UNIVERSITI SAINS MALAYSIA
GERAN PENYELIDIKAN UNIVERSITI PENYELIDIKAN
LAPORAN AKHIR

THE POTENTIAL USE OF CENTELLA ASIATICS AS ANTI-HELICOBACTER PYLORI AGENT

PENYELIDIK

PROF. MADYA DR. RAPEAH SUPPIAN

PENYELIDIK BERSAMA

PROF. MADYA DR. HASMAH ABDULLAH
PROF. MADYA DR. SITI SURAIYA MOHD NOR
DR. NOOR IZANI NOOR JAMIL

2016

UNIVERSITI SAINS MALAYSIA

UNIVERSITI SAINS MALAYSIA

RU GRANT
FINAL REPORT CHECKLIST

Please use this checklist to self-assess your report before submitting to RCMO.
Checklist should accompany the report.

NO.	ITEM	PLEASE CHECK (✓)		
		PI	JKPTJ	RCMO
1	Completed Final Report Form	✓	✓	✓
2	Project Financial Account Statement (e-Statement)	Tiada projek lama		
3	Asset/Inventory Return Form *(Borang Penyerahan Aset/Inventori)*	✓	✓	✓
4	A copy of the publications/proceedings listed in Section D(ii) (Research Output)	✓	✓	✓
5	Comprehensive Technical Report	✓	✓	✓
6	Other supporting documents, if any		✓	✓
7	Project Leader's Signature	✓	✓	✓
8	Endorsement of PTJ's Evaluation Committee		✓	✓
9	Endorsement of Dean/ Director of PTJ's		✓	✓

Project Code :
(for RCMO use only)

UNIVERSITI SAINS MALAYSIA

RU GRANT
FINAL REPORT FORM

Please email a softcopy of this report to rcmo@usm.my

A	**PROJECT DETAILS**
i	**Title of Research:** The potential use of *Centella asiatica* as anti-*Helicobacter pylori* agent
ii	
iii	**Name of Research Leader:** Rapeah Suppian
iv	**Name of Co-Researcher:** 1. Assoc. Prof. Dr. Hasmah Abdullah 2. Assoc. Prof. Dr. Siti Suraiya Mohd. Nor 3. Dr. Noor Izani Noor Jamil
v	**Duration of this research:** a) **Start Date** : 19 October 2007 b) **Completion Date** : 18 October 2010 c) **Duration** : 3 years d) **Revised Date** (*if any*) : Extension to 18 march 2011
B	**ABSTRACT OF RESEARCH**

(An abstract of between 100 and 200 words must be prepared in Bahasa Malaysia and in English. This abstract will be included in the Report of the Research and Innovation Section at a later date as a means of presenting the project findings of the researcher/s to the University and the community at large)

ABSTRACT

Centella asiatica (*C. asiatica*) is a medicinal plant that traditionally used for the treatment of variety disorder, including infections, inflammation and immune system deficiencies. This study was carried out to investigate the effects of different parts of *C. asiatica* (roots, petioles, leaves) extracted using methanol and water on yield, active compounds, antibacterial activity against *Helicobacter pylori* (*H. pylori*) as well as inflammatory activity of the *H. pylori*-infected macrophage. The highest yield of crude extract was obtained by the methanolic leaves extract (35.8%). Thin layer chromatography (TLC) analysis showed that, the two main compounds of *C. asiatica*; asiatic acid and asiaticoside were only detected in the methanolic extract of the plant parts with R_f values 0.97 cm for asiatic acid and 0.44 for asiaticoside respectively. High Performance Liquid Chromatography (HPLC) analysis confirmed the presence of these compounds in the methanolic

extracts of *C. asiatica* but not in the water extracts of the plant parts. The methanolic extract of the plant parts also exhibited good activity against *H. pylori* by inhibiting the growth of the bacteria either with Minimal Inhibitory Concentration (MIC), Minimal Bacteriocidal Concentration (MBC) or by disk diffusion assay. Among the plant parts, the methanolic leaves extract showed the highest inhibitory effect with inhibition zone ranging from 7 mm to 9 mm, and MIC and MBC at 5 mg/ml. The methanolic extracts of the plant parts also capable of increasing the inflammatory activity of *H. pylori* infected macrophage by stimulating higher phagocytic activity as well as the production of nitric oxide (NO). However, the extracts were significantly reduced the production of hydrogen peroxide (H_2O_2) in the infected cells. In conclusion, these findings suggested that the methanolic extracts of *C. asiatica* may have promising anti-*H. pylori* agent that can eliminate the bacteria directly or by modulating the innate defense mechanism of the host.

