a tertiary government hospital. Review of records was utilized to collect data. A census was conducted to obtain the target population of this study. The following inclusion criteria were followed: (1) patient was aged 60 years and above at the time of hospitalization; (2) patient had a diagnosis or at least a present working impression of osteoarthritis at the time of hospitalization; (3) patient had been prescribed with NSAIDs during admission; and, (4) patient had been admitted in the study hospital’s Pay Ward or Charity Ward anytime between September 1, 2009 and September 30, 2011. Patients who underwent joint replacement surgery or were prescribed NSAIDs for indications other than osteoarthritis were excluded. In such cases, it was assumed...
that their NSAID therapy was to manage conditions other than OA-related pain, such as post-operative pain or fever.

Prior to accessing the required medical records, the approval of the Expanded Hospital Research Office (EHRO) of the hospital was first secured for technical and ethical clearance. Data was collected from October 2011 to January of 2012. Retrieval of records began from the archived indices of in-patient cases, which are alphabetically arranged according to diagnosis upon discharge. Indices containing any form of osteoarthritis were selected. Individual case numbers of patients aged 60 and above who were admitted within the stated time period were then copied from each index to create locators for the retrieval. The Medical Records Division (MRD) staff used the locators to retrieve the actual records from the physical archive after verifying that these records are present in their computerized database. Upon retrieval, records were processed to determine inclusion, and electronic copies were made for the included cases. The electronic copy contained only the necessary information for the parameters in evaluating the NSAID utilization patterns. Compliance to standard clinical practice guidelines was determined from the physician’s orders, the nurse’s narrative notes and the therapeutic drug sheet of the patient records. A tally sheet was used to determine the frequency of the drug therapy problems encountered. Any medical records that the MRD staff failed to retrieve due to discrepancies between their physical archives and computer database were excluded.

Two instruments were employed to analyze the data taken from the patient medication charts. The first instrument was a table of drug therapy problems associated with NSAID use. The NSAIDs included were (1) those present in either the core list or complementary list of the Philippine National Drug Formulary (2008), and (2) drugs recommended by the 2009 American Geriatric Society Guidelines. The presence of any one of the drug therapy problems listed for a single drug signaled irrational NSAID therapy. Information used in the tool was gathered from multiple resources including the AGS Guidelines, A-Z Drug Facts and US FDA Professional Information from Drugs.com. The second instrument was a treatment algorithm that served as a guide to determine how well the patient’s prescribed therapy complied with standard clinical practices. This algorithm was adapted from the clinical practice guidelines of the Osteoarthritis Research Society International, the American College of Rheumatology, the American Geriatrics Society, and the Philippine Rheumatology Association. If the patient’s therapy did not comply with any of the steps outlined in the algorithm, this would also signal irrational NSAID therapy.

Microsoft Excel (Microsoft Corporation, 2010) was used to analyze the data obtained from the patient medication charts. Raw data was double checked against the soft data for consistency. Descriptive statistics such as percentages (of frequencies), mean, and mode values of each drug therapy problem were calculated. The number of patients who did and did not receive appropriate NSAID therapy with respect to their age and co-morbidities were also determined.
RESULTS AND DISCUSSION

The medical record indices used for the case search yielded 62 results for patients with OA aged at least 60 years old and admitted between September 1, 2009 and September 30, 2011. Further examination of the individual medical records led to the exclusion of 38 (61%) cases based on the inclusion/exclusion criteria. Of the excluded records, 8 (21%) had no documented NSAID use for OA and 19 (50%) had undergone either arthroplasty or joint replacement surgery. The 11 cases (29%) which the MRD staff failed to retrieve due to archive-database discrepancies were also excluded.

The final study population consisted of 24 cases including both male and female subjects. Majority of the subjects (67%) were female. The mean age was 73. Of these cases, 4 (17%) were charity patients while 24(83%) were pay patients. The average length of hospital stay for management of the disease is 7 days. The subtypes of osteoarthritis among the cases were as follows: 14 (58%) degenerative OA; 9 (37.5%) knee OA; and 4 (17%) OA involving both knees. There were 4 unclassified cases. Only 1 (4%) of the 24 patients had documented use of alternative therapy, specifically supplements. The use of paracetamol or paracetamol-tramadol, alone or in combination with other drugs, were noted in 12 cases (50%). Three (12.5%) were noted to have used topical NSAIDs during their hospital stay. In the two-year period covered by the study, there were only a small number of patients with OA admitted in the hospital. This may be because two-thirds of Filipinos with osteoarthritis prefer to purchase over-the-counter medications or avail of alternative therapies to manage their pain rather than visit a doctor or have themselves admitted (Lu et al., 2011).

Compliance to standard clinical practice guidelines was determined among the 24 patients that met the criteria. Only 7 (29%) were considered compliant. Table 1 shows the reasons for non-compliance and their corresponding number of cases. The use of COXIBs in patients with a history of hypertension was noted in 13(76%) cases, which violated the standard clinical guidelines in elderly patients with cardiovascular risk factors (McGettigan & Henry, 2006). The use of COXIBs may further increase the risk of cardiovascular complications in these patients. Among these 17 cases, 2 (12%) also had chronic kidney disease which makes the use of COXIBs less advisable. Of the cases with noted COXIB use, 5 were prescribed etoricoxib, which is not recommended for elderly patients with OA (National Institute for Health and Clinical Excellence, 2008).

<table>
<thead>
<tr>
<th>Issue of non-compliance</th>
<th>No. of Cases</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Use of COXIBs in patients with CVS risk factors</td>
<td>13</td>
<td>76.0</td>
</tr>
<tr>
<td>Use of COXIBs without prior use of paracetamol noted</td>
<td>11</td>
<td>65.0</td>
</tr>
<tr>
<td>Use of etoricoxib</td>
<td>5</td>
<td>29.0</td>
</tr>
<tr>
<td>Use of COXIBs in patients with renal risk factors</td>
<td>2</td>
<td>12.0</td>
</tr>
<tr>
<td>Use of naproxen without prior use of paracetamol noted</td>
<td>1</td>
<td>6.0</td>
</tr>
<tr>
<td>Use of NSAIDs in patients with renal risk factor</td>
<td>1</td>
<td>6.0</td>
</tr>
</tbody>
</table>

Among the drug therapy problems identified in the study (See Table 2), the most common was the over-utilization of the celecoxib, etoricoxib, and naproxen as seen in 16 (66.7%) cases. Encountered contraindications to NSAID therapy are listed in Table 3. There were 14 instances of NSAIDs prescribed to patients with pre-existing hypertension, accounting for half of the total number of NSAIDs prescribed in all cases. Hypertension is a relative contraindication for both selective and non-selective NSAIDs (American Geriatrics Society, 2009). Other contraindications are gastrointestinal bleeding and chronic kidney disease, for which 3 (10.7%) instances and 1 (3.6%) instance of NSAID prescription occurred, respectively. The latter condition is an absolute contraindication, in which case the patient should have been prescribed a drug from a different class of analgesics. Addressing pain while aggravating these conditions may be less beneficial for the elderly patient and even lead to further disability or death.
RESULTS AND DISCUSSION

The medical record indices used for the case search yielded 62 results for patients with OA aged at least 60 years old and admitted between September 1, 2009 and September 30, 2011. Further examination of the individual medical records led to the exclusion of 38 (61%) cases based on the inclusion/exclusion criteria. Of the excluded records, 8 (21%) had no documented NSAID use for OA and 19 (50%) had undergone either arthroplasty or joint replacement surgery. The 11 cases (29%) which the MRD staff failed to retrieve due to archive-database discrepancies were also excluded.

The final study population consisted of 24 cases including both male and female subjects. Majority of the subjects (67%) were female. The mean age was 73. Of these cases, 4 (17%) were charity patients while 24 (83%) were pay patients. The average length of hospital stay for management of the disease is 7 days. The subtypes of osteoarthritis among the cases were as follows: 14 (58%) degenerative OA; 9 (37.5%) knee OA; and 4 (17%) OA involving both knees. There were 4 unclassified cases. Only 1 (4%) of the 24 patients had documented use of alternative therapy, specifically supplements. The use of paracetamol or paracetamol-tramadol, alone or in combination with other drugs, were noted in 12 cases (50%). Three (12.5%) were noted to have used topical NSAIDs during their hospital stay. In the two-year period covered by the study, there were only a small number of patients with OA admitted in the hospital. This may be because two-thirds of Filipinos with osteoarthritis prefer to purchase over-the-counter medications or avail of alternative therapies to manage their pain rather than visit a doctor or have themselves admitted (Lu et al., 2011).

Compliance to standard clinical practice guidelines was determined among the 24 patients that met the criteria. Only 7 (29%) were considered compliant. Table 1 shows the reasons for non-compliance and their corresponding number of cases. The use of COXIBs in patients with a history of hypertension was noted in 13 (76%) cases, which violated the standard clinical guidelines in elderly patients with cardiovascular risk factors (McGettigan & Henry, 2006). The use of COXIBs may further increase the risk of cardiovascular complications in these patients. Among these 17 cases, 2 (12%) also had chronic kidney disease which makes the use of COXIBs less advisable. Of the cases with noted COXIB use, 5 were prescribed etoricoxib, which is not recommended for elderly patients with OA (National Institute for Health and Clinical Excellence, 2008).

Among the drug therapy problems identified in the study (See Table 2), the most common was the over-utilization of the celecoxib, etoricoxib, and naproxen as seen in 16 (66.7%) cases. Encountered contraindications to NSAID therapy are listed in Table 3. There were 14 instances of NSAIDs prescribed to patients with pre-existing hypertension, accounting for half of the total number of NSAIDs prescribed in all cases. Hypertension is a relative contraindication for both selective and non-selective NSAIDs (American Geriatrics Society, 2009). Other contraindications are gastrointestinal bleeding and chronic kidney disease, for which 3 (10.7%) instances and 1 (3.6%) instance of NSAID prescription occurred, respectively. The latter condition is an absolute contraindication, in which case the patient should have been prescribed a drug from a different class of analgesics. Addressing pain while aggravating these conditions may be less beneficial for the elderly patient and even lead to further disability or death.

Table 1. Non-compliance issues and the number of cases

<table>
<thead>
<tr>
<th>Issue of non-compliance</th>
<th>No. of Cases</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Use of COXIBs in patients with CVS risk factors</td>
<td>13</td>
<td>76.0</td>
</tr>
<tr>
<td>Use of COXIBs without prior use of paracetamol noted</td>
<td>11</td>
<td>65.0</td>
</tr>
<tr>
<td>Use of etoricoxib</td>
<td>5</td>
<td>29.0</td>
</tr>
<tr>
<td>Use of COXIBs in patients with renal risk factors</td>
<td>2</td>
<td>12.0</td>
</tr>
<tr>
<td>Use of naproxen without prior use of paracetamol noted</td>
<td>1</td>
<td>6.0</td>
</tr>
<tr>
<td>Use of NSAIDs in patients with renal risk factor</td>
<td>1</td>
<td>6.0</td>
</tr>
</tbody>
</table>

Table 2. Cases of drug therapy problems

<table>
<thead>
<tr>
<th>Drug Therapy Problem</th>
<th>No. of Cases</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Over-utilization</td>
<td>16</td>
<td>66.7</td>
</tr>
<tr>
<td>Contraindicated NSAID therapy</td>
<td>14</td>
<td>58.3</td>
</tr>
<tr>
<td>Adverse events related to NSAID use</td>
<td>6</td>
<td>25.0</td>
</tr>
<tr>
<td>Non-recommended drug therapy</td>
<td>4</td>
<td>16.7</td>
</tr>
</tbody>
</table>
Over-utilization of NSAIDs is another major concern. The recommended daily dose for celecoxib in the elderly should not exceed 100 mg (American Geriatrics Society, 2009). However, it was found that for the 12 times celecoxib was prescribed, the daily dose averaged 333.33 mg (See Table 4). This is 3.3 times the maximum daily dose, with some patients being given up to 400 mg per day. The average maximum daily dose for etoricoxib (in non-elderly populations) and naproxen also exceeded their recommended doses by 2 times and 2.3 times, respectively. It has been recommended in the AGS guidelines that once high doses of COXIBs are being used, a shift to opioid therapy should be considered since these patients may be at greater risk for NSAID-related adverse effects.

Table 3. Contraindications to NSAID therapy

<table>
<thead>
<tr>
<th>Contraindications</th>
<th>Related Drug/s</th>
<th>No. of Cases</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertension</td>
<td>Celecoxib, etoricoxib,</td>
<td>14</td>
<td>50.0</td>
</tr>
<tr>
<td></td>
<td>naproxen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chronic kidney disease</td>
<td>Celecoxib, etoricoxib,</td>
<td>3</td>
<td>10.7</td>
</tr>
<tr>
<td></td>
<td>naproxen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gastrointestinal bleeding</td>
<td>Celecoxib</td>
<td>1</td>
<td>3.6</td>
</tr>
</tbody>
</table>

Table 4. Average documented daily dose of administered NSAIDs

<table>
<thead>
<tr>
<th>Drug Used</th>
<th>Average Daily Dose</th>
<th>Recommended Maximum Daily Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Celecoxib</td>
<td>333.33 mg</td>
<td>100.00 mg</td>
</tr>
<tr>
<td>Paracetamol</td>
<td>2730.00 mg</td>
<td>4000.00 mg</td>
</tr>
<tr>
<td>Etoricoxib¹</td>
<td>120.00 mg</td>
<td>60 mg (Not recommended)</td>
</tr>
<tr>
<td>Naproxen¹</td>
<td>1 000 mg</td>
<td>440.00 mg</td>
</tr>
</tbody>
</table>

¹The doses and frequencies for some cases were not indicated in the medical records.

More than one factor could be at play in the non-compliance events. Firstly, health professionals may not be aware of the recommended maximum daily doses. It is often the case that hospitals do not have their own practice guidelines for pain management in elderly patients. The default reference then would be the American-based guidelines. A second possibility could be the aggressive pain management intended to decrease the patient’s stress, which may negatively affect their health perception and recovery instead. Healthcare providers should reconsider this practice. Although it may temporarily improve the patient’s quality of life, it might also initiate detrimental long-term effects on the cardiovascular system, especially if the patient already has an existing cardiovascular disease. Lastly, available dosage strengths of these drugs in the market far exceed the maximum daily doses recommended for geriatric populations.
Prescribers are encouraged to exercise more restraint in their therapeutic recommendations for the elderly while considering the dosage strengths present in the market.

Conditions which may be considered as adverse events related to the use of NSAIDs also surfaced during the hospital stay of some case patients. Hypertension is the most commonly occurring possible adverse event and may be associated with celecoxib use, occurring in one-third of the cases for which celecoxib was prescribed. Gastrointestinal bleeding associated with naproxen use is another possible adverse event which occurred in 1 out of the 2 instances it was prescribed. In addition, one of the patients who developed hypertension during the course of celecoxib therapy also developed anemia, which is another possible adverse event of celecoxib use. Another patient also developed anemia after having used the prescribed etoricoxib therapy.

At the first sign of adverse events in elderly patients on NSAID therapy, health practitioners should discontinue the suspected precipitating drug and prescribe alternatives associated with fewer risks. However, this retrospective study cannot establish the causal relationship between these adverse events and NSAID therapy.

Out of the 24 cases, 2 were admitted in the hospital for NSAID-induced gastropathy, which manifested as upper gastrointestinal bleeding resulting in melena, hematochezia, and hematemesis. The American College of Gastroenterology recommends a proton-pump inhibitor as the agent of choice in treating NSAID-induced gastropathy in situations where the NSAID cannot be discontinued because the therapy is necessary (Lanza et al., 1998). For those 2 cases, omeprazole was prescribed for concomitant administration with NSAID therapy, which means the clinicians handled those cases according to what the guidelines have recommended. One of the case patients was admitted for NSAID-induced nephropathy, probably induced by taking NSAIDs for established OA. The precipitating drug was not mentioned in the patient’s record, but during therapy only paracetamol was administered on an as-needed basis with four-hour spacing, indicating compliance with the existing guidelines.

