ACUTE TOXICITY EVALUATION OF *Holothuria scabra* Jaeger EXTRACT AND ITS EFFECTS ON INDUCED GASTRIC ULCER IN RATS

BY

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ABSTRACT

Holothuria scabra is one of edible marine animals which is widely consumed by Chinese and local Sabahan people, who believe in the medicinal properties offered by the species. This work was focusing on *H. scabra* in the attempt of finding new therapeutic properties, because of its large availability in the coastal area of Sabah and ability to be widely bred in hatcheries for restocking purpose. The study comprises of two types of study: anti-gastric ulcer and acute toxicity test, to investigate the existence of anti-gastric ulcer property in the species and its safety for human consumption. In the anti-gastric ulcer study, 150 mg/ml *H. scabra* water extract was administered into rats that had been induced with gastric ulcer by using acetic acid. Histology of the stomach was examined and the gastric mucous was estimated by using spectrophotometer. The toxicity test was conducted by giving *H. scabra* water extract with the doses of 5, 50, 300 and 2000 mg/kg into four groups of rats, whereas the control group received distilled water. Survival and toxicity signs were carefully observed for 14 days, in addition to performing the blood analyses and histopathological examination of liver and kidney tissues. For the anti-gastric ulcer study, the stomach tissues from both groups of treated and untreated were recovering, with no significant difference in terms of structural changes and inflammatory cell infiltrates. The treated rats did not produce significant higher amount of gastric mucous as compared to the non-treated rats, except on the day 12 of the experiment. In the toxicity test, no mortality and toxicity signs were observed throughout the observation period. Gross examination on liver and kidneys showed no changes in appearance, size, and colour of the organs when compared to the control group. Statistical analysis of the Kruskal-Wallis H test produced no significant difference in body weight between the control and all treated groups after the treatment, $x^2(4, N = 23) = 5.754$, $p = 0.218$. The haematological and biochemical blood parameters of the treated group of rats also showed no statistical difference as compared to the control. However, histopathological examination revealed presence of necrotic tissues and cellular damages in the liver and kidneys of all treated rats at all doses of extract. The study suggests that the effects of *H. scabra* water extract on the healing of induced gastric ulcer in rats were not conclusive. However, since blood analyses results and histopathological findings of the acute toxicity study were contradict, sub-acute toxicity is suggested to be conducted in future to further confirm the toxicity level of the extract.