ABSTRAK

Centella asiatica (*C. asiatica*) merupakan tumbuhan ubatan yang banyak digunakan secara tradisional untuk merawat pelbagai komplikasi termasuk jangkitan, inflamasi dan kelemahan sistem imun. Kajian ini telah dilakukan untuk mengetahui kesan ekstrak metanol dan air tiga bahagian *C. asiatica* yang berbeza (akar, batang dan daun) terhadap hasil ekstrak, pengesanan kompoun aktif, aktiviti antibakteria terhadap *Helicobacter pylori* (*H. pylori*) serta aktiviti inflamatori makrofaj yang dijangkiti *H. pylori*. Hasil ekstrak yang paling banyak diperolehi daripada bahagian daun yang diekstrak menggunakan metanol (35.8%). Analisis kromatografi lapisan nipis (TLC) menunjukkan kehadiran dua komponen utama *C. asiatica* iaitu asid asiatik dan asiatikosid hanya pada bahagian tumbuhan yang diekstrak menggunakan metanol dengan nilai R_f 0.97 cm untuk asid asiatik dan 0.44 untuk asiatikosid. Analisis kromatografi cecair berprestasi tinggi (HPLC) mengesahkan kehadiran asid asiatik dan asiatikosid dalam ekstrak metanol *C. asiatica* tetapi tidak di dalam ekstrak tumbuhan yang diekstrak menggunakan air. Bahagian tumbuhan yang diekstrak menggunakan metanol juga menunjukkan aktiviti perencatan pertumbuhan *H. pylori* yang baik sama ada pada perencetan kepekatan minimum (MIC), kepekatan bakterisidal minimum atau asai pembauran cakera. Antara ketiga-tiga bahagian tumbuhan yang dikaji, ekstrak metanol daun menunjukkan kesan perencetan tertinggi dengan zon perencatan daripada 7 mm hingga 9 mm, dan MIC dan MBC pada 5 mg/ml. Bahagian tumbuhan yang diekstrak menggunakan methanol juga telah menunjukkan peningkatan aktiviti inflamatori makrofaj dengan merangsang aktiviti fagositosis yang lebih tinggi serta meningkatkan penghasilan nitrik oksida (NO). Walaubagaimanapun, ekstrak tersebut telah menurunkan penghasilan hidrogen peroksida (H_2O_2) oleh sel makrofaj tersebut secara signifikan. Sebagai kesimpulan, penemuan ini mencadangkan bahawa ekstrak methanol *C. asiatica* mungkin mengandungi agen anti-*H. pylori* yang berpotensi yang dapat merencatkan pertumbuhan bakteria tersebut secara langsung atau dengan memodulasi mekanisme pertahanan imuniti semulajadi perumah.

C	**BUDGET & EXPENDITURE**

D **RESEARCH ACHIEVEMENTS**

i **Project Objectives** *(as stated/approved in the project proposal)*

No.	Project Objectives	Achievement
1	To develop different extracts of *C. asiatica*	Achieved
2	To determine the effect of the extract against *H. pylori* growth in *vitro*	Achieved
3	To determine the effect of the extract against *H. pylori*-infected macrophage	Achieved
4		
5		
6		

ii **Research Output**

a) Publications in ISI Web of Science/Scopus

No.	Publication (authors,title,journal,year,volume,pages,etc.)	Status of Publication (published/accepted/ under review)

b) Publications in Other Journals

No.	Publication (authors,title,journal,year,volume,pages,etc.)	Status of Publication (published/accepted/ under review)
1.	Norzaharaini, M.G., Wan Norshazwani, W.S., Hasmah, A., Nor Izani, N.J. & **Rapeah, S.** (2011). A preliminary study on the antibacterial activities of asiaticoside and asiatic asid against	Published

	selected Gram positive and Gram negative bacteria. *Health and the Environment Journal* 2(1): 23-27.	
2.	Hussein Ali Osman, Habsah Hasan, **Rapeah Suppian**, Nor Aizal Che Hamzah, Sharifah Emilia Tuan Sharif, Noorizan Abdul Majid and Bin-Alwi Zilfalil (2014). The Characteristics of *Helicobacter pylori* infection and clinical outcomes of patient with upper gastrointestinal bleeding admitted at Hospital Universiti Sains Malaysia. *World Applied Sciences Journal*, 32 (5): 747-751.	Published Collaborative paper with other related research grant

c) Other Publications
(book,chapters in book,monograph,magazine,etc.)

No.	Publication (authors,title,journal,year,volume,pages,etc.)	Status of Publication (published/accepted/ under review)
1.	**Rapeah Suppian** (2012). Persekitaran dan jangkitan *Helicobacter pylori* (dalam Haliza AR & Rohasliney H. Manusia dan kelestarian persekitaran), Penerbit Universiti Malaysia Terengganu, pp 111-119.	Published
2.	Hasmah A & **Rapeah S** (2012). Rawatan alternatif terhadap jangkitan *Helicobacter pylori* (dalam Haliza AR, Hasmah H. & Sakinah H. Isu persekitaran dan kesihatan di Malaysia), Penerbit Universiti Tun Hussein Onn Malaysia (UTHM), pp 51-70.	Published

d) Conference Proceeding

No.	Conference (conference name,date,place)	Title of Abstract/Article	Level (International/National)
1.	**Rapeah, S.** Siti Suraya, M.N. & Norhaslindawaty, A.R. (2008). Proceedings of the National Conference on Environment & Health 2008. School of Health Sciences, Universiti Sains Malaysia. pp 471-475. ISBN: 978-983-44173-0-7	Detection of *Helicobacter pylori* in paraffin-embedded gastric biopsy specimens by polymerase chain reaction (PCR).	National
2.	Nor zaharaini, M.G., Hasmah, A. & **Rapeah, S.** (2008). Proceedings of the National Conference on Environment & Health 2008. School of Health Sciences, Universiti Sains Malaysia. pp 442-447. ISBN: 978-983-44173-0-7	Therapeutic values of *Centella asiatica* in human health.	National

 # Please attach a full copy of the publication/proceeding listed above

iii.	**Other Research Ouput/Impact From This Project** *(patent, products, awards, copyright, external grant, networking, etc.)* 1. Networking/collaboration in *H. Pylori* project with Universiti Malaysia Terengganu 2. Collaboration in *H. Pylori* project with other researcher from USM

E HUMAN CAPITAL DEVELOPMENT

a) Graduated Human Capital

Student	Nationality (No.)		Name
	National	International	
PhD			1. 2.
MSc	1		1. Shalini Micheal 2.
Undergraduate	3		1. Wan Norshazwani Wan Shaffee 2. Nurul Hidayah Wahab 3. Nor Faradhiah Roslan

b) On-going Human Capital

Student	Nationality (No.)		Name
	National	International	
PhD			1. 2.
MSc	1		1. Nor zaharaini Mat Ghani 2.
Undergraduate			1. 2.