**CONCLUSION**

The use of NSAIDs in elderly patients is of concern to clinicians since improper use may lead to serious adverse drug reactions such as kidney failure, liver failure, ulcers and prolonged bleeding after an injury or surgery. It may also increase the risk for diseases such as congestive heart failure in patients who have existing cardiovascular risk factors. In this study, results revealed that the most commonly prescribed NSAID for osteoarthritis is celecoxib. It also has the highest number of drug therapy problems such as over-utilization, which occurred in all cases where it was prescribed. This is particularly dangerous since elderly patients have altered pharmacokinetics, increasing their susceptibility to adverse drug events with higher dosing. Of the 24 cases, only seven were considered compliant based on the standard clinical practice guidelines.

This study has recognizable limitations, particularly in its population coverage. Any information about a patient’s drug therapy prior to their hospital stay could also not be
determined if it was not declared by the patient and documented in the medical record by the healthcare provider. To increase the validity of the study’s results, a larger population of patients from many health institutions and spanning a timeframe longer than two years is recommended. The use of a centralized computer database for managing hospital records may also help make the retrieval more efficient and give more accurate and complete information in less time. Records from outpatient departments can also contribute more knowledge about the utilization of NSAIDs and its long-term effects, which cannot be observed in short hospital stays.

The results of this study may help raise awareness among clinicians and prescribers about the importance of international and local guidelines on NSAID therapy in the elderly, and the possible adverse effects of improper NSAID utilization. This may also serve as a rationale for the development of a local clinical practice guideline for elderly osteoarthritis patients.

REFERENCES


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The results of this study may help raise awareness among clinicians and prescribers about the importance of international and local guidelines on NSAID therapy in the elderly, and the possible adverse effects of improper NSAID utilization. This may also serve as a rationale for the development of a local clinical practice guideline for elderly osteoarthritis patients.

REFERENCES


Assuring quality when sourcing pharmaceutical excipients
Ray R. Marcuelo

ABSTRACT

The USP24 /NIF 19 lists 43 categories or functionality roles of excipients. Previously considered inert or inactive substances, excipients have gained global attention because of their significant role in drug absorption, stability and safety of drug products.

The paper identifies roles of the key players in the distribution chain. It stresses the need for approval mechanisms for excipients, the task of the excipient producer to establish impurity profile, the need for functionality-related internal specifications and control of manufacturing process changes. Some experiences drawn from developing countries are cited.

INTRODUCTION

The process of sourcing excipients is gaining immense global attention as primary component in assuring the quality, safety and efficacy of finished drug products.

These excipients constitute a bigger bulk by volume or by weight in formulations as compared to the amount of active ingredients. Production processes do not usually require any treatment such as further purification processes of the excipients and therefore, there is a great risk that any contamination present in the excipients will remain in the finished dosage form.

The well-known tragic incident in 1996, when 80 Haitian children died after ingestion of pediatric syrup was due to the use of glycerin that was contaminated with diethylene glycol, a toxic solvent used in motor anti-freeze.

The unfortunate incident triggered the need for better regulatory control of pharmaceutical excipients.

A review of controls applied to excipients a gap in the quality assurance chain, specifically during the transit time of excipients where distributors or even subdistributors are involved. The vendor qualification process and the GMP requirements for receipts, testing and release after the material reaches the drug manufacturer are valuable but are inadequate in detecting possible quality failures that could happen while the excipient is on transit.

SOURCING AND THE DISTRIBUTION CHAIN

The USA and Europe supply the majority of the excipients used by the companies in developing countries such as the Philippines, Indonesia, Thailand and Vietnam.

Some materials from Europe or the USA undergo transshipments to different countries such as Singapore, Japan and Taiwan. The materials may remain from 4 to 10 days in Singapore or Japan and at least 4 days in Taiwan while waiting for smaller ships to transport these materials to the
Philippines. Flavors that are used in small quantities are usually shipped by air and are delivered directly to the warehouse of the end user.

Common excipients manufactured in Vietnam, Indonesia and the Philippines are sugar and alcohol. Indonesia produces other excipients such as citric acid, magnesium stearate, glycerin, sodium citrate, sorbitol solution, sodium saccharin, stearic acid and sodium lauryl sulfate. Glycerin is also produced in the Philippines.

The modes of distribution in the Philippines do not differ significantly from other countries. Materials may be brought directly from the producer to the plant of the end user in bulk or in packaged units. Glycerin, for example is delivered in bulk direct to the storage tanks of the end user. Depending on agreed transactions, excipients may pass through distributors or several traders before they reached the end user.

Regulatory controls applied to distributors of excipients are not defined in many countries since excipients are not exclusively for pharmaceutical use. Several excipients find multiple uses also in food, cosmetic and other industrial applications. The excipients market for pharmaceutical use such as sugar is generally much smaller compared to the usage in the food industry. There is a requirement for distributors of pharmaceutical raw materials in Indonesia to have an analytical laboratory so that a license could be granted.

EXCIPIENTS REDEFINED

When one browses chapters of old pharmacy textbooks, excipients were viewed simply as pharmaceutical acids, pharmaceutic necessities or described as inactive ingredients. Owing to several technological advances, excipients have taken on myriad of functions from simple roles such as filler of diluent to more complex roles such as drug stabilizer, an enhancer or modifier of the activity of a drug in pharmaceutical dosage form. Flavors increase palatability while colors add aesthetic appeal. When flavors and colors are used creatively, formulators may help increase patient acceptability and sales such as that of a pediatric preparation. The USP/NF 19 lists 43 categories or functionality roles of excipients.

The International Pharmaceutical Excipients Council (IPEC), an umbrella organization comprised of IPEC-Americas, IPEC-Europe and IPEC-Japan has come out with the following comprehensive definition:

*Excipients are any substance other than the active drug substance or finished dosage form, that have been appropriately evaluated for safety and are included in drug delivery system to either aid the processing of the drug delivery system during its manufacture, protect, support or enhance stability, bioavailability, or patient acceptability, assist in product identification, or enhance any other attribute of the overall safety and effectiveness of the drug delivery system during storage or use.*
STRATEGIES IN ASSURING QUALITY

Following the basic concept of GMP, the quality of the excipients must be built into the excipient during its manufacture. Producers are responsible for supply of excipients that meet the quality, safety and purity specified in compendial monographs and/or manufacturer’s specifications. This original or producer’s quality must be protected and preserved during the handling, storage and transport of the material so that it is received by the end user without any alteration or deterioration in quality, safety and purity. The tragic deaths caused by contaminated glycerin in Haiti brought together the pharmaceutical and chemical industries and regulatory agencies to look closely at the manufacturing and distribution practices for excipients.

Assuring quality of pharmaceutical excipients depends on the collective performance of the key players, the producers, the distributors, traders, and the end users. Pharmaceutical organizations and regulatory agencies must assure that proper legislation and support systems are in place to protect public health and safety.

Control incoming shipments

Inspection and testing of incoming shipments is the responsibility of the end user. While this is a standard practice, it is a post-activity, meaning, it is done on a finished excipient and cannot change or improve the excipient’s original quality. Testing excipients prior to use, however, is of paramount importance in preventing the use of substandard or adulterated excipients in pharmaceutical formulations. The contamination of glycerin in Haiti incident would have been detected by proper testing prior to its use.

Establish an impurity profile

It is the duty of the manufacturer of the pharmaceutical excipients to identify and set appropriate limits for impurities.

IPEC’s definition of excipients has aptly required safety evaluation as a pre-requisite in the quality of the excipients, which are added to pharmaceutical formulations.

Highly relevant to the safety is the adequate and consistent purity of the excipient. The purity of the excipient can also affect the stability of some drug products.

Risk detection and the careful analysis of each of the processes used in the production will provide a sound basis for the excipient manufacturer to address safety issues and establish an impurity profile. Some impurities result from natural sources of excipients.

IPEC-Americas Significant Change Guide For Pharmaceutical Excipients specify the following items for inclusion in an impurity profile:

- All identified organic impurities
- Unidentified organic impurities at or above 0.1% whether specified or not
- Residual solvents
- Inorganic impurities
Toxic impurities

From an end user’s viewpoint on safety, a Type 4 DMF covering the Chemistry, Manufacturing and Control and type 5 Drug Master File which includes the impurity profile should be submitted. The impurity profile will be useful in setting objective criteria for determining the significance of a change in the processing of excipient.

Residual solvents have been addressed in the International Conference on the Harmonization (ICH) Guideline for Residual Solvent Q3C which includes a categorization of the residual solvents by risk assessment. Residual solvents are defined in the guideline as organic volatile chemicals that are used or produced in the manufacture of drug substance or excipients, or in preparation of drug products.

Analyzing impurities may range from a simple observation of a physical change to the use of more sophisticated chromatographic methods. Some laboratories especially in developing countries may not be equipped with capital-intensive instruments to conduct chromatographic tests for impurities. Producers of the excipients may provide assistance to the users acquiring standards and may relieve the lab of time-consuming tests for providing certificates of analysis that conform to the elements stated in the IPEC-Americas Certificate of Analysis Guide for Bulk Pharmaceutical Excipients.

Establish approval systems for excipients

In the USA, an excipient may be sold for use in food or pharmaceutical formulations by qualifying it through approval mechanisms available for components used in food and/or finished drug dosage forms (Title 21 CFR, Parts 182, 184, 186)

In Japan, a new evaluation system for excipients issued by the Ministry of Health and Welfare (WHW) became effective in July 1997. The General Rule for Preparations stated in the Japanese Pharmacopeia states that “excipients must be harmless at a regular dosage levels and not interfere with the therapeutic efficacy nor the testing of the preparations.” Excipients are therefore evaluated on the review of the drug product and not as an excipient per se.

Applications for the approval of drug products include:

1. The reasons for inclusion of the excipient in the drug product.
2. Any precedents of the use of the excipient.
3. Appropriate description of the quality standards.

Excipients considered safe are substances used domestically in Japan for pharmaceutical products, for identified route of administration and within the maximum dosage level of prior instance of use. New substances are considered new excipients. Existing excipients, even without prior instance of use, are considered new if the route of administration is different and dose levels exceed instances of prior use in pharmaceutical in Japan.
Establish functionality-related internal specifications

Formulators and process development scientists should identify parameters critical to the functionality role of the excipient. A consistent quality of excipients is an important element in the reproducibility of the production batches. Batch-to-batch or manufacturer-to-manufacturer variability of the excipients, particularly on the physical properties of the excipient such as particle size, particle shape, specific surface area, bulk density properties of excipients or viscosity has been reported to decrease manufacturing efficiency and may affect the characteristics of the final dosage forms such as hardness, dissolution or disintegration.

Assuring quality when sourcing pharmaceutical excipients

<table>
<thead>
<tr>
<th>Country of source</th>
<th>Particle size (µm)</th>
<th>Specific surface area (m²/g)</th>
<th>Effect on compressibility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Italy</td>
<td>2-80</td>
<td>1.01</td>
<td>Good flow, No Sticking</td>
</tr>
<tr>
<td>Germany</td>
<td>2-70</td>
<td>&lt;1.0</td>
<td>Poor flow, Sticking</td>
</tr>
<tr>
<td>Malaysia</td>
<td>4-40</td>
<td>5.035</td>
<td>Good flow, No Sticking</td>
</tr>
</tbody>
</table>

Testing of physical properties during the pre-formulation stage and establishing functionality-related specifications during process development is not new but has great value in optimizing production efficiency. The importance of establishing internal specifications can be illustrated by a familiar experience with Magnesium Stearate from three sourced. (figure 1 and table 1)

The three samples of Magnesium Stearate were mixed individually with granules of a single formulation of metoprolol tartrate tablet. Results indicated poor flow and sticking during compression of the granules using magnesium Stearate having specific surface area (SSA) of <1m²/g. Good flow and trouble free tableting operation were achieved in granules lubricated with Magnesium Stearate having SSA of 1.01m²/g and 5.035m²/g. Internal specific surface area specications were set at 1m²/g as a minimum and are used to evaluate new material source. Magnesium Stearate with SSA >3m²/g has been reported to have a goof lubricant properties.
Each drug product manufacturer, depending on their needs may set specific values for certain internal specifications related to the formulations, equipment, process and types of excipients applicable to their to their own plants. Labeling requirements indicated in official monographs such as specific surface area for Magnesium Stearate USP/ NF24 is a good guide for process development groups to identify critical parameters for the control of excipients. Efforts of IPEC and the Pharmacopeial Discussion Group to harmonize tests and test. Methods help reduced discrepancies and standardize interpretations of test results.

Established in-house specifications however, would need to be validated when new processes and equipment are used for the same formulations. It does not mean though that any change in the equipment or process would create changes in the functionality of an excipient. Data regarding a directly compressible lactose excipient in Table 2 show batch-to-batch variability in particle size cumulative distribution that had no effect on the compression, hardness and dissolution of a Salbutamol tablet. The results are to evaluate new sources. New sources which show comparable results to the numberweighted cumulative distribution are acceptable.

<table>
<thead>
<tr>
<th>Country of Source</th>
<th>Microscopic Description</th>
<th>Illustration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Italy</td>
<td>Crystals are mostly weakly birefringent crumpled thin plates which appear almost amorphous, few thin plates have smooth to wavy surface, some weakly birefringent needles and striated particles appear like wavy fibrous bundles; some particles have rounded air cavities.</td>
<td></td>
</tr>
<tr>
<td>Germany</td>
<td>20% of the particles are highly birefringent mot of the particles are thick plates with smooth wavy and moderately rough surfaces; some striated particles are present with wavy grooves and pits; few needles are present, some particles have rounded and elongated air cavities.</td>
<td></td>
</tr>
<tr>
<td>Malaysia</td>
<td>95 % of the particles are highly birefringents, mixture of fine plates and the needle chips and rounded to oval tablets; these tablets are elliptical with fine and coarse air cavities.</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1. Comparative photomicrographs of magnesium stearate samples from three sources.
Table 2. Particle size cumulative distribution batch-to-batch variability of directly compressible lactose.

<table>
<thead>
<tr>
<th>% Total Particle Number</th>
<th>Summary of Number-weighted Cumulative Distribution (microns)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L1</td>
</tr>
<tr>
<td>5%</td>
<td>&lt;5.29</td>
</tr>
<tr>
<td>10%</td>
<td>&lt;7.09</td>
</tr>
<tr>
<td>15%</td>
<td>&lt;9.03</td>
</tr>
<tr>
<td>20%</td>
<td>&lt;10.67</td>
</tr>
<tr>
<td>25%</td>
<td>&lt;12.37</td>
</tr>
<tr>
<td>30%</td>
<td>&lt;14.26</td>
</tr>
<tr>
<td>35%</td>
<td>&lt;16.39</td>
</tr>
<tr>
<td>40%</td>
<td>&lt;18.69</td>
</tr>
<tr>
<td>45%</td>
<td>&lt;21.30</td>
</tr>
<tr>
<td>50%</td>
<td>&lt;24.38</td>
</tr>
<tr>
<td>55%</td>
<td>&lt;27.95</td>
</tr>
<tr>
<td>60%</td>
<td>&lt;31.96</td>
</tr>
<tr>
<td>65%</td>
<td>&lt;36.58</td>
</tr>
<tr>
<td>70%</td>
<td>&lt;41.59</td>
</tr>
<tr>
<td>75%</td>
<td>&lt;47.63</td>
</tr>
<tr>
<td>80%</td>
<td>&lt;55.02</td>
</tr>
<tr>
<td>85%</td>
<td>&lt;65.19</td>
</tr>
<tr>
<td>90%</td>
<td>&lt;79.82</td>
</tr>
<tr>
<td>95%</td>
<td>&lt;107.52</td>
</tr>
<tr>
<td>99%</td>
<td>&lt;184.58</td>
</tr>
</tbody>
</table>

Control excipient manufacturing changes

Changes in the manufacturing processes of the excipient manufacturer may affect the manufacturability and stability of a pharmaceutical formulation. Some changes could modify the functionality of the excipient and affect critical product characteristics.