خلاصة البحث

إن الهولوثوريا سكابرا (*Holothuria* scabra) أو خيار البحر هو حيوان بحري صالح للأكل ويستهلك على نطاق واسع من قبل الصينين وسكان ولاية صباح الماليزية الذين يثقون بالخصائص الطبية في هذا النوع من الحيوانات. ركز هذا البحث على خيار البحر من نوع *H. scabra* في محاولة لإيجاد خصائص علاجية جديدة، بسبب توفره بشكل كبير في المنطقة الساحلية من ولاية صباح ولقدرته على التكاثر على نطاق واسع في مفارخ الأسماك لغرض إعادة التخزين. تهدف الدراسة إلى تحقيق أمرين: أولهما اختبار فعاليتها ضد القرحات المعدية والثاني اختبار السمية الحادة، وذلك للتحقيق في وجود خاصية معالجة قرحة المعدة في هذا النوع من خيار البحر وللتفقد من سلامته للاستهلاك البشري. لاختبار علاج قرحة المعدة تم إعطاء 150 ملغ/مل من المستخلصات المائية لخيار البحر إلى فئران تم اصابتها بقرحات معدية باستخدام حمض الخليك. تم فحص نسيج المعدة وتم تقدير كمية المخاط المعدي باستخدام المطياف الضوئي. أجري اختبار السمية بإعطاء المستخلصات المائية لخيار البحر على جرعات من 5، 50، 300، 2000 ملغم/كغم إلى أربع مجموعات من الفئران، في حين تلقت المجموعة الضابطة ماء مقطرا فقط. تمت مراقبة علامات السمية والنجاة بعناية لمدة 14 يوما، بالإضافة إلى إجراء تحاليل الدم والفحص النسيجي لأنسجة الأكباد والكلى. أما بالنسبة لدراسة مضاد القرحات المعدية، فكانت الملاظة أن أنسجة المعدة من كلا المجموعتين، المعالجة وغير المعالجة، في طريقها للتعافي، ولم يكن هناك فرق كبير من حيث التغيرات الهيكلية وتسللات الخلايا الالتهابية. لم تظهر الفئران المعالجة كمية كبيرة من المخاط المعدي مقارنة بالفئران غير المعالجة، إلا في اليوم 12 من التجربة. لوحظ في اختبار السمية عدم وجود أي حالة موت أو أي علامة للسمية طوال فترة المراقبة. لم يظهر الفحص الإجمالي للأكباد والكلى أي تغير في المظهر والحجم واللون مقارنة بالمجموعة الضابطة. لم ينتج التحليل الإحصائي لاختبار كروسكال–ووليس اتش أي فرق كبير في وزن الجسم بين المجموعة الضابطة وجميع المجموعات المعالجة بعد إعطاء المستخلصات، x^2 (4، ن = 23) = 5.754، p = 0.218. أظهرت نتائج فحص الدم والفحوص البيوكيميائية لمجموعة الفئران المعالجة عدم وجود أي فرق إحصائي مقارنة بالمجموعة الضابطة. كشف الفحص النسيجي المرضي وجود أنسجة ميتة وأضرار خلوية في الأكباد والكلى في جميع الفئران المعالجة على جميع الجرعات المعطاة. أشارت الدراسة إلى أن فعالية المستخلصات المائية لخيار البحر على علاج القرحات المعدية المصطنعة في الفئران لم تكن قاطعة. ومع ذلك، بما أن نتائج تحليل الدم ونتائج التحليل النسيجي لدراسة السمية الحادة كانت متناقضة، فإنه من المقترح القيام بفحص السمية شبه الحادة مستقبلا لمزيد من التحقق من مستوى سمية المستخلصات.

APPROVAL PAGE

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DECLARATION

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DEDICATION

To my beloved husband, children, mother and father

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4.35 Mean concentration of creatine phosphokinase (± S.E.M) in groups of rats: control, 5 mg/kg, 50 mg/kg, 300 mg/kg, and 60

2000 mg/kg doses of extract. n = 5 in 50, 300 and 2000 mg/kg groups and n = 4 in 5mg/kg and control groups. Means were not significantly different in all groups (Kruskal-Wallis H Test, p value less than 0.05 ($p < 0.05$) is the significant value

4.36 Liver tissues from control group of rats showed normal hepatocytes (H), and structures of central vein (CV) and part of portal triads (PT). (a) Magnification: 200X (b) Magnification: 400X 62

4.37 Liver tissues from rats treated with 5 mg/kg *H. scabra* extract showed increased mitotic figures (MF), degenerated hepatocytes (DC) and incomplete portal triads (PT). (a) Magnification: 400X (b) Magnification: 400X 63

4.38 Liver tissues from rats treated with 50 mg/kg *H. scabra* extract showed degenerated liver cells (DC). (a) Magnification: 200X (b) Magnification: 400X 63

4.39 Liver tissues from rats treated with 300 mg/kg *H. scabra* extract showed total disruption of central vein (CV) in (a) and degenerated liver cells (DC) in (b). (a) Magnification: 200X (b) Magnification: 400X 64

4.40 Liver tissues from rats treated with 2000 mg/kg *H. scabra* extract showed increased number of mitotic figures (MF) and areas with degenerated liver cells (DC). (a) Magnification: 200X (b) Magnification: 200X 64