c) Others Human Capital

Student	Nationality (No.)		Name
	National	International	
Post Doctoral Fellow			1. 2.
Research Officer			1. 2.
Research Assistant	2		1. Nor zaharaini Mat Ghani 2. Munirah Zakaria
Others (.Industrial training student)	2		1. Nik Sarah Syahidah Nik Sihamuddin (UIAM) 2. Wirdah Mohd Zain (UIAM)

F COMPREHENSIVE TECHNICAL REPORT

Applicants are required to prepare a comprehensive technical report explaining the project. The following format should be used (this report must be attached separately):

	• Introduction • Objectives • Methods • Results • Discussion • Conclusion and Suggestion • Acknowledgements • References

G	**PROBLEMS/CONSTRAINTS/CHALLENGES IF ANY**
	(Please provide issues arising from the project and how they were resolved) Difficulty in growing the *H. pylori* culture, easy to contiminate.
H	**RECOMMENDATION**
	(Please provide recommendations that can be used to improve the delivery of information, grant management, guidelines and policy, etc.) Improve collaboration with other related agencies such as Ministry of Health.

I	COMMENTS, IF ANY/ENDORSEMENT BY PTJ'S RESEARCH COMMITTEE

According to end database, this project is classified as 'beku' (frozen). However, Ag Rapeah (the PI) had successfully completed the project with very good outcomes, and had submitted the report.

Please evaluate the report and update her grant status in end database.

Appendix 1

UNIVERSITI SAINS MALAYSIA

BORANG PENYERAHAN ASET / INVENTORI

==========

A. BUTIR PENYELIDIK

1. NAMA PENYELIDIK : RAPEAH SUPPIAN
2. NO STAF : AM 50780
3. PTJ : SCHOOL OF HEALTH SCIENCES
4. KOD PROJEK : 1001/PPSP/8120163
5. TARIKH TAMAT PENYELIDIKAN : 18 MARCH 2011

B. MAKLUMAT ASET / INVENTORI

BIL	KETERANGAN ASET	NO HARTA	NO. SIRI	HARGA (RM)
1.	Soxlet apparatus (5000 ml)	AK000097620	2AK00009761PPS P 2009	28,500
2.	PC (Acer)	-	PTSA00C0468090 4BF62701 (CPU) 81206199542 (Monitor)	2,499

A Preliminary Study on the Antimicrobial Activities of Asiaticoside and Asiatic Acid against Selected Gram Positive and Gram Negative Bacteria

Norzaharaini MG[a], Wan Norshazwani WS[a], Hasmah A[a], Nor Izani NJ[a], Rapeah S[a]
[a]*School of Health Science, Health Campus, Universiti Sains Malaysia, 16150, Kubang Kerian, Kelantan.*

ABSTRACT: *Centella asiatica* is a medicinal plant traditionally used for the treatment of various disorders including infections. In this study, two bioactive compounds of *Centella asiatica* namely asiatic acid and asiaticoside were tested for antibacterial activity against two Gram negative and three Gram positive bacteria using disc diffusion method. They were *Helicobacter pylori*, *Escherichia coli*, *Streptococcus pneumoniae*, *Staphilococcus aureus* and *Pseudomonas aeroginosa*. The antibacterial activities were assessed by the presence or absence of inhibition zones. The result of this preliminary study demonstrated that asiatic acid has antibacterial activity against all the bacteria except *Pseudomonas aeruginosa* with inhibition zones ranged from 7–12 mm. No inhibition activity was observed for asiaticoside against all the bacteria. This data revealed that asiatic acid is responsible for the antibacterial activity of *Centella asiatica*.

Keywords: Asiatic acid, anti-bacteria, asiaticoside, *Centella asiatica*

Introduction

The use of medicinal plants and their derivatives as source for antimicrobial drugs has become more important nowadays due to the growing incidences of drug-resistant pathogens (Hammer et al., 1999; Martin and Ernst, 2003; Samy and Gopalakrishnakone, 2010). Various plants including *Centella asiatica* (*C. asiatica*) and their derivatives have been used as therapeutic agents for various human diseases since prehistoric times (Henkel et al., 1999; Nostro et al., 2000; Taemchuay et al., 2008). *C. asiatica*, popularly known in Malaysia as pegaga, is a small herbaceous annual plant with small-sized leaves and short petiole stem. It grows in damp swampy areas in tropical and sub-tropical regions including Malaysia (Verma et al., 1999). This plant has been used to cure various conditions including headache, asthma, eczemas, ulcers and wound healing, and has been commercialized for various medicinal and cosmetic purposes (Ullah, 2009). Furthermore, it is claimed to possess a wide range of pharmacological effects such as anti-oxidant, anti-ulcer, anti-stress, anti-cancer, anti-microbial and wound healing effect.

Corresponding Author:
Norzaharaini MG
School of Health Sciences,
Universiti Sains Malaysia,
16150 Kubang Kerian, Kelantan
Email: zaharaini@gmail.com

Published 31 Jan 2011

C. asiatica is also believed to increase energy and sexual potency as well as maintains and prolongs health life, and has been used as tonic in Ayurvedic medication (Chakraborty et al., 1996; Sharma et al., 1996; Kimura et al., 2008).

C. asiatica contains four main bioactive compounds known as asiatic acid, asiaticoside, madecassicacid and madecassoside (Matsuda et al., 2001). Among them, asiaticoside and asiatic acid are the most important bioactive compounds which are responsible for the medicinal values of this plant (Hausen, 1993; Lu et al., 2004; Zheng and Qin, 2007; Pittella et al., 2009). Thus, the extraction and isolation of these compounds have drawn public interest. Many researchers around the world have been conducted extensive study to investigate other potential values of these compounds for the benefit of human's health. Though the therapeutic values of asiaticoside and asiatic acid have been shown in various fields, knowledge with regard to their potential as antibacterial agent is still lacking. Most studies on antibacterial effects of this plant mainly focus on plant extracts. Thus, this study was conducted to evaluate the antibacterial activity of asiaticoside and asiatic acid against selected Gram negative and Gram positive bacteria including *Helicobacter pylori (H. pylori)*, *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*), *Streptococcus pneumoniae* (*S. pneumoniae*) and *Pseudomonas aeruginosa* (*P. aeruginosa*).