IPEC-Americas has developed a Significant Change Guide for Bulk Pharmaceutical Excipients. The guide defines significant change as: “any change that alters an norm or that is likely to alter the excipient performance in the dosage form.

This document enables the excipient manufacturer of bulk pharmaceutical excipients (BPE) to evaluate the significance of change for the purpose of informing the customer and/or regulatory authorities. Types of changes include the site, scale, equipment, process, packaging and specification. Change risk fall into three levels: level 1 – Minor Change, level 2 Might be Significant, and level 3 – Always Significant. Examples of changes and their levels are given for a better understanding of the guidance.

A diligent and prompt compliance of excipient manufacturers to the requirements of the guidance document is vital to enable the end user to plan resources and manage effectively any possible technical or cost issues brought by any significant change.
Establish a portfolio of certified suppliers

Due to budget limitations, very few external audits on excipient manufacturers are performed since the majority of suppliers are located in foreign countries. Most often, the reliability of suppliers in based on initial plant visits and documentation audits through a supplier survey questionnaire. As a matter of policy, several companies prefer suppliers who have obtained ISO 9001 or ISO 9002 certification and those who can furnish recent regulatory audit or 2nd party audit reports. We have considered it an effective practice to put technical personnel who have a good understanding of quality requirements in the logistics and procurement group. Two pharmacists originally with the quality assurance group now assume responsibility for purchasing research and sourcing management programs. These technical personnel are able to perform as audit team leaders or members. Quality-related issues in the sourcing process are also resolved faster. Another advantage is the elimination of some risk wherein some technical data or information from the lab goes out of context when personnel receiving the information or message do not fully understand quality needs or concerns.

Developing countries watch with interests IPEC-Americas’ supplier assessment programs, which involves an audit of the processes and manufacturing practices of suppliers and distributors. The use of the following references will strengthen auditing systems that are currently used:

d. GMP Audit Guideline For Distributors of Bulk Pharmaceutical Excipients, IPEC, 2000
e. Guidelines For Handling and Distribution of Propylene Glycol USP/EP, CEFIC

A rigorous supplier assessment program will put manufacturers, distributors and traders on the GMP track. The GMP Audit Guideline for Distributors prepared by IPED has adequately addressed risks encountered in the Haiti glycerin case. Distributors are expected to include in their quality systems good practices in storage, handling, labeling and relabeling of excipients. IPEC’s 3rd party auditing has created a new dimension in the field of auditing. When IPEC audit reports become available and hopefully at optimal costs, developing countries faced with inadequate resources may opt for 3rd party auditing and will benefit from a continuous update of their portfolio of certified suppliers.

Consider the use of a Product Clearance Test

Any contamination or adulteration of a finished drug product is the responsibility of the drug manufacturer whether it was due to poor distribution or manufacturing practices. Considering this serious responsibility, an internal product clearance test was developed. This procedure does not replace any of the GMP controls required for components and the finished drug product. The idea of conducting a test that will assure as of the safety of finished products came after the Tylenol incident in 1992. Pesticides and insecticides in the plants of excipients producers and
distributors’ warehouses could also be a source of contamination if not handled or stores properly.

CONCLUSION

Assuring quality during sourcing involves the commitment of key players in the distribution chain.

At the core of the tasks of assuring quality of excipients are people who will make use of available modern production processes, sophisticated analytical methods, harmonized or improved specifications, GMPs, good distribution practices, auditing techniques and regulations. All these support systems become useful only when people choose to use them and use them properly. This is a great lesson that all of us can learn from the 1996 tragic incident in Haiti. The willingness of all sectors from the pharmaceutical and chemical industries, governments, manufacturers and users and other organizations to work together despite several impediments has brought remarkable advances in harmonization. The notable achievements of IPEC, ICH, the Pharmacopoeial Discussion Group, WHO, CEFIC, FECC and other organizations are worthy of great recognition.

Working together and sharing in the fruits of common endeavor is a custom known in the Philippines as “bayanihan”. “Bayan” means country or community while “anihan” means harvesting. This community of action that reaps common benefits known as “goton-royong” in the Indonesia. Let us continue to keep that “bayanihan” spirit alive to assure safety, strength, purity and quality of drug products.

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CONCLUSION

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At the core of the tasks of assuring quality of excipients are people who will make use of available modern production processes, sophisticated analytical methods, harmonized or improved specifications, GMPs, good distribution practices, auditing techniques and regulations. All these support systems become useful only when people choose to use them and use them properly. This is a great lesson that all of us can learn from the 1996 tragic incident in Haiti.

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REFERENCES:

Evaluation of 5-HT$_{2A}$ Receptor Agonistic Property of Elemicin

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ABSTRACT

Canarium luzonicum L. (Burseraceae), also known as Manila elemi and, in the Philippines, as sahing or pili is a tree commonly harvested for its oleoresin which is processed further into oil for export. The oil contains elemicin, which is said to have psychedelic properties due to the activation of the 5-HT$_{2A}$ receptors. 5-HT$_{2A}$ receptor agonists increase motor behaviors and cognitive learning and are also associated with mania while 5-HT$_{2A}$ receptor antagonists decrease motor behaviors and cognitive learning and other features of depression. In rats administered intraperitoneally with corn oil, immobility time was higher by an average of 97 to 152 seconds in tail-suspension test and lower in the number of head-twitches (19 to 8), number of rearing (20 to 5), ambulation (70 to 18), and speed (0.70 to 0.19 squares per minute) in open-field test compared to the elemicin (80mg/kg) group. Higher motor behaviors, namely mobility time, rearing, ambulation and speed and occurrence of head-twitches in rodents signify 5-HT$_{2A}$ receptor activation. To confirm the 5-HT$_{2A}$ receptor agonistic property of elemicin, clozapine, a 5-HT$_{2A}$ receptor antagonist was administered to block the effects of 5-HT$_{2A}$ receptor activation. In rats administered intraperitoneally with clozapine (10mg/kg) prior to elemicin, immobility time was higher by an average of 97 to 199 seconds in tail suspension test and lower in the number of head-twitches (19 to 2), number of rearing (20 to 5), ambulation (70 to 19), and speed (0.70 to 0.19 squares per minute) in the open-field test compared to the elemicin group. The effects of elemicin were abolished when clozapine was administered prior to elemicin, which demonstrates that clozapine prevents elemicin from binding to the 5-HT$_{2A}$ receptor. All these results serve to demonstrate the 5-HT$_{2A}$ receptor agonistic effect of elemicin.

Keywords: elemicin, 5-HT$_{2A}$ receptor, elemi oil
INTRODUCTION

Eliceminc, a phenylpropene, is a component of the oil extracted from the oleoresin produced by the tree bark of Canarium luzonicum (Burseraceae), locally known as pili which is a tree native only to the Philippines. It is acknowledged for possible psychedelic or hallucinatory effects when taken internally (Shulgin, Sargent & Naranjo, n.d.). The tree’s saheng or oleoresin despite being traditionally used as a stimulant, rubefacient, and an anti-rheumatic, is commonly harvested for export wherein it is utilized as a vital component for the manufacture of cosmetic products (Gamil, 2011). Whereas due to its claimed exotic scent and skin rejuvenating properties, the elemi oil is currently receiving increasing demands from international buyers to integrate it into their new line of products—specifically for aromatics, perfumes, soaps and facial moisturizers.

5-HT2A is a subtype of the 5-HT2 receptor belonging to the serotonin receptor family that is stated to have the highest influence for the hallucinogenic effects of known psychedelics.

5-HT2A receptor agonists are associated increased dopamine release (Artigas et al., 2005), as such, increase motor and are also associated with mania, the effects of which can only be ameliorated by 5-HT2A receptor 2 antagonists. Oppositely, 5-HT2A receptor antagonists decrease motor behaviors are also associated with depression.

This study was conducted to determine the 5-HT2A receptor agonistic properties of elemicin in animal models. The motor behavior was determined to assess this effect of elemicin. Open field test and tail suspension test were conducted to evaluate the effect of elemicin on the motor behaviour. Head-twitch test was performed to confirm the activation of 5-HT2A receptor. This research is directed at providing evidence to substantiate the 5-HT2A receptor agonistic property of elemicin.

METHODOLOGY

The research conducted determined the possible psychedelic effect of elemicin from Elemi oil. The research assessed the relation of Eliceminc as a 5-HT2A receptor agonist through its locomotor activity. The rats were divided into four groups namely the rats administered with elemicin, the rats administered with clozapine, the rats administered with clozapine prior to elemicin and the rats administered with corn oil. They were subjected to tests that determined their locomotor activity.

Animals

24 adult male Sprague-Dawley rats (JOSELITO C. PLEGARIA Biological & Zoological Supply) were used in the study. They were housed in Thomas Aquinas Research Center and were divided into 4 test groups each consisting of 6 rats, namely: Group A (Eliceminc Group), Group B...
Materials
98+% Elemicin was ordered from Advanced Technology and Industrial Co., LTD. located in Kowloon, Hong Kong. A certificate of analysis was supplied by the company for authentication of the chemical. Clozapine (Leponex) 25mg was obtained from Mother Teresa of Calcutta Medical Center (MTCMC), San Fernando, Pampanga.

Analysis
For data analysis for Open Field Test, the following parameters were measured: ambulation (frequency the animal crosses the dividing line with four limbs), speed (squares/min) and rearing (frequency the animal stood on hind legs) (Bertges et al., 2011; Walsh & Cummins, 1976). The results from the experimental group were compared to the control group. For data analysis for Head Twitch Test and Tail-Suspension Test, data were analysed by independent t-test through the use of the SPSS program.

OPEN FIELD TEST
Set-up
The open field apparatus consisted of a 45cm-long square acrylic apparatus, closed with 40-cm high walls. The floor was divided into 16 11.25cm-long squares to enable the number of explored squares to be counted. In order to prevent possible biasing effects of odor trials, the apparatus was cleaned with 70% isopropyl alcohol after each test. A video camera was set 2.5 meters above the apparatus for recording.

Procedure
Elemicin (80mg/kg), clozapine (10mg/kg) or corn oil was administered to the rats intraperitoneally and returned to the cage. After 60 minutes, the animal was placed at the side of the open field and filmed for 10 minutes with a digital camera, which captured the images and send them straight to a computer for analysis. (Bertges et al., 2011).
Figure 1. Graph showing the effects of drugs on the average number of ambulation and rearing exhibited by rats in a 10-minute period during the open-field test. Data are presented as means±SEM. Elemicin (80mg/kg) treatment has significantly higher number of ambulation and rearing (p<0.05) compared to the pre-treatment of Clozapine (10mg/kg), indicating antagonism (p<0.05).

Figure 2. Graph showing the effects of drugs on the speed (squares per minute) exhibited by rats in a 10-minute period during the open-field test. Data are presented as means±SEM. Elemicin (80mg/kg) treatment has significantly higher speed (p<0.05) compared to the pre-treatment of Clozapine (10mg/kg), indicating antagonism (p<0.05).

Results and Discussion

Significant difference between the groups was expressed by their p-value obtained from independent t-test (see appendix D). There was a significant difference with rats administered with elemicin from rats administered with clozapine (p<0.05), rats administered with clozapine prior to elemicin (p<0.05) and rats administered with corn oil (p<0.05). The rats administered with clozapine had no significant difference from rats administered with clozapine prior to...
elemicin (p>0.05) and a significant difference from rats administered with corn oil (p<0.05). There was a significant difference with rats administered with clozapine prior to elemicin from rats administered with corn oil (p<0.05).

The mean frequency of ambulation and rearing exhibited by rats in a 10-minute period represented by each group during the open-field test is summarized in Figure 1. Elemicin induction showed a significantly higher average frequency of ambulation and rearing in relation to the control group that suggests increased locomotor activity and 5-HT$_{2A}$ receptor activation. Pre-treatment with clozapine had a general depressing effect which resulted to a lower average number of ambulation and rearing nearly to the same number of rats treated only with clozapine. 5-HT$_{2A}$ receptor activation is related to increased locomotor activity (Geyer et al., 2013). Ambulation and rearing are some of the parameters measured pertaining to the locomotor activity of rats in the open-field test. As such rats administered with elemicin have higher frequency of ambulation and rearing compared to rats administered with clozapine. Likewise, rats administered with clozapine prior to elemicin have lower frequency of ambulation and rearing due to clozapine antagonizing the 5-HT$_{2A}$ receptor.

Speed, as seen in figure 2, is computed by dividing ambulation over the duration the rat was left inside the open-field apparatus.

**Figure 3.** Graph showing the effects of drugs on the average immobility time exhibited by rats during a 6-minute period. Data are presented as means±SEM. Elemicin (80mg/kg) treatment has significantly lower immobility time (p<0.01) compared to the pre-treatment of Clozapine (10mg/kg), indicating antagonism (p<0.01).
TAIL-SUSPENSION TEST

Set-up
A plastic enclosure (40×25×30 cm) fitted with a ceiling hook

Procedure
Elemicin (80mg/kg), clozapine (10mg/kg) or corn oil was administered to the rat intraperitoneally and returned to the cage. Adhesive tape was wrapped around the animal’s tail in a constant position three quarters of the distance from the base of the tail. The animals were suspended by passing the suspension hook through the adhesive tape so that the animal hangs with its tail in a straight line. The duration of immobility was measured continuously for 5 minutes. (Castagne et al., 2009; Chermat et al., 1976)

Results and Discussion
Significant difference between the groups was expressed by their p-value obtained from independent t-test (see appendix D). There was a significant difference with rats administered with elemicin from rats administered with clozapine (p<0.01), rats administered with clozapine prior to elemicin (p<0.01) and rats administered with corn oil (p<0.01). The rats administered with clozapine had no significant difference from rats administered with clozapine prior to elemicin (p>0.01) and a significant difference from rats administered with corn oil (p<0.01). There was a significant difference with rats administered with clozapine prior to elemicin from rats administered with corn oil (p<0.01).

The mean duration of immobility time exhibited by rats in a 6-minute period represented by each group during the tail-suspension test is summarized in Figure 3. Elemicin induction showed a significantly lower average duration of immobility time in relation to the control group which suggests increased locomotor activity and 5-HT$_{2A}$ receptor activation. Pre-treatment with clozapine had a general depressing effect which resulted to a lower average duration of immobility time nearly to the same duration of rats treated only with clozapine. The clinical diagnosis of depression requires the presence of several “core” symptoms (depressed mood, decreased pleasure) that is clearly not possible to reproduce in animals (Castagne et al., 2009). However resignation (termed as “behavioural despair” or “learned helplessness”) is used as a behavioural parameter. In the tail suspension test, inability to escape from a stressful situation serves as an indicator of resignation. Prolonged and repeated stress is probably necessary for inducing a lasting change that could be construed as a “depressive state” (Castagne et al., 2009). Immobility is then interpreted as resignation wherein the subject has given up hope of escaping. 5-HT$_{2A}$ receptor activation is related with increased dopamine release (Artigas et al., 2005) that is associated with mania. As such rats administered with elemicin is observed to have less immobility time than rats administered with clozapine which decreases dopamine levels. Likewise, rats administered with clozapine prior to elemicin did not exhibit decreased immobility time due to clozapine antagonizing the 5-HT$_{2A}$ receptor.
**Figure 4.** Graph showing the effects of drugs on the average number of head-twitches exhibited by rats in a 10-minute period during the open-field test. Data is presented as means±SEM. Elemicin (80mg/kg) treatment has significantly higher number of head-twitches (p<0.05) compared to the pre-treatment of Clozapine (10mg/kg), indicating antagonism (p<0.05).