4.41 Kidney tissues from control group of rats showed glomeruli (G), Bowman capsule (BC), proximal convoluted (PC) and distal convoluted (DC) tubules. (a) Magnification: 200X (b) Magnification: 400X 65

4.42 Kidney tissues from rats treated with 5 mg/kg *H. scabra* extract showed changes in tubular structure (TC). (a) Magnification: 200X (b) Magnification: 400X 65

4.43 Kidney tissues from rats treated with 50 mg/kg *H. scabra* extract showed structural changes in tubules (TC) and glomerulus (GC). (a) Magnification: 200X (b) Magnification: 200X 66

4.44 Glomerular loss (GL) and tubular changes (TC) in kidney tissues from rats treated with 300 mg/kg *H. scabra* extract. (a) Magnification: 200X (b) Magnification: 200X 66

4.45 Kidney tissues from rats treated with 2000 mg/kg *H. scabra* extract showed degenerated cells (DC). (a) Magnification: 200X (b) Magnification: 200X 67

LIST OF ABBREVIATIONS

AA	Arachidonic acid
ALP	Alkaline phosphatase
ALT	Alanine transaminase
AST	Aspartate aminotransferase
CK-MB	Creatine kinase-MB
CPK	Creatine phosphokinase
DHA	Docosahexaenoic acid
ED	Effective Dose
EPA	Eicosapentaenoic acid
ER	Estrogen receptor
et al.	(*et alia*): and others
GFR	Glomerular filtration rate
GGT/ɣ-GT	Gamma-glutamyl transferase
GHS	Globally Harmonised Classification System
HDL	High-density lipoprotein
HGB	Haemoglobin
IL	Interleukin
LD	Lethal Dose
LDH	Lactate dehydrogenase
LDL	Low-density lipoprotein
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
MOSTI	Ministry of Science, Technology and Innovation
mRNA	Messenger ribonucleic acid
NADH	Nicotinamide adenine dinucleotide (reduced form)
NSAIDs	Non-steroidal anti-inflammatory drugs
OECD	Organization for Economic Cooperation and Development
PCV	Packed cell volume
PLT	Platelet
PMNs	Polymorphonuclear neutrophils
PR	Progesteron receptor
PUFA	Polyunsaturated fatty acid
RBC	Red blood cell
RDW	RBC distribution width
VLDL	Very low-density lipoprotein
WBC	White blood cell

LIST OF SYMBOLS

α	-	alpha
β	-	beta
ɣ	-	gamma
g	-	gram
kg	-	kilogram
mg	-	miligram
µg	-	microgram
L	-	litre
ml	-	mililitre
µl	-	microlitre
M	-	molar
rpm	-	revolutions per minute
ω	-	omega

CHAPTER ONE

INTRODUCTION

BACKGROUND OF STUDY

Throughout several decades, extensive studies and works have been conducted to find biologically active compounds from natural sources like terrestrial plants and microorganisms, which can potentially be developed into new drugs and pharmaceutical products. It has been documented that between 2000 and 2005, 23 new drugs derived from natural sources were successfully brought into the market (Chin, Balunas, Chai & Kinghorn, 2006), whereas 19 natural product-based drugs were launched on the market between 2005 and 2010 (Mishra & Tiwari, 2011).

Marine organisms have also drawn attention of scientists to find and isolate new compounds. Many of them have been attributed to certain biological activities, but eventually, very few have been marketed or are under development (Murti & Agrawal, 2010). The utilization of marine organisms as one of the natural sources for isolating bioactive compounds began in the early 1950s when spongouridine and spongothymidine were firstly isolated from the Carribean sponge (*Cryptotheca crypta*). Both are used until today, respectively as an anticancer drug and antiviral drug, following its approval 15 years after the findings (Chin et al., 2006). Apart from sponges, shellfish, oyster, squid, and sea cucumber are other organisms of interest that have been explored by researchers, for their potential therapeutic effects for the treatment or prevention of various diseases in human.