Material and Methods

Bacteria culture

Two Gram negative bacteria (*Escherichia coli* ATCC 29952, *Helicobacter pylori* ATCC 45903, *Pseudomonas aeruginosa*) and three Gram positive bacteria (*Streptococcus pneumoniae*, *Staphylococcus aureus*) obtained from the Culture Laboratory of the School of Health Sciences, Universiti Sains Malaysia were used in this study. *H. pylori* was cultured onto Colombia blood agar or Muller Hinton agar (BBL USA) with 5% sheep blood in an anaerobic jar supplemented with microaerobic gas pack BR 0056A (Oxoid, UK) at 37°C for 48 to 72 hours. *S. pneumoniae* was cultured on Colombia blood agar or Muller Hinton agar with 5% sheep blood and incubated in a tin jar provided with candle for one to two days. The colony was preserved in Tryptic Soy Broth (Oxoid, UK) with 20% glyserol in -80°C prior to use. *E. coli*, *S. aureus* and *P. aeruginosa* were cultured onto Muller Hinton agar overnight at 37°C.

Preparation of bacterial suspensions

H. pylori was inoculated in Tryptic Soy Broth with 5% fetal bovine serum and incubated in microaerobic environment at 37°C for 48-78 hours. The inoculum was adjusted to a 0.5 McFarland turbidity standard (Uyub et al., 2010). *S. pneumoniae*, *E. coli*, *S. aureus* and *P. aeruginosa* were inoculated in Muller Hinton broth and incubated at 37°C for 14 to 16 hours, the inocula were then adjusted to a 0.5 McFarland standard of turbidity.

Disk diffusion assay

The disk diffusion assay suggested by national committee for clinical laboratory standard (NCCLS) was used for antimicrobial test with slight modifications. Six mm sterilized filter paper disks (Whatmann, UK) were impregnated with 20 µl of asiaticoside and asiatic acid (Sigma, USA) diluted to a concentration of 1000 µg/ml, 500 µg/ml, 250 µg/ml, 125 µg/ml thus each disc contained 20 µg, 10 µg, 5 µg, 2.5 µg, respectively. DMSO was used as the negative control and standard antibiotics were used as the positive control. Amoxicillin (10 µg) was used as the positive control for *E. coli*, gentamycin (5 µg) for *S. pneumoniae*, *S. aureus* and *P. aeruginosa*, while clarithromycin (10 ug) was used as the positive standard for *H. pylori*. The experiment was performed in triplicate and the mean diameters of the inhibition zones were measured based on the nearest millimeter of the clear zone surrounding the discs.

Results

The antibacterial activities of asiaticoside and asiatic acid against five different bacteria are presented in TABLE 1.

TABLE 1- Antibacterial activities of asiaticoside and asiatic acid of *Centella asiatica* against 5 different bacteria

Bacteria strain	Asiaticoside	Asiatic acid	+ve control
Helicobacter pylori ATCC 45903	NZ	12 (20) 8 (10)	NZ (10)
Escherechia coli ATCC 29952	NZ	7 (20)	20.5 (10)
Staphilococcus aureus	NZ	8 (20)	24 (5)
Streptococcus pneumoniae	NZ	7 (20)	42 (5)
Pseudomonas aeruginosa	NZ	NZ	15 (5)

Inhibition zone (mm) (Concentration, µg /disc)
*NZ –no inhibition zone

Asiatic acid inhibited the growth of all the bacteria except *P. aeruginosa* with various inhibition effects ranged from 7-12 mm at 20 µg. The compound also inhibited the growth of *H. pylori* at 10 µg with an inhibition zone of 8 mm. However, no inhibition effects were detected for all the bacteria against asiaticoside. No inhibitory zones were observed for all the bacteria against asiatic acid at concentration less than 20 µg and for *H. pylori* at concentration less than 10 µg. Inhibition zones were observed for standard antiobiotics except for clarithromycin.

Discussion

Asiaticoside and asiatic acid are the most important bioactive compounds presence in *C. asiatica* which responsible for its medicinal values (Hausen, 1993; Lu et al., 2004; Zheng and Qin, 2007; Pittella et al., 2009).

In this preliminary study, we evaluated the inhibition effects of asiaticoside and asiatic acid against selected Gram negative and Gram positive bacteria. Although asiatic acid, an active metabolite of

asiaticoside, has not been reported in the scientific literature as an antibacterial agent, the result presented in this preliminary study shows that this bioactive compound capable of inhibiting the growth of all the bacteria except *P. aeruginosa* with inhibition zones ranged from 7-12 mm at 20 µg. Inhibitory effect was also observed for *H. pylori* against 10 µg asiatic acid with diameter of the inhibition zone at 8 mm. Surprisingly, no inhibition zone was detected for all the bacteria against asiaticoside. This data is not in agreement with previous report showing that asiaticoside has antibacterial and fungicidal effects against various pathogens and fungi (Hausen et al., 1993; Taemchuay et al., 2008). We believed that this phenomenon might be the result of the extraction method used to extract or synthesis the compound. The extraction of bioactive compounds from medicinal plants is influenced by various factors including the method and solvent used for the extraction (Kim et al., 2009). Furthermore, in this study, the inhibitory effects of these compounds were only evaluated by disc diffusion method. This method seems to be limited and influenced by other factors such as concentration of extract, duration of exposure and the bacteria tested (Okoli and Iroegbu 2005). Thus, further study should be carried out to confirm this data with other method such as minimum inhibition concentration (MIC) and minimum bacterial concentration (MBC).