**HEAD-TWITCH TEST**

**Set-up**
The experimental apparatus used was from the open field test. The open field apparatus consisted of a 45cm-long square wooden apparatus, closed with 40-cm high walls (Walsh & Cummins, 1976). In order to prevent possible biasing effects of odor trials, the apparatus was cleaned with 70% isopropyl alcohol after each test.

**Procedure**
Elemicin (80mg/kg), clozapine (10mg/kg) or corn oil was administered to the rat intraperitoneally and returned to the cage. 60 minutes after injection, the rat placed inside the experimental apparatus. At least two observers counted the head-twitch response in a 10 minute period. The observers’ scores were then be averaged before analysis.

**Results and Discussion**
Significant difference between the groups was expressed by their p-value obtained from independent t-test (see appendix D). There was a significant difference with rats administered with elemicin from rats administered with clozapine (p<0.05), rats administered with clozapine prior to elemicin (p<0.05) and rats administered with corn oil (p<0.05). The rats administered with clozapine had no significant difference from rats administered with clozapine prior to
Elemicin (p>0.05) and a significant difference from rats administered with corn oil (p<0.05). There was a significant difference with rats administered with clozapine prior to elemicin from rats administered with corn oil (p<0.05).

The mean number of head-twitch response exhibited by rats in a 10-minute period represented by each group during the open-field test is summarized in Figure 4. Activation of the 5-HT$_{2A}$ receptor modulates head-twitch response (Canal et al., 2010). Elemicin induction showed a significantly higher average frequency of head-twitches in relation to the control group that indicates 5-HT$_{2A}$ receptor activation. Pre-treatment with clozapine had a general depressing effect which resulted to a lower average frequency of head-twitches nearly to the same frequency of rats treated only with clozapine. This indicates that the effects produced by elemicin were abolished by clozapine confirming that elemicin exerts its agonistic effect by 5-HT$_{2A}$ receptor activation.

**SUMMARY OF RESULTS**

5-HT$_{2A}$ receptor activation is related to increased locomotor activity. Significant difference (p<0.05) in parameters such as ambulation, rearing and speed in rats administered with elemicin from rats administered with clozapine prior to elemicin suggested the possible agonistic effect of elemicin to the 5-HT$_{2A}$ receptor. This was further tested by utilizing the 5-HT$_{2A}$ receptor’s ability in increasing dopamine levels which results into increased mood levels through the tail-suspension test. Rats administered with elemicin had a significant difference (p<0.01) in immobility time compared to rats administered with clozapine prior to elemicin, also suggesting the possible agonistic effect of elemicin to the 5-HT$_{2A}$ receptor. To confirm the findings, the head-twitch test was conducted due to its direct relation to 5-HT$_{2A}$ receptor activation. Rats administered with elemicin had a significant difference (p<0.05) in the frequency of head-twitches from rats administered with clozapine prior to elemicin.

**Conclusion**

Elemicin exhibited significant 5-HT$_{2A}$ receptor agonistic property as seen from the head-twitch test, open field test and tail suspension test. The 5-HT$_{2A}$ agonistic property was shown when the data obtained from the different tests of rats administered with elemicin, rats administered with clozapine, rats administered with clozapine prior to elemicin and rats administered with corn oil were compared.
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Preparation and Characterization of Chitosan/Polycaprolactone/k-Carrageenan Scaffolds

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ABSTRACT

One application of tissue engineering is developing extracellular matrices such as scaffolds. These are biocompatible polymers used as frameworks and temporary substitutes for tissue regeneration developed through several different approaches, serving as an environment for cell migration and attachment. In this study, kappa-carrageenan (k-C) and chitosan (CHT) have been mixed with polycaprolactone (PCL) in different amounts. PCL:CHT:k-C ratios were all prepared using a freeze-drying method. The resulting products were characterized using SEM, FTIR, and DSC to check the products' functional groups, homogeneity, morphology, porosity, crystallinity, and thermal properties. Moreover, swelling ratio test was conducted to further assess the absorption rate of the product. The SEM images show pores ranging from 3-6 µm and rough surface morphology. No new functional groups were formed as shown in the FTIR spectra of the products, indicating that no interaction occurred at the molecular level between the polymeric components. The swelling ratio test indicated that the increasing chitosan content increased its ability for absorption capacity and swelling capability.

In vitro and in vivo techniques would further verify the applicability of the products formulated in tissue engineering.

Keywords: chitosan, -carrageenan, polycaprolactone, scaffold, tissue engineering, polymers
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ABSTRACT

One application of tissue engineering is developing extracellular matrices such as scaffolds. These are biocompatible polymers used as frameworks and temporary substitutes for tissue regeneration developed through several different approaches, serving as an environment for cell migration and attachment. In this study, kappa-carrageenan (k-C) and chitosan (CHT) have been mixed with polycaprolactone (PCL) in different amounts. PCL:CHT:k-C ratios were all prepared using a freeze-drying method. The resulting products were characterized using SEM, FTIR, and DSC to check the products’ functional groups, homogeneity, morphology, porosity, crystallinity, and thermal properties. Moreover, swelling ratio test was conducted to further assess the absorption rate of the product. The SEM images show pores ranging from 3-6 µm and rough surface morphology. No new functional groups were formed as shown in the FTIR spectra of the products, indicating that no interaction occurred at the molecular level between the polymeric components. The swelling ratio test indicated that the increasing chitosan content increased its ability for absorption capacity and swelling capability. In vitro and in vivo techniques would further verify the applicability of the products formulated in tissue engineering.

Keywords: chitosan, κ-carrageenan, polycaprolactone, scaffold, tissue engineering, polymers
**Introduction**

Biomedical engineering connects the fields of engineering and medicine. It incorporates the physical science approach of engineering principles and designs with the biological science background of medical practice in order to enhance the quality of life.

Tissue engineering, a major branch of biomedical engineering, has been an emerging and multidisciplinary area that includes engineering and several sciences like biology and medicine, also pharmaceutics and pharmacology with regard to its therapeutic action. Tissue engineering makes use of biomaterials, biomolecules, design aspects of biomechanics and engineering, and cells aiming to develop biological substitutes to restore, replace or regenerate defective tissues. One application of tissue engineering is by developing extracellular matrices such as scaffolds, biocompatible polymers used as frameworks, and temporary substitutes for tissue regeneration. Scaffolds have been developed through several different approaches, for example, electrospinning, gas foaming, emulsification, and solvent casting with the use of either natural or synthetic materials. The scaffold should promote transportation of fluids and endure external mechanical stress until regeneration of the functional new tissue is complete.

The scaffold is the filler of void in tissue (Lewandowska-Szumiel, 2011). To attain the goal of tissue reconstruction, scaffolds must be porous with adequate pore size; to facilitate cell seeding and diffusion throughout the whole structure of both cells and nutrients (Khaled et al., 2011). Considering the materials for scaffolding, they should be non-toxic, sterile, biodegradable and biocompatible (Danis’ovic’ et al., 2012).

**Experimental**

**Preparation of Scaffolds**

Chitosan (CHT – 85% degree of deacetylation, medium weight) and polycaprolactone (PCL) were purchased from Sigma-Aldrich. Kappa-Carrageenan (k-C) as well as carboxymethyl κ-Carrageenan (CMκC) were bought from Mioka. Glacial acetic acid, 80% (v/v) acetic acid, deionized water, and distilled water together with CMκC were made available at the Organic Synthesis Laboratory of Research Center for Natural and Applied Sciences (RCNAS). Deionized water without Ca+/Mg+ was used.

**Chitosan/Polycaprolactone/k-Carrageenan blend**

The method used is a modification of the solvent casting method (Bibat, C., et. al., 2011) and the freeze drying method. Four ratios were prepared in making the complex blends of PCL, CHT and k-C. These were 33k-C:33CHT:33PCL, 20k-C: 40CHT:40PCL, 20k-C: 30CHT:50PCL and 20k-C: 50CHT: 30PCL. The ratios’ names were shortened based on the chitosan mass as 33CHT, 40CHT, 30CHT and 50CHT respectively.
Chitosan and κ-carrageenan were dissolved in 80% acetic acid to prepare a 1% (w/v) solution and 2% (w/v) solution respectively while polycaprolactone was dissolved in glacial acetic acid to have a 2% (w/v) solution. PCL solution was first mixed slowly to CHT solution and stirred for 2-3 minutes. The resulting mixture was combined gradually to the κ-carrageenan solution and stirred for 2-3 minutes. The same procedure was done with the other ratios. The resulting mixture was mixed until a homogenous mixture was obtained. The homogenous solution was frozen for 8 hours and then lyophilized. The mixture was poured on a tray covered with foil and air-dried for 4-5 days at room temperature (30°C).

**Preparation of Samples for Characteriation**

Scaffold samples were sterilized with phosphate buffer solution with penicillin streptomycin and washed with deionized water.

**Scanning Electron Microscopy (SEM) Analysis**

SEM analysis of the films were carried out in a scanning electron microscope with a third party at the Surface Physics Laboratory of DLSU Science and Technology Research Center with a JEOL scanning, microscopy model no. 5380. All specimens were coated with a conductive layer of sputtered gold. Its micrograph was taken at an accelerating voltage of 5 kV to ensure a suitable image resolution for 10μm, 5μm and 1μm shots.

**Differential Scanning Calorimetry (DSC) Analysis**

In order to determine the properties of the films with respect to temperature, the samples will go through DSC scans using the DSC Q10 machine of the Institute of Chemistry of the University of the Philippine. Weights of the samples ranging from 2.7-3mg. were sealed in aluminum plates and placed inside the machine with a reference plan. A working temperature ranging from 4°C-150°C were used to catalyze the glass transition temperature, melting temperature, and heat flow of the sample.

**Fourier Transform Infrared Spectroscopy (FTIR) Analysis**

FTIR Spectra of the samples were recorded at the Chem. Lab. of DLSU using Nicolet 6700 FTIR spectrophotometer. The samples were mixed with KBr. The mixture was compressed into thin film using a pelletizer. The spectrophotometer was calibrated by simply scanning the air that was in the sample area before placing the pellet. This revealed the functional groups present in the samples.
Results and Discussion

**Surface morphological analysis of the scaffolds**

The surface morphology of the scaffolds were determined by Scanning Electron Microscope (SEM) in increasing magnification of 30µm, 20µm and 10µm to determine if the scaffolds obtained were porous or not and to determine if the surface was rough or smooth. Figure 1 shows SEM micrograph of 60: 40 (PCL: CHT) scaffold in 30µm magnification.

![SEM micrograph of 60:40 (PCL: CHT) scaffold in 30µm magnification](image1)

**Figure 1.** SEM micrograph of 60:40 (PCL: CHT) scaffold in 30µm magnification

The standard ratio, 60:40 (PCL:CHT), scaffold has a rough surface as shown in Figure 1. The surface looks like craters in a rough surface. Pores are present on the film’s surface with pore size ranging between 4.5µm to 9µm. Homogeneity of the blend was not concluded by mere observation of the surface morphology. Figure 2 shows SEM micrograph of 60:35:5 (PCL: CHT: κ-C) scaffold in 30µm, 20µm and 10µm (from left to right) magnification.

![SEM micrograph of 60:35:5 (PCL: CHT: k-C) scaffold in 30µm, 20µm and 10µm (from left to right) magnifications](image2)

**Figure 2.** SEM micrograph of 60: 35:5 (PCL: CHT: k-C) scaffold in 30µm, 20µm and 10µm (from left to right) magnifications

The 60:35:5 (PCL: CHT: κ-C) scaffold as shown in Figure 2, has a very rough surface with visible round and semi-round contours. There was very minimal number of pores visible. The visible pores have sizes ranging between 1.5µm to 3.5µm. Figure 3 shows SEM micrograph of 60: 30:10 (PCL: CHT: k-C) scaffold in 30µm, 20µm and 10 µm (from left to right) magnification.

![SEM micrograph of 60:30:10 (PCL: CHT: k-C) scaffold in 30µm, 20µm and 10µm (from left to right) magnification](image3)

The 60:30:10 (PCL: CHT: κ-C) scaffold as shown in Figure 3, has a rough exterior. A mixture of round, semi-round and irregular shaped contours are what comprises the surface of the scaffold. The scaffold produced was porous since pores can be seen on the scans with pores having sizes from 1.5 µm to 4 µm. Figure 4 shows SEM micrograph of 60:20:20 (PCL: CHT: k-C) scaffold in 30µm, 20µm and 10µm (from left to right) magnification.

![SEM micrograph of 60:20:20 (PCL: CHT: k-C) scaffold in 30µm, 20µm and 10µm (from left to right) magnification](image4)

The 60:20:20 ((PCL:CHT: κ-C) scaffold as shown in Figure 4, has a rough surface. The surface is predominated by round and semi-round contours. There are also few pores visible. The pore sizes ranged between 1.5 µm and 8 µm. As surface morphology is concerned, it can be seen that with an increase in the amount of κ-carrageenan, it produced rougher surfaces, but a decrease in the number of pores visible.
Results and Discussion

Surface morphological analysis of the scaffolds

The surface morphology of the scaffolds were determined by Scanning Electron Microscope (SEM) in increasing magnification of 30 µm, 20 µm and 10 µm to determine if the scaffolds obtained were porous or not and to determine if the surface was rough or smooth.

Figure 1 shows SEM micrograph of 60:40 (PCL:CHT) scaffold in 30 µm magnification.

Figure 1. SEM micrograph of 60:40 (PCL:CHT) scaffold in 30 µm magnification

The standard ratio, 60:40 (PCL:CHT), scaffold has a rough surface as shown in Figure 1. The surface looks like craters in a rough surface. Pores are present on the film's surface with pore size ranging between 4.5 µm to 9 µm. Homogeneity of the blend was not concluded by mere observation of the surface morphology. Figure 2 shows SEM micrograph of 60:35:5 (PCL:CHT:κ-C) scaffold in 30 µm, 20 µm and 10 µm (from left to right) magnification.

Figure 2. SEM micrograph of 60:35:5 (PCL:CHT:κ-C) scaffold in 30 µm, 20 µm and 10 µm (from left to right) magnifications

The 60:35:5 (PCL:CHT:κ-C) scaffold as shown in Figure 2, has a very rough surface with visible round and semi-round contours. There was very minimal number of pores visible. The visible pores have sizes ranging between 1.5 µm to 3.5 µm. Figure 3 shows SEM micrograph of 60:30:10 (PCL:CHT:κ-C) scaffold in 30 µm, 20 µm and 10 µm (from left to right) magnification.

Figure 3. SEM micrograph of 60:30:10 (PCL:CHT:κ-C) scaffold in 30 µm, 20 µm and 10 µm (from left to right) magnification

The 60:30:10 (PCL:CHT:κ-C) scaffold as shown in Figure 3, has a rough exterior. A mixture of round, semi-round and irregular shaped contours are what comprises the surface of the scaffold. The scaffold produced was porous since pores can be seen on the scans with pores having sizes from 1.5 µm to 4 µm. Figure 4. SEM micrograph of 60:20:20 (PCL:CHT:κ-C) scaffold in 30 µm, 20 µm and 10 µm (from left to right) magnification.

Figure 4. SEM micrograph of 60:20:20 (PCL:CHT:κ-C) scaffold in 30 µm, 20 µm and 10 µm (from left to right) magnification

The 60:20:20 (PCL:CHT:κ-C) scaffold as shown in Figure 4, has a rough surface. The surface is predominated by round and semi-round contours. There are also few pores visible. The pore sizes ranged between 1.5 µm and 8 µm.

As surface morphology is concerned, it can be seen that with an increase in the amount of κ-carrageenan, it produced rougher surfaces, but a decrease in the number of pores visible. Material surface roughness (or topography) is another important factor influencing cell adhesion and behavior. Indeed, roughness modulates the biological response of tissues in contact with the
implant. Material surface roughness has a direct influence in vitro as well as in vivo on cellular morphology, proliferation, and phenotype expression. Literature papers have been reported that cells grown on micro rough surfaces were stimulated towards differentiation; as shown by their gene expression in comparison with cells growing on smooth surfaces. They have implied that cells prefer to stretch on rough surfaces. It was also observed that the proliferation of cells was better on rough surfaces compared to smoother surfaces. The presence of pores smaller than 160µm, produced by salt leaching, has been reported to be optimal for attachment of human skin fibroblasts (Hsin-I Chang and Yiwei Wang, 2011).