Ridzwan (2007) mentioned distinct uses of sea cucumbers in two different regions in Malaysia. In the Peninsular of Malaysia, certain sea cucumbers from the

genus *Stichopus*, which are also known as *gamat* among the locals, are widely consumed for its medicinal properties such as for the treatment of internal injuries. However in Sabah, some species of sea cucumbers are taken as food as well as used in some practices on the basis of folks and traditional beliefs. In addition, the marine animal has long been consumed by the Chinese as part of their cuisine, and is traditionally believed to offer a wide range of medicinal properties against various diseases as well as promote better human health. The traditional Chinese medicine believes that sea cucumber nourishes blood, vital essence, kidneys and reduces intestinal dryness; hence is commonly used to treat weakness, impotence and weakness of the aged, constipation, and incontinence (Chen, 2003).

Correspondingly, it also has been demonstrated in previous researches and studies that certain sea cucumbers possess such properties as antiangiogenic (Tong, Zhang, Tian, Yi, Xu, Li, Tong, Lin & Ding, 2005; Tian, Zhu, Zhang, Xie, Xin, Yi, Lin, Geng & Ding, 2007), antimicrobial (Abraham, 2002), antioxidant and antiproliferative (Osama, 2009), antinociceptive (Ridzwan, Leong & Syed Zahir, 2003), for wound healing agent (Fredalina, Ridzwan, Zainal Abidin, Kaswandi, Zaiton, Zali, Kittakoop & Mat Jais, 1999) and osteoclastogenesis inhibitor (Kariya, Mulloy, Imai, Tominaga, Kaneko, Asari, Suzuki, Masuda, Kyogashima & Ishii 2004). The beneficial properties exhibited by sea cucumbers are ascribed by the presence of numerous high-value bioactive compounds. These include high level of good-quality protein such as glycine, glutamic acid, and arginine amino acids which play significant role in immune regulation; phenolics and free radical scavengers for anticancer agents; fatty acids for tissue repair and wound healing properties; and mucopolysaccharides and chondroitins for treating arthritis and joint pain.

Out of many species of sea cucumbers found in the coastal area of Malaysia, *Holothuria scabra* Jaeger is one of the edible sea cucumber species which can be found in the coastal area of Sabah. The Brunei Malays who live in Labuan Federal Territory use the marine animal for curing piles (Ridzwan, 2007). In addition, the marine animal also has remarkable commercial value (Conand, 2006), where it is mainly sold in the form of dried *trepang* or *beche-de-mer* in the Asian market (Hamel, Conand, Pawson & Mercier, 2001). In China, the dried product of *H. scabra* is in high demand and is typically used in the Chinese cuisines. *H. scabra* is also one of special dishes among part of local Sabahan people (Ridzwan, 2010).

The claims on the potential health benefits of sea cucumbers by the Chinese and high commercial value of *H. scabra* have triggered the work on this species. Besides, the other basis of conducting this study is because of the population reduction of one of the common sea cucumber species in Malaysia, *Stichopus hermanii* due to overfishing (Ridzwan, 2010). *S. hermanii*, which current name is *Stichopus horrens* Selenka, is generally known to have significant role in the healing of wound and internal injury (Ridzwan, 2010). Therefore, there is a need to find another locally available sea cucumber species that may substitute *S. horrens* and offer promising good health effects. *H. scabra* is one of the best options because of its high availability in the coastal area of Sabah (Ridzwan, 2007) and ability to be largely produced in hatcheries for restocking purpose (Nurzafirah & Ridzwan, 2015).

The importance of this study can also be viewed in the aspect of consumption of certain commercial synthetic drugs for particular treatments may have undesirable and uncomfortable side effects in patients. For example, in peptic ulcer disease, the drugs used include ranitidine, cimetidine, omeprazole, and bismuth chelate. Even though unwanted effects are rare and uncommon, patients who consume cimetidine