Even though asiatic acid is capable of inhibiting the growth of 4 out of 5 bacteria tested in the present study, the inhibition zones were less than the reference antibiotics and at higher concentration (20 µg), thus indicating that asiatic acid only possess weak antibacterial property against all the tested bacteria in comparison with the standard antibiotics. For *H. pylori*, no inhibition zone was detected against standard antibiotics, clarithromycin at 10 µg. No inhibition was also observed for *H. pylori* against 10 µg amoxicillin or 5 µg gentamycin (data not shown). We assumed that this bacterium might be resistant to those antibiotics.

In conclusion, the results of this preliminary study indicate that asiatic acid has antibacterial effects against *H. pylori*, *E. coli*, *S. aureus* and *S. pneumonia*. Further research with other bacteria and method of evaluation is needed to support this finding.

Acknowledgments

This study was supported by Research University grant 1001/PPSP/8120163 and the author is being funded by USM fellowship. We also acknowledge the kind help provided by En. Nordin Senik and En. Anizan Bakhtiar at the Culture Laboratory of School of Health Sciences, Universiti Sains Malaysia.

References

1. Chakraborty, T., Sinha, S.P., and Sukul, N.C. (1996). Preliminary evidence of antifilarial effect of *Centella asiatica* on *canine dirolfilariasis*. *Fitoterapia*, 67: 110-112.
2. Grimaldi, R., De Ponti, F., DûAngelo, L., Caravaggi, M., Guidi, G., Lecchin, S., Frigo, G. M. and Crema A. (1990). Pharmacokinetics of the total triterpenic fraction of *Centella asiatica* after single and multiple administrations to healthy volunteers: a new assay for asiatic acid. *Journal of Ethnopharmacology*. 28: 235-241.
3. Hammer, K.A., Carson, C.F. and Riley, T.V. (1999). Antimicrobial activity of essential oils and other plants extracts. *Journal of Applied Microbiogy*. 86: 985–990.
4. Hausen, B.M. (1993). *Centellaasiatica* (Indian pennywort), an effective therapeutic but a weak sensitizer. *Contact Dermatitis*, 29:175-179.
5. Henkel, T., Brunne, R.M., Muller, H. and Reichel, F. (1999). Statistical investigation into the structural complementarity of natural products and synthetic compounds, *Angewandte Chemie-International Edition*, 38:643-647.
6. Kim, W.J., Kim, J., Veriansyah, B., Kim J.D., Lee, Y. ., Oh, S.G. and Tjandrawinata, R.R. (2009). Extraction of bioactive components from *Centella asiatica* using subcritical water. *Journal of Supercritical Fluids*, 48:11-216.
7. Kimura, Y., Sumiyoshi, M., Samukawa, K., Satake, N. and Sakanaka M. (2008). Facilitating action of asiaticoside at low doses on burn wound repair and its mechanism. *European Journal of Pharmacology*, 584: 415 – 423.
8. Lu, L., Ying, K., Wei, S., Fang, Y., Liu, Y., Lin, H., Ma, L. and Mao, Y. (2004). Asiaticoside induction for cell-cycle progression, proliferation and collagen synthesis in human dermal fibroblasts. *Inernational Journal of Dermatology*. 43(11): 801-807.
9. Martin, K.W. and Ernst, E. (2003). Herbal medicines for treatment of bacterial infections: a review of controlled clinical trials. *Journal of Antimicrobial Chemotherapy*, 51: 241–246.
10. Matsuda, H., Morikawa, T., Ueda, H. and Yoshikawa, M. (2001). Medicinal foodstuffs. XXVII. Saponin constituents of gotukola (2): Structures of new ursane-andoleanane- type triterpeneoligoglycosides, centellasaponins B, C, and D, from *Centella asiatica* cultivated in Sri Lanka. *Chemical and Pharmaceutical Bulletin*, 49: 1368-1371.
11. Nostro, A., Germano, M.P., D'Angelo, V., Marino, A. and Cannatelli, M.A. (2000). Extraction methods and bioautography for

evaluation of medicinal plant antimicrobial activity. *Letters in Applied Microbiology*. 30(5): 379.

12. Okoli, S. and Iroegbu, C.U. (2005). *In vitro* antibacterial activity of *Synclisa scabrida* whole root extracts. *African Journal of Biotechnology*, 4 (9):946-952.
13. Pittella, F., Dutra R.C., Junior, D.D., Lopes. M. T. P. and Barbosa Nádia, R. (2009) Antioxidant and cytotoxic activities of *Centella asiatica* (L) Urb. *International Journal of Molecular Sciences*. 10(9): 3713–3721.
14. Rush, W.R., Murray, G. R. and Graham, D.J. M. (1993). The comparative steady-state bioavailability of the active ingredients of madecassol. *Eurupeon Journal of Drug Metabolisme and Pharmacokinetics*. 18: 323-326.
15. Samy, R.P. and Gopalakrishnakone, P. (2010). Therapeutic potential of plants as anti-microbials for drug discovery. *eCAM*, 7(3):283-294.
16. Sharma, D.N.K., Khosa, R.L., Chansauria, P. N. and Sahai, M. (1996). Antistress activity of *Tinospora cordifolia and Centella asiatica*. *Phytother. Res.*, 10: 181-183.
17. Taemchuay, D., Rukkwamsuk, T., Sukpuara, T. and Ruangwises, N. (2008). A study on antibacterial activity of crude extracts of asiatic Pennywort and water Pennywort against *Staphylococcus aureus*. *KMITL Science Journal*. 8(2): 1-4.
18. Ullah, M.O., Sultana, S., Haque A. and Tasnim, S. (2009). Antimicrobial, cytotoxic, and antioxidant activity of *Centella asiatica*. *European Journal of Scientific Research*. 30(2): 260-264.
19. Uyub, A.M., Nwachukwu, I.N., Azlan, A.A. and Fariza, S.S. (2010). *In-vitro* antibacterial activity and cytotoxicity of selected medicinal plant extracts from Penang Island Malaysia on metronidazole-resistant- *Helicobacter pylori* and some pathogenic bacteria. *Ethnobotany Research and Application*. 8: 95-106.
20. Verma, R.K., Bhartariya, K.G, Gupta, M.M. and Kumar, S. (1999). Reverse-phase high performance liquid chromatography if asiaticoseide in *Centella* asiatica. *Phytochem. Anal.*, 10: 191–193.
21. Zheng, C.J. and Qin, L.P. (2007). Chemical components of *Centella asiatica* and their bioactivities. *Journal of Chinese Integrative Medicine*, 5(3): 348-351.