3.2 Functional Groups

The FTIR spectra of PCL:CHT standard ratio and a PCL:CHT:κ-C ratio are compared in Figures 5 and 6 to investigate the presence and possible interaction of their functional groups.

![Figure 5. FTIR spectrum of PCL:CHT](image)

Several absorption bands specific for PCL, CHT and κ-C were visible. The most prominent distinguishing feature is the O-H strong broad stretch overlapping the N-H stretch centered between 3355- 3440 cm⁻¹ and strong carbonyl group stretch present at 1726- 1729 cm⁻¹.

![Figure 6. FTIR spectrum of PCL:CHT:κ-C](image)
The peaks at 1296-1297 cm\(^{-1}\) indicate the backbone C-C and C-O stretching modes in the crystalline PCL. The functional group amine with peaks at 1636-1637 cm\(^{-1}\) indicates the presence of CHT. Functional groups of alkanes and carbonyls were also present. The summary of the peaks are found in Table 1.

**Table 1.** Major Frequency Readings of the standard and a blend ratio and their corresponding type of absorption

<table>
<thead>
<tr>
<th>Frequency (cm(^{-1}))</th>
<th>Type of Adsorption</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCL:CHT</td>
<td>PCL: CHT: (\kappa)-C</td>
</tr>
<tr>
<td>3355.32</td>
<td>3440.19</td>
</tr>
<tr>
<td>2946.39</td>
<td>2949.29</td>
</tr>
<tr>
<td>1726.29</td>
<td>1729</td>
</tr>
<tr>
<td>1636.67</td>
<td>1636</td>
</tr>
<tr>
<td>1296.22</td>
<td>1297.18</td>
</tr>
<tr>
<td>1242.21</td>
<td>1243.18</td>
</tr>
<tr>
<td>1067.65</td>
<td>1069.88</td>
</tr>
<tr>
<td>1047.39</td>
<td>1044.50</td>
</tr>
</tbody>
</table>

**Thermal Properties**

DSC scan of \(\kappa\)-c shown in figure 7 has a melting peak at around 77°C while the DSC scan of CHT shown in figure 8 has a melting peak at around 78°C. According to DSC scans shown in Figure 9, the PCL is shown to have a melting peak at around 66°C. Based on the data of Bibat et al., pure PCL melts at 60°C. Based on these data, the peak of PCL was around 60°C - 66°C.

![Figure 7. DSC Scan of \(\kappa\)-C](image)
In figure 10, the DSC scan of PCL:CHT showed the peak at 61°C implying the presence of PCL, while the CHT peak was also present at around 78°C. Lastly, in figure 11, with the DSC scan of PCL:CHT:k-C, the peak was observed to be at 59°C which is to be the PCL content while the peak of k-C and CHT were seen at around 77°C and 78°C respectively.
Swelling Properties

Diffusion and exchange of nutrients (e.g., oxygen) and waste throughout the entire scaffold are related to the swelling properties of the scaffold (Correia, C, et al, 2011). As shown in Table 2, the swelling ratio of all the samples increased in the 30 minute and 2 hour intervals except that of the 60:20:20 ratio. The highest swelling ratio was that of the PCL: CHT blend and lowest is the k-carrageenan content. The swelling ratio with the less k-C component has a greater swelling ratio and may be due to the hydrophilic property of the CHT, reducing it and adding k-C made the absorption capacity of the scaffold to decrease.

Table 2. Swelling Ratio of the Different Samples at 30 minutes and 2 hours

<table>
<thead>
<tr>
<th>Ratio</th>
<th>30 minutes</th>
<th>2 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>60:35:05</td>
<td>1.4</td>
<td>4.1</td>
</tr>
<tr>
<td>60:30:10</td>
<td>1.5</td>
<td>4.9</td>
</tr>
<tr>
<td>60:20:20</td>
<td>1.4</td>
<td>4</td>
</tr>
<tr>
<td>60:40:00</td>
<td>1.9</td>
<td>11.3</td>
</tr>
</tbody>
</table>
Conclusion

In summary, all blends produced rough surfaces with the 60:35:5 (PCL:CHT:κC) and 60:20:20 (PCL:CHT:κC) producing more contours and protrusion while the 60:40 (PCL:CHT) and 60:30:10 (PCL:CHT:κ-C) producing a more porous scaffold. As surface morphology is concerned, it can be seen that with an increase in the amount of κ-carrageenan, it produced rougher surfaces but a decrease in the number of pores visible. The pores present are of small in quantity and uneven in size based on initial screening. The presence of such pores and rough surfaces can better aid in the proliferation of cells.

The FTIR scans showed functional groups that are present in the components. The DSC scans showed the melting temperatures of the samples analyzed and the pure PCL component matched with that of the scaffolds made. Peaks of the components observed are present indicating good compatibility with each other.

In the swelling ratio test, the highest swelling ratio was observed to be that of the 60:40 PCL:CHT sample. The sample ratio with the less κ-C component has a greater swelling ratio and may be due to the hydrophilic property of the CHT, reducing it and adding κ-C made the absorption capacity of the scaffold to decrease.

The best samples appear to be both the 60:30:10 and the 60:35:5 in line with the surface morphology seen on the SEM image and the swelling ratio test done.

References


In summary, all blends produced rough surfaces with the 60:35:5 (PCL:CHT:κC) and 60:20:20 (PCL:CHT:κC) producing more contours and protrusion while the 60:40 (PCL:CHT) and 60:30:10 (PCL:CHT:κ-C) producing a more porous scaffold. As surface morphology is concerned, it can be seen that with an increase in the amount of κ-carrageenan, it produced rougher surfaces but a decrease in the number of pores visible. The pores present are of small in quantity and uneven in size based on initial screening. The presence of such pores and rough surfaces can better aid in the proliferation of cells.

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References


The Efficacy of *Musca Domestica* (Muscidae) Larval Secretions in Debridement of Necrotic Wounds in Diabetic Rats

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Abstract

Maggot Debridement Therapy (MDT) utilizes live, sterilized fly larvae as primary treatment in healing necrotic wounds especially in diabetic patients. The efficacy of the larval secretions of *Musca domestica* (Muscidae) in wound debridement of diabetic rats was evaluated in comparison to MEBO® (Moist Exposed Burn Ointment) and to Phosphate Buffer Solution, the vehicle of the secretions. This study on the topical application of *Musca domestica* larval secretions showed that with the P-value of 0.193261 (>0.05) the rate of wound healing of the secretions is as effective as that of the MEBO® treatment. Results also showed that the quality of wound healing after treatment with the larval secretions of *Musca domestica* with a P-value of 0.00117762 (<0.05) was better than that of the MEBO treatment. Thus, it can be concluded that the larval secretions of *Musca domestica* showed efficacy on the debridement of necrotic wounds in Diabetic rats.

Keywords: debridement, diabetes, secretion, MEBO®
The Efficacy of Musca Domestica (Muscidae) Larval Secretions in Debridement of Necrotic Wounds in Diabetic Rats

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Abstract

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Keywords: debridement, diabetes, secretion, MEBO®

Introduction

Maggots have been known for centuries to help healing of wounds, specifically to hasten clotting period. Despite of the abhorrent medium, maggot therapy proved more effective and efficient in the stimulation of wound healing of multi-drug resistant ulcers caused by diabetes. According to historical medical records, Civil War surgeons noted that soldiers whose wounds harbored maggots seemed to recover faster. Maggot Debridement Therapy (MDT) is an alternative therapy for wounds quietly championed since the early 1990s but whose advancement was significantly hindered by the advent of antibiotics. Now, with the steady development of antibiotic-resistant infections, MDT is making a comeback. In January 2004, the U.S. Food and Drug Administration (FDA) began regulating medicinal maggots, and allowed the production and marketing of one particular strain, Phaenicia sericata larvae, marketed under the brand name Medical Maggots™. By February of 2004, the British National Health Service (NHS) permitted its doctors to prescribe maggot therapy. Patients no longer have to be referred to one of a few regional wound-specialty hospitals to get maggot treatments.

Maggot Debridement Therapy (MDT) is the medical use of live maggots (fly larvae) for treating non-healing wounds. In maggot debridement therapy (also known as maggot therapy, larva therapy, biodebridement or biosurgery), disinfected fly larvae are applied to the wound for 2 to 3 days within special dressings to keep them from migrating. Medical-grade maggots basically chew away dead flesh, leaving healthy tissue unharmed. It has also been found to disinfect the wound and stimulate healing. Maggots do not appear all by themselves (de novo), as was believed 150 years ago; they hatch from eggs, laid by adult female flies. The flies used most often for the purpose of maggot therapy are "blow flies" (Calliphoridae); and the most commonly used species is Phaenicia sericata, the green blow fly.

Research shows people with diabetes experience more difficulty with wound healing than non-diabetic people do. Due to poor healing capacity, these ulcers can easily become infected and bacteria can enter the body’s system. Standard treatments for stimulating healing in people with
diabetic ulcers include unmedicated dry or saline-moistened dressings, medicated dressings with wound-healing medicines, topical antibacterial treatment, and surgical debridement. Thus, led to prevailing evidence that debridement occurred significantly faster in wounds treated with maggot therapy compared to standard treatments. MDT proved as an efficient, low cost alternative method to cleanse and promote the healing of chronic soft tissue wounds before they progress to a stage where amputation is the only alternative.

Existing studies have established the healing capability of maggots is attributable to its secretion of proteolytic enzymes on exogenous materials present in the wound. This knowledge opted the researchers to utilize the larval secretions of *Musca domestica* in the possibility of promoting wound healing and debridement when using live larvae.

**Materials and Methods**

*Maggots of Musca domestica*

Moist and repugnant conditions were initially observed in this process of the experiment to attract *Musca domestica* flies but sterilized conditions were assumed to ensure that the collection would be free from contaminants.

*Collection and Larval Development of Musca domestica*

*Musca domestica* (common house fly) were trapped in observation cages. At the age of 1–2 weeks, the flies can lay eggs (Greenberg, 1973). Decaying beef meat was used as bait and grounds for the flies to lay their eggs. The observation cages were covered with perforated covers to create a protected area with proper ventilation. The observation cages were then incubated at 37° C for 24 hours to allow the development of eggs and hatching into larvae. The larvae were isolated and placed in a sterile jar using wooden tongue depressors and forceps.

*Sterilization of Musca domestica larvae*

The larva were soaked in 2% chlorhexidine solution for 10 minutes, decanted and soaked in 70% ethanol for another 10 minutes. The larvae-ethanol mixture was strained and the larva were washed with sterile water several times to free it from any residual alcohol, followed by washing and soaking in sterile saline solution to normalize its pH and prevent the escape of the maggots from the container. Surviving larva was used and the remaining ones were discarded. The larvae were kept in a sterile glass bottle.
Collection of the Secretions of the Maggots

The larva-sterile saline mixture was sterile strained and the one-day sterile and starved larvae were placed in a tared sterile weighing bottle. Nine grams of maggots were transferred on a fine-mesh sieve over a funnel fixed onto the neck of a flask. The larvae were washed three times with 180mL of phosphate buffer solution (PBS) with pH 7.0 to stimulate larval secretion and were allowed to be soaked in the same solution for 10 minutes. The mixture was sterile filtered and the process was repeated to collect further. The secretion/PBS mix was stored in 5 ± 3°C.

Animals

The study utilized healthy male Sprague Dawley rats that were eventually induced with diabetes. The rats were individually housed and maintained on standard pellet food and distilled water ad libitum throughout the experiment. The animals were allowed to acclimatize for 5 days at room conditions. The animals that were handled underwent necessary physical examination to determine inclusion before the experiments. Animals that exhibited evidence of variances from the goals of study were immediately replaced. Randomly selected rats were distributed to the negative control group, positive control group and treatment group; each consisting of 5, all of which used the excision and necrotic incision wound models. The study was approved by the Research Center for the Natural and Applied Sciences Institutional Animal Care and Use Committee of the University of Santo Tomas.

Induction of Diabetes in Sprague Dawley rats

All animals were weighed and their fasting blood glucose levels were determined before inducing Diabetes. The animals were injected with a single dose of streptozotocin (60mg/kg) in distilled water administered intraperitoneally. Fasting blood glucose levels were measured after 12 hours to confirm that the rats are diabetic. Blood was drawn from the tail vein for this purpose.

Wound Healing Activity Tests

Animals were anesthetized using Ketamine (80 mg/Kg) administered intraperitoneally before inflicting the excision and necrotic incision wound models. The wound areas were applied according to the group treatment and the wound dressing were changed every other day for all the groups. The day of scab falling, after wounding, without any residual raw wound will be considered as the period of epithelialization.
Incision Wound Model

One straight para-vertebral incision of 6 cm length, with 3 sutures was made throughout the entire thickness of the skin on the right side of the rat’s body. The sutures were done by a single person using silk 2.0 thread. The breaking strength of the wound was determined after the rats have been euthanized; the wounds were isolated from the body and the breaking strength was determined using a Tensiometer.

Figure 1. Incision Wound Model

Excision Wound Model

Rats were inflicted with excision wounds according to the method of Morton and Malone (1972). The area of the wound was outlined on the left side of the back of the animals with methylene blue using a circular stainless steel stencil. A full thick excision wound of circular area 300 mm² was created along the markings. The entire wound was left open and was measured using planimetry every two days.

Figure 2. Excision Wound Model
Induction of Necrotizing Wounds

A flat circular polymer disc with a diameter of 3.81cm was inserted, sutured inside the incision wound model. The discs were removed after 12 hours to allow ischemia of wound to take place and the wound was closed again with sutures. Treatment was started after 24 hours to provide time for wound contraction.

Statistical Treatment

In the incision wound model, the data regarding the tensile strength was used while in the excision wound model, the day when the wound was completely healed was used. One-way ANOVA was used because only one variable was being compared and Tukey’s post-hoc test was used to determine whether there is a significant change between the rates of healing of the wounds and quality of the wound healed between the three groups. Data was analyzed using the application, Microsoft Excel, and the P value was set to <0.05 for all analyses.

Results

![Blood Glucose Levels Before And After Administration of Streptozotocin](image)

Figure 3. Graph showing the blood glucose of fifteen rats before and after administration of Streptozotocin
The data above shows the blood glucose levels in mg/dL of the fifteen rats before and after administration of Streptozotocin. All rats demonstrated normal blood glucose levels (70-140 mg/dL) prior to administration of Streptozotocin. A blood glucose level of greater than or equal to 200 mg/dL indicates diabetic status. The blood glucose levels of the subjects were measured again after administration of streptozotocin and determined to be greater than 200 mg/dL which was indicative of diabetes.

Figure 4. Graph Showing the Trend of the Mean Rate Wound Closure of the Three Treatment Groups

The data above shows the trend on how fast the wounds healed per treatment group over a period of 21 days.
The data above shows the blood glucose levels in mg/dL of the fifteen rats before and after administration of Streptozotocin. All rats demonstrated normal blood glucose levels (70 - 140 mg/dL) prior to administration of Streptozotocin. A blood glucose level of greater than or equal to 200 mg/dL indicates diabetic status. The blood glucose levels of the subjects were measured again after administration of streptozotocin and determined to be greater than 200 mg/dL which was indicative of diabetes.

Figure 4. Graph Showing the Trend of the Mean Rate Wound Closure of the Three Treatment Groups

The data above shows the trend on how fast the wounds healed per treatment group over a period of 21 days.

Figure 5. Graph Showing the Mean Rate of Wound Healing in Days of the Three Treatment Groups

The data above shows the mean rate of wound healing in days of the three treatment groups. This was based on the recorded time of healing of the excision wound models. The P-value of 0.193261 (<0.05) and the computed F value of 1.890909 (> F critical value, 3.885294) suggested that there was no significant difference in the time of healing of the three groups and further post-hoc analysis was not required.

Figure 6. Graph Showing the Mean Tensile Strength of the Three Treatment Groups
The data above shows the mean tensile strength of the three treatment groups. Tensile strength was computed by dividing the breaking strength of the wound in grams (g) by the cross sectional area of the skin in square millimeters (mm²). The P-value of 0.001117762 (<0.05) and the computed F value of 12.6248616 (>F critical value, 3.885293835) suggest that there is a significant difference between the three treatment groups. The mean difference of the Phosphate and Maggot group, and MEBO and Maggot group is 0.45732 and 0.43548 respectively (>Critical Value, difference between the three treatment groups. The mean difference of the Phosphate and Maggot group, and MEBO and Maggot group is 0.45732 and 0.43548 respectively (>Critical Value, 0.221337435). Therefore, it can be concluded that there is a significant difference between the tensile strength between the Phosphate and Maggot groups, and between MEBO and Maggot groups.

Discussion

The purpose of the study was to determine the efficacy of the larval secretion of Musca domestica in the debridement of wound in diabetic rats. To accomplish that goal, it was necessary to reach prerequisite goals. Measuring the rate of wound healing determined high degree of importance during the literature review conducted in this study. Related to that effort, it became necessary to reach an understanding about the mechanisms of wound debridement and management, Musca domestica life cycle and larval digestive enzyme activity. Once these fundamental steps were achieved, this research was able to go forward.

All test subjects were ensured to be diabetic prior to the experiment proper. ANOVA and further post-hoc analysis for the incision wound models testify that there is a significant difference between the computed tensile strength of Phosphate and Maggot group, and between the MEBO and Maggot group. The ANOVA for the excision wound models indicated no significant difference among the groups thus, requiring no further post-hoc analysis.

Conclusion

Based on the study, it can be concluded that the quality of wound healing after treatment with the larval secretion of Musca domestica was better than that of the MEBO treatment, as shown by statistical analysis of the measured tensile strength of the incision wound models, while the recorded rate of healing of the excision wound models showed that the larval secretion treatment is only as effective as that of the MEBO treatment. Thus, it can be established that the larval secretion of Musca domestica showed efficacy on the debridement of necrotic wounds.

References

The purpose of the study was to determine the efficacy of the larval secretion of Musca domestica and its potential role in wound healing. The study included three treatment groups: Phosphate, MEBO, and Maggot. The mean difference of the Phosphate and Maggot groups, and between MEBO and Maggot groups, was computed and an F value of 12.6248616 was obtained, which is greater than the F critical value of 3.885293835. This suggests that there is a significant difference between the three treatment groups.

The data above shows the mean tensile strength of the three treatment groups. Tensile strength was computed by dividing the breaking strength of the wound in grams (g) by the cross-sectional area of the skin in square millimeters (mm²). The P-value of 0.001117762 is less than 0.05, indicating a significant difference.

The quality of wound healing after treatment with the larval secretion of Musca domestica was better than that of the MEBO treatment, as shown by the recorded tensile strength. This suggests that the larval secretion treatment is only as effective as MEBO in promoting wound healing.

Based on the study, it can be concluded that the quality of wound healing after treatment with the larval secretion of Musca domestica showed efficacy on the debridement of necrotic wounds.


THE CHEMOPREVENTIVE POTENTIAL OF CRUDE LEAF EXTRACT OF PAK-CHOI (Brassica rapa L. cv. Pak-choi Family Brassicaceae) IN 7, 12-DIMETHYLBENZ(α)ANTHRACENE (DMBA)/CROTON OIL-INDUCED IN VIVO TWO-STAGE SKIN TUMORIGENESIS IN MALE ICR MICE

Chua, Sharmaine S.; Cobar, Flordelyn C.; Cosas, Reysan S.; Diona, Ellaine Angelli A.; Francisco, Jonelle Prince M.; Lauron, John Paul V.; Lingat, Jean Desiree P.
Mrs. Mylene S. Andal, R.Ph., MS Pharm.

Abstract
Pak-choi (Brassica rapa L. cv. Pak-choi) has been traditionally used in Chinese and Filipino cuisines. Pak-choi belongs to the Brassicaceae Family and it has created a lot of interest in medicinal therapy due to its potential cancer fighting compound, specifically, glucosinolates. However, extensive research done on the plant is still limited. Therefore, the present study was designed to evaluate the chemopreventive potential of Pak-choi leaves crude extract (PLCE) on 7, 12-dimethylbenz(α)anthracene (DMBA)-initiated skin tumorigenesis in ICR mice with topical application of croton oil twice weekly for 10 weeks. Results showed significant difference between treatment groups (mice treated with 0.5% w/v, 2% w/v and 5% w/v of PLCE extract; denoted as group IV, V and VI, respectively) and control groups for tumor incidence and tumor volume (P<0.05). Significant reduction in tumor incidence (30%), tumor volume (0.50±0.43) and delayed latency period of tumor formation (8th Week) was observed in Group IV (0.5% w/v) in comparison to Carcinogen Control. This study indicates that PLCE extract has a chemopreventive potential at a lower dosage of 0.5% w/v. Further studies are required to elucidate the underlying mechanism(s) leading to this effect.

Keywords: Chemopreventive, Pak-choi, Two-Stage Skin Tumorigenesis, Glucosinolates, Curcumin
Introduction

The integument or skin is the largest organ of the body, making up 16% of body weight (Gawkrodger, 2002). It is a physical barrier to the environment, allowing and limiting the inward and outward passage of water, electrolytes and various substances while providing protection against micro-organisms, ultraviolet radiation, toxic agents and mechanical insults. By allowing these agents to penetrate and damage the skin, several diseases may arise such as skin cancer. According to Skin Cancer Foundation Statistics, one in every three cancers being diagnosed is a skin cancer (World Health Organization, 2013) with its occurrence more prevalent in males than in females (American Cancer Society, 2013).

Skin cancer represents approximately two to four percent of all cancers in Asians. (Gloster, et al, 2006)

Skin cancer arises primarily from sun-exposed body site and is associated with repeated sun exposure (Standford Medicine, 2013). Hence, an approach aimed at preventing or protecting cells from ultraviolet radiation-induced cellular damages has considered as an effective strategy for the management of skin cancer. Chemoprevention, a novel pharmacologic strategy, depicts the strategy of blocking or retarding the initiation of pre-malignant tumors with non-toxic chemical resources – natural, synthetic, or biological agents (Surh, 2003).

Outstanding sources of cancer chemopreventive substances include natural agents from dietary plants. Bioactive components of dietary plants had been demonstrated their effectiveness in preventing cancer through epidemiological studies and laboratory researches. Potential novel studies and researches provide powerful resource for the future acceptance of natural dietary compounds as chemopreventive agents. In contrast to conventional skin cancer treatments, chemopreventive agents can inhibit, reverse or suppress skin carcinogenesis with less undesirable effects for an inexpensive price. Chemoprevention by dietary phytochemicals has been regarded as new, safe, efficient and economic strategy for cancer treatment.

Numerous models and standards have been established to investigate and examine novel treatments in skin cancer studies which include animal models, human cell lines, tissue cultures, oncogenes and tumor suppressor genes, and pertinent clinical data. An established commonly used animal model is a two-stage carcinogenesis induced by 7, 12-dimethylbenz(α)anthracene (DMBA) and croton oil which functions as a carcinogen initiator and promoter, respectively. The DMBA initiates the cancer (initiation period), on the other hand, additional mutations generated by croton oil (promotion period) are necessary for cancer to develop (Filler et al, 2007). In skin cancer chemoprevention studies, the in vivo two-stage carcinogenesis in animal model has been extensively used for its ability to mimic human physiological response in skin carcinogenesis (Das et al, 2004).

The researchers have found a potential plant of interest to further study its significance in medicinal therapy. Thus, this study used the plant Pak-choi (Brassica rapa L.cv. Pak-choi Family Brassicaceae) as their potential chemopreventive agent in skin cancer chemoprevention study. The Brassicaceae family is widely considered to be healthy foods, high in vitamin C and
soluble fiber, and contains multiple nutrients and phytochemicals such as glucosinolates. Among the species of the Genus Brassica are cabbage, cauliflower, arugula, kale, turnip, broccoli, and brussel sprouts; Pak-choi has limited well-established studies and has restricted proven particular importance in research and therapeutics. As a result, the researchers wanted to find out its therapeutic importance, curative and beneficial effects, its cost-effectiveness as an alternative therapy, and further study the chemopreventive potential of the crude leaf extract of Pak-choi.

**Materials and Methods**

**Preparation of extract from plant**

Pak-choi leaves were collected at a local supermarket in Manila during the month of July 2013. A voucher specimen was submitted to the Bureau of Plant Industry, San Andres Malate, Manila for identification. The leaves of Pak-choi were cut into smaller pieces and dried at 40°C for three days. Dried leaves were grounded using a laboratory mill and macerated using 80% aqueous ethanol as solvent. The crude extract was kept in a suitable air-tight container ready for physical testing, phytochemical and biological testing.

The leaves of Pak-choi were pulverized and dried. About 1000g of the plant sample was weighed and placed in a suitable air-tight container. Then, the grounded leaves were macerated in 80% aqueous ethanol for 48 hours. The ethanolic extract was concentrated in a rotary evaporator under reduced pressure. To completely eliminate the solvent, the extract was further concentrated in a desiccator cabinet until a solid mass was obtained. The concentrated crude extract was measured and stored in a tightly-stoppered container ready for physical testing, phytochemical screening and biological testing.

**Animals**

Eight to ten weeks old male ICR mice, weighing 35-50g were obtained from the Department of Science and Technology and kept at the animal house of Centro Escolar University (CEU), Mendiola Manila, with ethical approval from Institutional Animal Care and Use Committees (IACUC) of Centro Escolar University. The mice were housed 5 per cage and acclimatized for one week prior to the commencement of experiment. The test animals were fed on standard laboratory diet with free access to water. Three days before treatment, the mice were dorsally shaved with an electric hair clipper, for an approximately 2 cm x 2 cm area (about 1 cm off tail). Each will be grouped separately for their proper treatment.

**Chemicals**

All chemicals were purchased prior to the study. 7, 12 – dimethylbenz (α) anthracene (DMBA), Croton oil and Curcumin were purchased from Sigma-Aldrich Co. (United States) while acetone was purchased from Labscan (Poland). All other reagents were commercially available. DMBA, a tumor initiator, was dissolved at a concentration of 100μg/100μL in Acetone. Croton oil which served as a tumor promoter was dissolved in Acetone to give 1%
Croton oil solution. Curcumin, used as a positive control, was dissolved in acetone to produce 0.2% w/v topical solution.

**In vivo two-stage skin tumorigenesis study**

All mice were divided into 6 groups (n=10). Group I served as the vehicle control treated only with 200 μL of Acetone. In groups II to VI, mice were initiated with a single topical application of 100 μg DMBA in 100 μL acetone solution one week prior to the promotion period. Group II served as the Carcinogen Control treated only with 100 μL of 1% croton oil solution throughout the promotion period. Group III served as the positive control group treated with 200 μL of 0.2%w/v topical solution of Curcumin, 30 minutes before the croton oil application. Groups IV, V and VI were treated with 200 μL of Pak-choi crude leaf extract at a concentration of 0.5%w/v, 2%w/v and 5%w/v topical solutions, respectively, 30 minutes before croton oil application. All treatments were applied topically, twice a week for 10 weeks of promotion period. (Rosilda et al, 2011)

**Morphological Assessment**

Body weight, latency period of tumor formation, percentage of tumor incidence, tumor burden and tumor volume were observed and measured at a 7-day interval. Only tumors which persisted more than one week with diameter greater than 1 mm were taken into consideration for data analysis. Latency period of tumor formation was determined when the first tumor appeared. Percentage of tumor incidence was calculated by dividing the number of tumor-bearing mice with the total number of mice in a particular group and multiplied by 100%. Tumor burden was obtained by dividing the total number of tumors with the number of tumor-bearing mice in a group. Tumor volume was measured by multiplying π/6 to the length, width and height of the tumor. (Girit et al, 2008)

**Histopathological Analysis**

The experiment was terminated at the end of the 10th week of tumor promotion and mice were sacrificed for histopathological analysis. Skin samples obtained from dissection were fixed in 10% formalin before being processed in an automatic tissue processor by standard protocols. Processed tissues were embedded in paraffin wax, sectioned with microtome at a thickness of 4 μm and stained with Haematoxylin and Eosin (H&E) using routine protocol. Stained slides were observed under light microscope and digital micrographs of the slides were taken.

**Statistical Analysis**

All data were statistically treated by one-way analysis of variance (ANOVA) following post-hoc studies using Tukey’s Honestly Significant Difference to assess significant differences of mean between groups. SPSS 22.0 software was used for the calculations and all values were expressed as mean ± S.E.M. (standard error of mean) at 5% significant level. Values with $P<0.05$ were considered statistically significant.
Results

Results of physicochemical analyses showed that PLCE was brownish green in color, had an ammoniacal odor, and solid mass in appearance. PLCE was soluble in water and 80% ethyl alcohol, slightly soluble in ether and acetone, and insoluble in benzene. Results of phytochemical showed that PLCE was positive in the presence of alkaloids, steroids, tannins and quaternary bases.

Table 1 and Figures 1 and 2 show summary of the chemopreventive potential of PLCE on DMBA/Croton Oil – induced in vivo two stage mouse skin tumorigenesis. No toxic effect had been observed with the selected dose of PLCE, as evident by body weight, skin texture and overall morphological appearance of the mice. No significant rise of the average body weight of all groups was observed at the termination of the experiment. Tumors begin to appear on the skin from week 5 to 8 during the promotion period. The latency period of tumor formation was greatly delayed to the 8th week in Group IV (0.5%w/v topical solution of PLCE) in contrast to the Carcinogen Control, which started to develop tumor in the sixth week. Tumor formation was prompted one week earlier to the fifth week in Group VI (5%w/v topical solution of PLCE) in comparison to the Carcinogen Control. Both Group V (2% w/v topical solution of PLCE) and the Positive Control (0.2% w/v topical solution of Curcumin) had a latency period of seven weeks.

![Graph](a) Percentage Tumor Incidence

![Graph](b) Tumor Burden

Results of histopathological analysis showed varying degree of hyperplasia, keratin horn pearls and malignant lesions in all treated groups. Differences in mitotic activity and...
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Tumor incidence was significantly different between treatment groups at $P<0.05$. At the end of the study, Group VI and the Carcinogen Control obtained a tumor incidence of 70%. Group IV (30%) was found to have a high significance difference level of tumor incidence in contrast to the Carcinogen Control and Group VI. No significant difference was observed between Group IV and the Positive Control (40%).

Statistical analysis showed that there is no significant difference between control and treatment groups for tumor burden. Group VI found to have the highest tumor burden of $0.21\pm0.09$ while Group V found to have the lowest of only $0.16\pm0.07$. Groups II, III and IV have a tumor burden of $0.20\pm0.06$, $0.20\pm0.09$ and $0.21\pm0.09$, respectively.

There is a significant difference between groups in tumor volume ($P<0.05$). Among all groups, Carcinogen Control has found to have the highest tumor volume of $4.19\pm0.93$ while Group IV has found to have the lowest of $0.50\pm0.43$. Group VI also exhibited a high tumor volume of $3.23\pm1.01$ among all experimental groups but lower than Carcinogen Control. Group IV, having a tumor volume of $0.50\pm0.43$, is lower than the Positive Control. Group V has found to have a tumor volume of $0.65\pm0.27$. As in tumor incidence, tumor volume of Group IV has found to be significantly different from the Carcinogen Control at $P<0.05$. Significant difference was also observed between Groups IV and VI and no significant difference was observed between Group IV and the Positive Control, and Group VI and the Carcinogen Control.