RESEARCH ARTICLE

Evaluation of the Atlas *Helicobacter pylori* Stool Antigen Test for Diagnosis of Infection in Adult Patients

Hussein Ali Osman[1], Habsah Hasan[1], Rapeah Suppian[2], Norhaniza Bahar[3], Nurzam Suhaila Che Hussin[4], Amry Abdul Rahim[5], Syed Hassan[6], Dzulkarnaen Zakaria Andee[6], Bin-Alwi Zilfalil[7]*

Abstract

Background: *Helicobacter pylori* (*H.pylori*) is one of the most important causes of dyspepsia and gastric cancer and diagnosis can be made by invasive or non-invasive methods. The Atlas *Helicobacter pylori* antigen test is a new rapid non-invasive method which is simple to conduct. The aim of this study was to determine its sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy. Materials and Methods: This prospective study was conducted between July 2012 and December 2013. Stool samples of 59 dyspeptic patients who underwent upper endoscopy were evaluated for *H. pylori* stool antigen. Results: From the 59 patients who participated in this study, there were 36 (61%) males and 23 (39%) females. *H. pylori* was diagnosed in 24 (40.7%) gastric biopsies, 22 (91.7 %) of these being positive for the Atlas *H. pylori* antigen test. The sensitivity, specificity, PPV, NPV and accuracy were 91.7%, 100%, 100%, 94.6% and 96.6% respectively. Conclusions: The Atlas *H. pylori* antigen test is a new non-invasive method which is simple to perform and avails reliable results in a few minutes. Thus it can be the best option for the diagnosis of *H. pylori* infection due to its high sensitivity and specificity.

Keywords: *Helicobacter pylori*- sensitivity - specificity - Atlas *H. pylori* antigen test

Asian Pac J Cancer Prev, 15 (13), 5245-5247

Introduction

Helicobacter pylori(*H. pylori*) is a Gram-negative microaerophilic bacterium and one of the most common bacterial pathogens of humans that infects more than half of the world's population (Amjad et al., 2010; Zhang et al., 2014). The bacteria has worldwide distribution and the prevalence ranges from 25% in developed countries to more than 90% in developing areas, but not all infected individuals eventually developed the disease (Miernyk et al., 2011; Ghotaslou et al., 2013).

The prevalence of *H. pylori* infection varies widely by geographic area, age, race, and socioeconomic status (Brown et al., 2002). *H. pylori* infection is associated with chronic gastritis, gastric or duodenal ulcer, gastric cancer and MALT-lymphoma (Ben Mansour et al., 2010; Zhao et al., 2012). *H. pylori* was classified as a class I carcinogen in humans by a working group of the World Health Organization International Agency for Research on Cancer (IARC) based on various epidemiological studies (Khalilpour et al., 2013)

There seems to be no firm agreement as to which method should be used as gold standard for the detection of *H. pylori* infection (Redeen et al., 2011). Gastric biopsy based tests which include culture, histology and the rapid urease test (RUT) are considered the standard diagnostic tests (Al-Humayed et al., 2008; Kalem et al., 2010). However, these tests necessitate an upper gastrointestinal endoscopy and are considered invasive tests.

Non-invasive tests include the urea breath tests (UBT) and serology and stool antigen test (Bhewa et al., 2007; Redeen et al., 2011). Urea breath tests and stool antigen test can detect active infection while serology test does not differentiate between active infection and exposure to *H. pylori* (Ricci et al., 2007; Peng et al., 2009).

The choice of a given testing strategy is influenced by sensitivity, specificity, the clinical circumstances and the cost-effectiveness of the test (Peng et al., 2009). In the last years, many studies have focused on noninvasive methods; *H. pylori* stool antigen test provides a simple alternative to the urea breath test and is appropriate for diagnosis and follow-up of infection (Gisbert and Pajares,

*[1]Department of Medical Microbiology and Parasitology, School of Medical Sciences, [2]Department of Biomedical Science, School of Health Sciences, Universiti Sains Malaysia, Kelantan, [3]Department of Medicine, [4]Department of Pathology, Hospital Kuala Lumpur, Kuala Lumpur, [5]Department of Medicine, [6]Department of Surgery, [7]Department of Paediatrics, School of Medical Sciences, Universiti Sains Malaysia, Kelantan, Malaysia; *For correspondence: zilfalil2@hotmail.com*

2004; Shimoyama et al., 2009).

The aim of this study was to assess the efficacy of new Atlas *Helicobacter pylori* Antigen Test for the detection of *H. pylori* infection in dyspeptic patients and find its sensitivity, specificity and negative and positive predictive values and accuracy in the diagnosis of *H. pylori* infection.

Materials and Methods

Patients

This is a prospective study conducted at Hospital Universiti Sains Malaysia and Hospital Kuala Lumpur among 59 adult dyspeptic patients between July 2012 to December 2013. The patients were selected from patients who presented with gastrointestinal symptoms at the endoscopy unit of Universiti Sains Hospital, Kubang Kerian, Kelantan and Hospital Kuala lumpur, Malaysia. After receiving a full explanation of the purpose of the study, each patient gave informed consent and was enrolled into the study.

Gastric antral biopsies were collected for rapid urease test as well as stool sample for the detection of *H. pylori* antigen from stool. The diagnosis of infection was based on the RUT. Patients were considered *H. pylori*-positive when the results of RUT were positive. This test was performed with a homemade solution with 1 mL distilled water, one drop 1% phenol red, and 100 mg urea. One antral sample were placed in the solution and maintained at room temperature. The test was considered positive when the color changed from yellow to red within 24 hours (Pourakbari et al., 2011).

Inclusion and exclusion criteria

Patients were excluded from the study if they had received treatment with antibiotics, proton pump inhibitors, H2 receptor antagonists and bismuth compounds within the last four weeks. Patients with previous gastric surgery, long-term use of corticosteroid and immunosuppressant, a history of bleeding or active gastrointestinal bleeding and diarrhoea were also excluded from the study.

This study was approved by the Human Research Ethics Committee, Universiti Sains Malaysia, Kubang Kerian, Kelantan, Malaysia and National Medical Research Resgistry (NMRR).

Detection of H. pylori by Atlas H. pylori antigen test (Atlas Medical, UK)

Atlas *Helicobacter pylori* Antigen Test (Atlas medical, UK) is a rapid immunoassay using a monoclonal anti-*H. pylori* antibody on a strip for the detection of *H. pylori* infections in stool specimens. The *Helicobacter pylori* antigen reacts with the conjugate-Pink Red latex particles sensibilized with anti-*H. pylori* monoclonal antibody coated to the membrane of the strip. The formed H. pylori-conjugate complex, which migrates upward the membrane by capillarity, binds to the specific antibody molecules fixed to the reaction zone.

The stool is collected in a clean container and the test done as soon as possible or stored at 2-8°C for a longer period of time. The test device and sample are put at room temperature (15-30°C) prior to testing. The test was performed according to the manufacturer's instructions. By using the applicator stick of the provided sample diluent vial, a small portion of stool specimen is transferred into the sample diluent and mixed well by shaking gently. The tip of the vial was broken off and four drops were added to the sample well in the test device.

The test was read after 5 minutes of incubation. A positive test result is indicated by appearance of green band at (control line) and red band in the zone marked T (result line). The sample is considered negative when only one green band (control line) appears in the white central zone of the strip. If no colored bands appear or only one band appears in the T zone the result is regarded as invalid and if an inconclusive result is obtained, the test is repeated with a new strip.

Table 1. Sensitivity, Specificity, Positive and Negative Predictive values, and Accuracy of Atlas *Helicobacter pylori* Antigen test in the Detection of *Helicobacter pylori* Infection Atlas *Helicobacter pylori* Antigen Test (n=59)

	Atlas *Helicobacter pylori* antigen test
True positive	22
True negative	35
False positive	0
False negative	2
Sensitivity (95% CI)	91.7% (72.9-98.7)
Specificity (95% CI)	100% (89.9-100)
Positive predictive value (PPV) (%)	100% (84.4-100)
Negative predictive value (NPV) (%)	94.6% (81.8-99.2)
Accuracy	96.6

*95% CI=95% confidence interval

Statistical analysis

The sensitivity, specificity and positive and negative predictive values of the Atlas *Helicobacter pylori* antigen test were calculated against the gold standard for diagnosis of *H. pylori* infection by two by two standard method. Calculations of 95% confidence intervals (CI) were conducted for proportions of these values.

Results

A total of 59 patients, who consisted of 36 (61%) males and 23 (39%) females with a mean age of 51.2±13.3 years and ranging from 26-80 years were recruited into the study.

Out of the total, 24 patients were *H. pylori* positive and 35 were *H. pylori* negative by the gold standard method. Atlas *Helicobacter pylori* antigen test was positive in 22 patients and negative in 35. Thus the sensitivity, specificity, PPV and NPV of Atlas *Helicobacter pylori* Antigen Test were 91.7%, 100%, 100% and 94.6% respectively. The diagnostic accuracy was 96.6% (Table 1).

Discussion

H. pylori is acquired in childhood and survives in the human stomach, the only niche known to date (Tan and Wong, 2011; Valliani et al., 2013). Noninvasive testing for *H. pylori* has been strongly recommended as it is less expensive and more patient-friendly than invasive testing

that requires endoscopy (Manes et al., 2001) and does not need a very complicated laboratory facility.

To the best of our knowledge, this is the first prospective study to find out the efficacy of Atlas *Helicobacter pylori* antigen test in the diagnosis of *H. pylori* infection.

The most important finding of this study was that the new stool antigen test showed high sensitivity (91.7%) and specificity (100%) (Table 1). Our result is similar to other studies done on monoclonal stool antigen test based on immune chromatography, study done In Brazil, found the sensitivity and specificity as 88.0% and 87.5%, respectively (Silva et al., 2010). Similar study done in Turkey showed the sensitivity and specificity of HpSA test as 68.9% and 100% respectively (Ceken et al., 2011). In addition to this, study done in Korea, found the sensitivity and specificity of *Helicobacter pylori* stool antigen immunochromatographic assay (S-ICT test as 84.5% and 96.2% respectively (Jekarl et al., 2013).

The advantages of Atlas *Helicobacter pylori* antigen test over stool antigen test especially HpSA test which has been validated and widely used is that it is easy and takes less than 10 minutes. HpSA test based on enzyme immunoassay tests takes over 1 hour to avail the result, Therefore the new Atlas *Helicobacter pylori* Antigen test is more convenient and saves patient time.