In overall, Group IV (0.5% w/v) showed better effects than the Carcinogen Control, in terms of tumor incidence, tumor volume and the delayed latency of tumor formation. Group IV showed comparable effect to the Positive Control. Group VI (5% w/v), nevertheless, turned out to be comparable in tumor incidence, tumor volume and latency period of tumor formation in comparison to the Carcinogen Control.

Results of histopathological analysis showed varying degree of hyperplasia, keratin horn pearls and malignant lesions in all treated groups. Differences in mitotic activity and...
vascularization were observed. The epidermis is less hyperplastic in Group IV in contrast to Carcinogen Control, whereas Group V has more keratinized horn pearls with comparable hyperplastic epidermis to the Carcinogen Control. Mitotic activity and vascularization of Group IV is less in contrast to the Carcinogen Control, and comparable to the Positive Control. Groups V and VI has the same mitotic activity and vascularization to Carcinogen Control. This suggests that Group IV has the least proliferating activity of cancer cells. Formed tumors are malignant papillomas which are indicative of basal cell carcinoma that completely breached the dermis layer of the skin.

Figure 2. Results of Histopathological Analysis Obtained From Control and Treatment Groups at the End of the Study

Arrows showed hyperplasia of epidermis (black), keratinized horn pearls (yellow) and malignant lesions where basement membrane was breached (red). (H&E stained micrographs taken at 40x magnifications)

<table>
<thead>
<tr>
<th>Group</th>
<th>Initial Effect</th>
<th>Final Effect</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (Negative)</td>
<td>10</td>
<td>10</td>
<td>0.59</td>
</tr>
</tbody>
</table>
| II (Carcinogen Control) | 10 | 10 | 1.18 | *Treatment groups refer to Group IV (0.5% w/v), Group V (2%w/v) and Group VI (5%w/v)
| III (Curcumin) | 10 | 10 | 0.99 | *Treatment groups refer to Group IV (0.5% w/v), Group V (2%w/v) and Group VI (5%w/v)
| IV (0.5%w/v PLCE) | 10 | 10 | 0.92 | *Treatment groups refer to Group IV (0.5% w/v), Group V (2%w/v) and Group VI (5%w/v)
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Table 1. Chemopreventive Effect of Pak-Choi Leaf Crude Extract on DMBA/CO-induced Two-Stage in vivo Mouse Skin Tumorigenesis

<table>
<thead>
<tr>
<th></th>
<th>Initial</th>
<th>Effective</th>
<th>Initial</th>
<th>Final</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>I (Negative)</td>
<td>10</td>
<td>10</td>
<td>38.84 ± 0.59</td>
<td>41.53 ± 0.99</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>II (Carcinogen Control)</td>
<td>10</td>
<td>10</td>
<td>40.82 ± 0.84</td>
<td>42.13 ± 1.18</td>
<td>6</td>
<td>70</td>
<td>0.20±0.06</td>
<td>4.19±0.93</td>
</tr>
<tr>
<td>III (Curcumin)</td>
<td>10</td>
<td>10</td>
<td>39.75 ± 0.88</td>
<td>41.73 ± 1.10</td>
<td>7</td>
<td>40</td>
<td>0.20±0.09</td>
<td>0.65±0.27</td>
</tr>
<tr>
<td>IV (0.5%w/v PLCE)</td>
<td>10</td>
<td>10</td>
<td>39.10 ± 1.43</td>
<td>40.49 ± 1.45</td>
<td>8</td>
<td>30</td>
<td>0.17±0.09</td>
<td>0.50±0.43</td>
</tr>
<tr>
<td>V (2%w/v PLCE)</td>
<td>10</td>
<td>10</td>
<td>40.44 ± 0.81</td>
<td>42.37 ± 0.81</td>
<td>7</td>
<td>50</td>
<td>0.16±0.07</td>
<td>1.70±0.83</td>
</tr>
<tr>
<td>VI (5%w/v PLCE)</td>
<td>10</td>
<td>10</td>
<td>35.47 ± 0.37</td>
<td>40.61 ± 0.92</td>
<td>5</td>
<td>70</td>
<td>0.21±0.09</td>
<td>3.23±1.01</td>
</tr>
</tbody>
</table>

Values expressed as mean ± S.E.M. 1Significant levels between treated groups and carcinogen control group (Group II) at $P<0.05$; 2Significant levels between treated groups and positive control group (Group III) at $P<0.05$; *Treatment groups refer to Group IV (0.5%w/v), Group V (2%w/v) and Group VI (5%w/v)
Discussion

Many years of research revealed that cancer is easier prevented than treated. Whereas chemotherapy is designed to destroy cancer after it appears, chemoprevention involves the abrogation or delay in the onset of cancer (Aggarwal et al., 2004). Chemoprevention by phytochemicals appeared to be one of the most practical means in controlling cancer (Sengupta et al., 2004). Pak-choi, belonging to Brassicaceae Family, uniquely contains glucosinolates at approximately 20μmol/g dry mass of vegetable (Kushad et al., 1999) and is thought that this phytochemicals are primarily responsible for the putative cancer chemoprevention. (Johnson, 2002)

Two-stage in vivo mouse skin tumorigenesis model involves the cancer formation processes initiation and promotion. Inhibition of tumor promotion is a better strategy in cancer chemoprevention than inhibition of tumor initiation because initiation is a short irreversible event whereas promotion is a long cumulative process, which is reversible during the initial stage (Agarwal and Mukhtar, 1991; DiGiovanni, 1992). The present study showed the cancer chemopreventive effect in mice treated with Pak-choi leaf crude extract (PLCE) at low dose (0.5% w/v), whereas at high dose (5% w/v), PLCE enhances tumorigenesis. Glucosinolates present in PLCE is effective at lower doses and sufficient doses to produce its therapeutic action, but exert its toxicity at high dose. (Fahey et al., 1997). Other studies suggest the ability of certain phytochemicals to exert cancer chemopreventive effect at low doses (Rao and Shen, 2002; Russo, 2007).

The mechanism of action for cancer chemoprevention of glucosinolates is elucidated in the study by Hayes et al., (2009). Inhibition of carcinogenesis by glucosinolates is not primarily attributable to this class of compound, but rather it appears to be due to certain breakdown metabolites of glucosinolates. Hydrolysis of these phytochemicals is catalysed by an enzyme called myrosinase. Myrosinase cleaves glucosinolates at the thioglycoside linkage to produce glucose and an unstable aglycone that spontaneously rearranges to yield several breakdown products including isothiocyanates, thiocyanates, nitriles, cyanoepithioalkanes and indoles. These products have the abilities to induce cytoprotective genes, mediated by the Nrf2(NF-E2 related factor 2) and AhR (aryl hydrocarbon receptor) transcription factors, and their abilities to repress NF-jB (nuclear factor-jB) activity, inhibit histone deacetylase, and inhibit cytochrome P450. Isothiocyanates appear to alter gene expression through modification of critical thiols in regulatory proteins such as Keap1 (Kelch-like ECH-associated protein1) or IKK (IjB kinase), causing activation of Nrf2 and inactivation of NF-jB, respectively. Certain indoles act as ligands for AhR. Isothiocyanates and indoles are also capable of affecting cell cycle which arrest and stimulate apoptosis. (Hayes et al., 2009)

Despite effectiveness of PLCE at low dosage, high dosage of PLCE (5%w/v) demonstrates tumor promoting effect. Studies suggest that at high concentration of glucosinolates breakdown products, certain types may initiate mutagenic, cytotoxic and carcinogenic properties. (Williamson, 2008)
**Conclusion**

This current study revealed that Pak-choi crude leaf extract has chemopreventive potential in DMBA-initiated and croton-oil promoted *in vivo* two-stage skin tumorigenesis in male ICR mice during the promotion period, particularly at low dosage (0.5% w/v).

**Recommendation**

Additional study is required to characterize specific glucosinolates present in Pak-choi. Elucidation of the exact mechanism underlying this chemopreventive potential must also be studied. Different Pak-choi cultivars, maturity of the plant, and conditions where it is cultivated should be taken into consideration. Other models of carcinogenesis, whether *in vivo* or *in vitro* and other forms of cancer is suggested to be studied. Other routes of administration for the plant extract are also considered. Formulation of appropriate dosage forms is also suggested.

**References:**


The Renoprotective Property of the Flavonoids from the Bulb of Sibuyas Tagalog (Allium cepa, L. cv. group aggregatum, family Alliaceae) on Streptozotocin-Induced Diabetic Nephropathy in Male Wistar Rats

1,2,3,4,5,6,7 Centro Escolar University, Manila, Philippines

ABSTRACT

This study was designed to determine the renoprotective property of the flavonoids from the bulb of sibuyas tagalog on the basis of body weight, biochemical and histopathological parameters in streptozotocin-induced diabetic nephropathy in male wistar rats. Flavonoids were obtained through maceration with hot methanol and further extraction with diethyl ether and ethyl acetate. These were identified through physical, chemical and instrumental tests.

Streptozotocin at a dose of 60 mg/kg body weight dissolved in 0.1 M citrate buffer was intraperitoneally administered to rats to induce diabetic nephropathy. The flavonoids extract were used to treat Wistar rats for three months at doses 100mg/kg, 300mg/kg and 500mg/kg. Aminoguanidine was used as the positive control, which was given orally at a dose of 150mg/kg. After eighty four (84) days, all rats were sacrificed via cervical dislocation.

KEYWORDS: Renoprotective, Flavonoids, Streptozotocin, Diabetic Nephropathy, Aminoguanidine
INTRODUCTION

World Health Organization (WHO) defines diabetes as a chronic disease that occurs when the pancreas does not produce enough insulin or when the body cannot efficiently utilize the use of insulin it synthesizes (WHO, 2013). As of March 2013, WHO states that there are 347 million people who were diabetics, and projects that it will be the 7th leading cause of death in the year 2030. About 80% of deaths related to diabetes arise in low- and middle-income countries, including the Philippines. Among the consequences associated with worsen diabetes are risks of cardiovascular diseases, retinopathy, neuropathy, and nephropathy (Diabetes Journals, 2012).

Diabetic nephropathy, the leading cause of chronic kidney disease, is a complication associated with the inability of the kidneys to function well due to damage induced by diabetes (Medscape, 2012). High blood sugar from diabetes destroys glomerulus that filters wastes from the blood, causing glomerular thickening and scar. About 30-40% of patients with diabetes develop evidence of nephropathy (Clinical Key, 2012). When diabetic nephropathy is left untreated, it can result in end–stage renal disease (ESRD) requiring dialysis or kidney transplantation (Palo Alto Medical Foundation, 2010).

Sibuyas Tagalog, a native and abundant species of onion in the Philippines, found to contain secondary metabolites, specifically flavonoids, that have the potential renoprotective property. With this, the researchers aimed at determining the renoprotective property of the flavonoid constituents of the bulb of Sibuyas Tagalog on streptozotocin-induced diabetic nephropathy in rats.

MATERIALS AND METHODS

Plant Material: Fresh sibuyas tagalog bulbs were collected from the market of Divisoria, Manila (supplies came from Ilocos Norte) during the months of June to July 2013. Samples were authenticated at the Botany Division of the National Museum.

Chemicals: All chemicals used in the study were obtained from the CEU Physical Sciences Department, except for Streptozotocin and Aminoguanidine hydrochloride which were purchased from Belman Laboratories.

Extraction: About 1000 grams of fresh sibuyas tagalog bulb was macerated for 48 hours using hot methanol and was subjected to rotary evaporator to remove the methanolic solvent. Ethyl acetate and diethyl ether were also used for further re-extraction of the flavonoid and were separated from the flavonoid extract using separatory funnel and rotary evaporator.

Animals: Fifteen (15) Male Wistar Rats weighing 250-400 grams were purchased from Department of Science and Technology-IACUC. The rats were housed in the CEU Animal Facility in a 470mmX355mmX270mm (LxWxH) plastic cage with shredded paper as bedding. The CEU Animal Facility is an air conditioned room, and laboratory animals were placed under 12h dark/12h light condition.
**Animal Treatment Model:** The rats were divided into 5 groups, with three rats each: (I) Negative Control, (II) Positive Control, (III, IV, V) Experimental Groups – pretreated with flavonoids extract. The biological test was done for a period of 84 days. The first week of the biological testing was acclimatization of the rats.

Group I: Streptozotocin (60mg/kg) on the 8th day.
Group II: Streptozotocin (60mg/kg) on the 8th day treated with Aminoguanidine (150mg/kg) on 15th to 84th day.
Group III: Streptozotocin (60mg/kg) on the 8th day treated with flavonoids extract (100mg/kg) on 15th to 84th day.
Group IV: Streptozotocin (60mg/kg) on the 8th day treated with flavonoids extract (300mg/kg) on 15th to 84th day.
Group V: Streptozotocin (60mg/kg) on the 8th day treated with flavonoids extract (500mg/kg) on 15th to 84th day.

**Diabetic Nephropathy Inducer and Positive Control:** Diabetic nephropathy in rats was induced by a single intraperitoneal injection with 60 mg/kg Streptozotocin on the 8th day of the biological test. 5% sucrose solution was given 24 hours after induction to prevent fatal hypoglycemia. Aminoguanidine hydrochloride dissolved in distilled water given orally at a dose of 150 mg/kg was used as the positive control. It was administered one week after inducing streptozotocin on once a day basis via oral gavage for a period of 70 days.

**Analysis of Results:** Body weights, biochemical test results and photomicrographs of the kidney from the histopathological study served as basis for evaluating the renoprotective property of the flavonoids extracted from Sibuyas Tagalog bulb. The level of serum and urinary albumin, serum and urinary creatinine, blood and urine urea nitrogen and serum lipid profile were determined. For histopathological studies, the result from Group I was observed to confirm the induction of diabetic nephropathy. The results from the experimental groups (Groups III, IV and V) were compared with that of the negative control group (Group I) to determine whether the flavonoids extract provided renoprotection against Streptozotocin-induced diabetic nephropathy. The results from the experimental groups were also compared to that of the positive control (Group II), to determine if the extent of renoprotection is comparable to a known and widely used renoprotective agent, Aminoguanidine.

**Statistical Treatment:** Statistical analysis was performed by One-way ANOVA followed by Dunnet's test to compare the positive and negative control groups. The statistical difference between the normal and diseased was analyzed by Un-paired t-test. The results were considered statistically significant, if p < 0.05.
RESULTS AND DISCUSSION

The percentage yield of the sibuyas tagalog extract was 8.02%. Results of the physical, chemical and instrumental tests all confirmed the presence of flavonoids.

Physical properties of the flavonoids extract
The flavonoids extract obtained was semi-solid, dark bloody red and ethyl acetate odor. It is very soluble in water, ethanol 80% and 95%, and methanol, soluble in chloroform and insoluble in ether and hexane.

Chemical tests for identification of flavonoids
The chemical test for the presence of flavonoids namely Bate Smith and Metcalf Test, Wilstatter-Cyanidin Test, Shinoda Test, Lead Acetate Test and Ammonia Test all produced a positive result.

Instrumental Analysis for the Presence of Flavonoids

FTIR was used to identify the functional groups present in the flavonoids extract. Results show that it has strong, broad phenolic functional group, as well as weak to strong aromatic carbons, a characteristic common to flavonoids.

Instrumental test using the High Performance Liquid Chromatography shows the notable peak that has a retention time of 19.216 minutes and an area of 367.68 mAU•s. The data obtained from the extract illustrates comparability with the standard Quercetin, having a retention time of 19.529 minutes. Based from the values given, the resulting concentration of the quercetin from the 500mg/mL extract was found to be 9.73 x 10⁻³ mg/mL.

Effects of Flavonoids Extract on Body Weight

Figure 1. Body Weights of the Fifteen (15) Male Wistar Rats

Table 1. Dunnett’s Procedure for Body Weight

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>DIFFERENCE OF MEANS</th>
<th>D-VALUE</th>
<th>VERBAL INTERPRETATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>II – III</td>
<td>73.77</td>
<td>61.79</td>
<td>SIGNIFICANT</td>
</tr>
<tr>
<td>II – IV</td>
<td>129.20</td>
<td>61.79</td>
<td>SIGNIFICANT</td>
</tr>
<tr>
<td>II – V</td>
<td>135.90</td>
<td>61.79</td>
<td>SIGNIFICANT</td>
</tr>
</tbody>
</table>

Based from Figure 1, only group 1 showed a decrease in body weight while that of the remaining groups increased. Dunnett’s procedure showed significant difference of groups III, IV and V from group II. This implies that obtained values from the experimental group III, IV and V were comparable to the positive control, group II.

Effects of Flavonoids Extract on Blood Glucose

Figure 2. Blood Glucose Level of Group I-V

Table 2. Dunnett’s Procedure for Blood Glucose Level

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>DIFFERENCE OF MEANS</th>
<th>D-VALUE</th>
<th>VERBAL INTERPRETATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>II – II</td>
<td>191.67</td>
<td>113.21</td>
<td>SIGNIFICANT</td>
</tr>
<tr>
<td>II – IV</td>
<td>4.33</td>
<td>113.21</td>
<td>NOT SIGNIFICANT</td>
</tr>
<tr>
<td>II – V</td>
<td>181.33</td>
<td>113.21</td>
<td>SIGNIFICANT</td>
</tr>
</tbody>
</table>

In diabetic rats, the blood glucose level was significantly high (~ 600 mg/dl) throughout the study period. The diabetic rats that were treated with Aminoguanidine (group II) showed a significant decrease in the blood glucose level, but the experimental groups III and V elicited significant increase in blood glucose level. Table 2 shows that there is no significant difference in the blood glucose level for group IV as compared the positive control group.
RESULTS AND DISCUSSION

The percentage yield of the sibuyas tagalog extract was 8.02%. Results of the physical, chemical and instrumental tests all confirmed the presence of flavonoids.

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Based from the values given, the resulting concentration of the quercetin from the 500mg/mL extract was found to be 9.73 x 10^{-3} mg/mL.

Effects of Flavonoids Extract on Body Weight

![Figure 1. Body Weights of the Fifteen (15) Male Wistar Rats](image)

Table 1. Dunnett’s Procedure for Body Weight-Positive Control Group vs. Experimental Groups

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>DIFFERENCE OF MEANS</th>
<th>D-VALUE</th>
<th>VERBAL INTERPRETATION</th>
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<tr>
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<td>73.77</td>
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<td>129.20</td>
<td>61.79</td>
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</table>

Based from Figure 1, only group 1 showed a decrease in body weight while that of the remaining groups increased. Dunnett’s procedure showed significant difference of groups III, IV and V from group II. This implies that obtained values from the experimental group III, IV and V were comparable to the positive control, group II.

Effects of Flavonoids Extract on Blood Glucose

![Figure 2. Blood Glucose Level of Group I-V](image)

Table 2. Dunnett’s Procedure for Blood Glucose Level- Positive Control vs. Experimental Groups

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>DIFFERENCE OF MEANS</th>
<th>D-VALUE</th>
<th>VERBAL INTERPRETATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>II - III</td>
<td>191.67</td>
<td>113.21</td>
<td>SIGNIFICANT</td>
</tr>
<tr>
<td>II - IV</td>
<td>4.33</td>
<td>113.21</td>
<td>NOT SIGNIFICANT</td>
</tr>
<tr>
<td>II - V</td>
<td>181.33</td>
<td>113.21</td>
<td>SIGNIFICANT</td>
</tr>
</tbody>
</table>

In diabetic rats, the blood glucose level was significantly high (~ 600 mg/dl) throughout the study period. The diabetic rats that were treated with Aminoguanidine (group II) showed a significant decrease in the blood glucose level, but the experimental groups III and V elicited significant increase in blood glucose level. Table 2 shows that there is no significant difference in the blood glucose level for group IV as compared the positive control group.
Effects of Flavonoids Extract on HDL, LDL, Cholesterol and Triglycerides

Figure 3. Serum Cholesterol, Triglycerides, High-Density Lipoprotein and Low-Density Lipoprotein of Group I-V

The changes in the lipid level of animals in all the four groups are indicated in figure above. All values obtained were not significant.

Effects of Flavonoids Extract on Serum and Urinary Albumin

Figure 4. Serum and Urinary Albumin Level of Group I-V

Table 3. Dunnett’s Procedure for Serum Albumin Level-Positive Control Group vs. Experimental Groups

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>DIFFERENCE OF MEANS</th>
<th>D-VALUE</th>
<th>VERBAL INTERPRETATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>II – III</td>
<td>0.29</td>
<td>0.51</td>
<td>NOT SIGNIFICANT</td>
</tr>
<tr>
<td>II – IV</td>
<td>0.62</td>
<td>0.51</td>
<td>SIGNIFICANT</td>
</tr>
<tr>
<td>II – V</td>
<td>0.15</td>
<td>0.51</td>
<td>NOT SIGNIFICANT</td>
</tr>
</tbody>
</table>

Table 4. Dunnett’s Procedure for Urinary Albumin Level-Positive Control Group vs. Experimental Groups

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>DIFFERENCE OF MEANS</th>
<th>D-VALUE</th>
<th>VERBAL INTERPRETATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>II – III</td>
<td>1.78</td>
<td>0.84</td>
<td>SIGNIFICANT</td>
</tr>
<tr>
<td>II – IV</td>
<td>0.02</td>
<td>0.84</td>
<td>NOT SIGNIFICANT</td>
</tr>
<tr>
<td>II – V</td>
<td>0.39</td>
<td>0.84</td>
<td>NOT SIGNIFICANT</td>
</tr>
</tbody>
</table>

Diabetic nephropathy is being diagnosed clinically by an increase in the level of albumin in urine (albuminuria), accompanied by a decrease in its level in serum. In the study, the diabetic rats showed a significant increase in the albumin content in urine with a decrease in the level of albumin in serum. This confirmed that the rats induced with diabetes have progressed to DNP. After treatment with Flavonoids extract, groups III and V showed no significant difference with that of group II for the serum albumin level. However, as seen on figure 4, group IV exhibited the highest level of albumin in the serum, showing better result than the positive control. For the urinary albumin, groups IV and V showed no significant difference with that of group II, meaning the values obtained are comparable to the value of the positive control. However, based from figure 4, group IV exhibits the lowest amount of albumin excreted in the urine as compared to the other experimental groups. At the end of the study, the albumin level of the diabetic rats supplemented with Flavonoids extract was brought back to normal, which proved that Flavonoids extract has the potential to attenuate DNP.

Effects of Flavonoids Extract on Serum and Urinary Creatinine

Figure 5. Serum and Urinary Creatinine Level of Group I-V

Table 5. Dunnett’s Procedure for Serum Creatinine Level-Positive Control Group vs. Experimental Groups

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>DIFFERENCE OF MEANS</th>
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</thead>
<tbody>
<tr>
<td>II – III</td>
<td>0.06</td>
<td>0.23</td>
<td>NOT SIGNIFICANT</td>
</tr>
<tr>
<td>II – IV</td>
<td>0.35</td>
<td>0.23</td>
<td>SIGNIFICANT</td>
</tr>
<tr>
<td>II – V</td>
<td>0.17</td>
<td>0.23</td>
<td>NOT SIGNIFICANT</td>
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</table>

Table 6. Dunnett’s Procedure for Urinary Creatinine Level-Positive Control Group vs. Experimental Groups

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</thead>
<tbody>
<tr>
<td>II – III</td>
<td>1.91</td>
<td>0.97</td>
<td>SIGNIFICANT</td>
</tr>
<tr>
<td>II – IV</td>
<td>0.29</td>
<td>0.97</td>
<td>NOT SIGNIFICANT</td>
</tr>
<tr>
<td>II – V</td>
<td>0.86</td>
<td>0.97</td>
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Any kidney damage is reflected by a decrease in the creatinine content in urine, and DNP is no exception. The diabetic rats showed a significant decrease in the level of creatinine in urine with a significant increase in serum which proved that the animals have encountered kidney damage. However, after treatment with Flavonoids extract, group IV showed that the creatinine content decreased significantly in serum. Table 5 showed that groups III and V showed no significant difference with that of group II meaning the values obtained are comparable to the positive group. However, based on figure 5, group IV exhibited the lowest level of serum albumin, better than the positive control. For the urinary creatinine, table 6 showed that groups IV and V showed no significant difference with that of group II. Group IV exhibited the highest level of creatinine in the urine, indicative of a healthier kidney as compared to the positive control. This demonstrated that the Flavonoids extract, particularly the experimental group IV, has improved the kidney damage in diabetic rats.

Effects of Flavonoids extract on Serum and Urinary Urea Nitrogen

![Figure 6. Serum and Urinary Urea Nitrogen Level of Group I-V](image)

Table 7. Dunnett’s Procedure for Serum Urea Nitrogen Level-Positive Control Group vs. Experimental Groups

<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>II - III</td>
<td>9.00</td>
<td>10.07</td>
<td>NOT SIGNIFICANT</td>
</tr>
<tr>
<td>II - IV</td>
<td>6.03</td>
<td>10.07</td>
<td>NOT SIGNIFICANT</td>
</tr>
<tr>
<td>II - V</td>
<td>15.13</td>
<td>10.07</td>
<td>SIGNIFICANT</td>
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Table 8. Dunnett’s Procedure for Urinary Urea Nitrogen Level-Positive Control Group vs. Experimental Groups

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</tr>
</thead>
<tbody>
<tr>
<td>II - III</td>
<td>16.16</td>
<td>10.06</td>
<td>SIGNIFICANT</td>
</tr>
<tr>
<td>II - IV</td>
<td>1.47</td>
<td>10.06</td>
<td>NOT SIGNIFICANT</td>
</tr>
<tr>
<td>II - V</td>
<td>14.70</td>
<td>10.06</td>
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### Effects of Flavonoids extract on Serum and Urinary Urea Nitrogen

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Table 7. Dunnett’s Procedure for Serum Urea Nitrogen Level

- Positive Control Group vs. Experimental Groups

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</tr>
</tbody>
</table>

Table 8. Dunnett’s Procedure for Urinary Urea Nitrogen Level

- Positive Control Group vs. Experimental Groups

In diabetic nephropathy condition, there was an increase in the level of blood urea nitrogen content with a decrease in its level in urine. The diabetic rats showed a significant increase in the blood urea nitrogen content and a significant decrease in the urine urea nitrogen content, which confirmed that the animals have progressed to diabetic nephropathy. However, the animals treated with Flavonoids extract, specifically group IV, showed a significant decrease in the blood urea nitrogen content and a significant increase in the urine urea nitrogen content, which was similar to that observed in Aminoguanidine-treated animals. Table 7 showed that groups III and IV exhibited no significant difference with that of group II. Serum level of urea nitrogen of groups III and IV were comparable to the positive group and based on figure 8, group IV has the lowest level of urea nitrogen in the blood. For the urinary urea nitrogen levels, table 8 showed that only group IV showed a comparable value to group II but as seen on figure 6, group IV has higher level of urea nitrogen excreted in the urine as compared to the positive control. This potentially proves that Flavonoids extract could be used as a renoprotectant in diabetic animals.

### Histopathological Study

![Figure 7. Kidney histology from Control and Diabetic rats](image)

Histopathology images of kidney from control and diabetic rats.

(1A): diabetic rats, PAS (2A,2B,2C) positive control rats

Group 1 showed general disruption of cortical architecture, fewer tubules, glomerular and tubule degradation, glomerulosclerosis, basement membrane thickening. It showed the presence of positive hemispherical deposit. Group 2A, 2B, 2C showed mild mesangial expansion with no change in the glomerular basement membrane thickening.

![Figure 8. Kidney histology from Experimental Groups (100mg/kg, 300mg/kg, 500mg/kg)](image)

Histopathology images of kidney from experimental rats

(3A,3B,3C):(2A,2B,2C) positive control rats
Group 3A, 3B, 3C showed 60% loss of tubular detail, moderate tubular degeneration/tubulonephrosis, confluent and spreading. Group 4A, 4B, 4C showed 60% loss of tubular details, mild to moderate tubular degeneration/tubulonephrosis. Group 5A, 5B, 5C showed 50% loss of tubular details, moderate tubular degeneration/tubulonephrosis.

Figures 7 and 8 illustrate the changes in the kidney histology of the animals in all groups. The diabetic rats showed changes such as hypercellularity, glycosuria, and proteinuria in tubules with mild mesangial expansion and proliferation in the glomeruli. Prominent nodular glomerulosclerosis with glomerular basement membrane thickening was also observed. However, minimal changes were observed in the diabetic animals that were supplemented with flavonoids extract and aminoguanidine, which proves that the flavonoids extract from sibuyastagaloghas the potential to ameliorate diabetic nephropathy.

CONCLUSION

Based on the data obtained, the researchers have concluded that the flavonoids extract obtained from Sibuyas Tagalog bulb with the dose of 300 mg/kg has a renoprotective property on Streptozotocin-induced diabetic nephropathy in male Wistar rats.

RECOMMENDATIONS

1. To develop a better method of extraction of the flavonoids from the Sibuyas Tagalog bulb;
2. To perform additional HPLC tests using other standard flavonoids to identify the other probable flavonoids present in the sibuyas tagalog bulb extract;
3. To identify the mechanism by which the flavonoids from Sibuyas Tagalog bulb exhibits renoprotection;
4. To perform biological test in a larger group of experimental animals; and
5. To formulate dosage form from the Sibuyas Tagalog flavonoids extract.

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**REFERENCES**

T. M. Shiju et al. (2013). Renoprotective effect of aged garlic extract in streptozotocin-induced diabetic rats. Renal Research Lab, Biomedical Research Center, School of Bio Sciences and Technology, VIT University, Vellore - 632 014, India.
INSTRUCTIONS TO AUTHORS

The JPPhA accepts manuscripts of the following type:

1. Original Research Reports on Pharmaceutical Sciences, Pharmacy Education and Pharmacy Practice
2. Abstracts of oral and poster presentations in international and local scientific conferences
3. Drug use reviews

Manuscript Format:

1. Full-length articles must be in English. They should not exceed ten (10) double-spaced pages in length, including the cover page, abstract, references, tables and figures
2. Author names, their respective affiliations and the corresponding author must be included
3. All manuscripts must include an abstract containing a maximum of 180 words typed on a separate page. The abstract must contain the background, objectives, materials and methods, results and conclusions/recommendations of the study.
4. After the abstract, provide at least three key words or identifiers.
5. Graphic files are welcome if supplied as JPEG files. When possible, place symbol legends below the figure image instead of to the side.
6. The article title must be written in Times New Roman, font size 14, centered. Subtitles with same font type, font size 12, left justified.
7. The paragraph in the body of the article should be written in Times New Roman, font size 11, justified
8. The title of the table should be written in sentence format on top of the table with table number written before the title. Example is - Table 1. Demographic characteristics of study participants.
9. Referencing style must follow Harvard style of presentation—both appearing on the text and reference list at the bottom of the article. In the text of the article, only the author name(s) and year of publication should appear enclosed in parenthesis. In the reference list, the first line of each reference should have hanging indent of five spaces in the following order of information:

   Journal: authors (last name, first name), title of article, name of the journal, volume, number or issue number, pages, year of publication

   Book: author/s, year of publication, title of book, edition, place of publication, publisher, pages

   Internet site: http:author and title of article at www.____ accessed on (date) and time

Important Reminder:

1. Submission of Manuscripts for the next publication will start on June 1, 2014 and will end on September 30, 2014
2. Review of manuscript will be scheduled on October 2014, subject to the availability of reviewers.
3. The next release of the JPPhA will be scheduled on December 2014
4. You can email your manuscripts to philpharm@surfshop.net, copy furnish bryanposadasrph@gmail.com