The limitations of this study were small sample size and the fact that we only investigated the performance of the test during initial diagnosis of *H. pylori* infection and not in the post treatment setting.

In conclusion, the Atlas *H. pylori* antigen test is a new non-invasive method which is simple to perform and avails the result in few minutes. Our results have shown that, it has high sensitivity, specificity and diagnostic accuracy and can be used as an alternative method in the diagnosis of *H. pylori* infection in adults. However, there is a need for further studies with a greater number of different patients and to find also its effectiveness in the post treatment setting.

Acknowledgements

The authors of this study wish to thank the endoscopy and the records division staff for their support. This study was supported by the Research University Grant number 1001/PPSP/812108 and 304/PPSP/61312082 from USM.

References

Al-Humayed SM, Ahmed ME, Bello CS, Tayyar MA (2008). Comparison of 4 laboratory methods for detection of Helicobacter pylori. *Saudi Med J*, **29**, 530-2.

Amjad N, Osman HA, Razak NA, et al (2010). Clinical significance of *Helicobacter pylori* cagA and iceA genotype status. *World J Gastroenterol*, **16**, 4443-7.

Ben Mansour K, Fendri C, Zribi M, et al (2010). Prevalence of *Helicobacter pylori* vacA, cagA, iceA and oipA genotypes in Tunisian patients. *Ann Clin Microbiol Antimicrob*, **9**, 10.

Bhewa Y, Hilmi I, Cheah PL, Navaratnam P, Goh KL (2007). Evaluation of the monoclonal stool antigen test for *Helicobacter pylori* in an Asian population with dyspepsia. *J Dig Dis*, **8**, 207-10.

Brown LM, Thomas TL, Ma JL, et al (2002). *Helicobacter pylori* infection in rural China: demographic, lifestyle and environmental factors. *Int J Epidemiol*, **31**, 638-45.

Ceken N, Yurtsever SG, Baran N, et al (2011). Comparison of *Helicobacter pylori* antibody detection in stool with other diagnostic tests for infection. *Asian Pac J Cancer Prev*, **12**, 1077-81.

Ghotaslou R, Milani M, Akhi MT, et al (2013). Diversity of *Helicobacter pylori* cagA and vacA genes and its relationship with clinical outcomes in Azerbaijan, Iran. *Adv Pharm Bull*, **3**, 57-62.

Gisbert JP, Pajares JM (2004). Stool antigen test for the diagnosis of *Helicobacter pylori*infection: a systematic review. Helicobacter, **9**, 347-68.

Jekarl DW, An YJ, Lee S, et al (2013). Evaluation of a newly developed rapid stool antigen test using an immunochromatographic assay to detect *Helicobacter pylori*. *Jpn J Infect Dis*, **66**, 60-4.

Kalem F, Ozdemir M, Baysal B (2010). Investigation of the presence of *Helicobacter pylori* by different methods in patients with dyspeptic complaints. *Mikrobiyol Bul*, **44**, 29-34.

Khalilpour A, Santhanam A, Wei LC, et al (2013). Antigenic proteins of *Helicobacter pylori*of potential diagnostic value. *Asian Pac J Cancer Prev*, **14**, 1635-42.

Manes G, Balzano A, Iaquinto G, et al (2001). Accuracy of the stool antigen test in the diagnosis of *Helicobacter pylori*infection before treatment and in patients on omeprazole therapy. *Aliment Pharmacol Ther*, **15**, 73-9.

Miernyk K, Morris J, Bruden D, et al (2011). Characterization of *Helicobacter pylori*cagA and vacA genotypes among Alaskans and their correlation with clinical disease. *J Clin Microbiol*, **49**, 3114-21.

Peng NJ, Lai KH, Lo GH, Hsu PI (2009). Comparison of noninvasive diagnostic tests for *Helicobacter pylori* infection. *Med Princ Pract*, **18**, 57-61.

Pourakbari B, Mirsalehian A, Maleknejad P, et al (2011). Evaluation of a new antigen for diagnosis of *Helicobacter pylori* infection in stool of adult and children. *Helicobacter*, **16**, 42-6.

Redeen S, Petersson F, Tornkrantz E, et al (2011). Reliability of diagnostic tests for *Helicobacter pylori* infection. *Gastroenterol Res Pract*, **2011**, 940650.

Ricci C, Holton J, Vaira D (2007). Diagnosis of *Helicobacter pylori*: invasive and non-invasive tests. *Best Pract Res Clin Gastroenterol*, **21**, 299-313.

Shimoyama T, Kato C, Kodama M, Kobayashi I, Fukuda Y (2009). Applicability of a monoclonal antibody-based stool antigen test to evaluate the results of *Helicobacter pylori* eradication therapy. *Jpn J Infect Dis*, **62**, 225-7.

Silva JM, Villares CA, Monteiro Mdo S, et al (2010). Validation of a rapid stool antigen test for diagnosis of *Helicobacter pylori*infection. *Rev Inst Med Trop Sao Paulo*, **52**, 125-8.

Tan VP, Wong BC (2011). *Helicobacter pylori* and gastritis: Untangling a complex relationship 27 years on. *J Gastroenterol Hepatol*, **26**, 42-5.

Valliani A, Khan F, Chagani B, et al (2013). Factors associated with *Helicobacter pylori* infection, results from a developing country - Pakistan. *Asian Pac J Cancer Prev*, **14**, 53-6.

Zhang M, Zhou YZ, Li XY, et al (2014). Seroepidemiology of *Helicobacter pylori* infection in elderly people in the Beijing region, China. *World J Gastroenterol*, **20**, 3635-9.

Zhao Y, Wang J, Tanaka T, et al (2012). Association between HLA-DQ genotypes and haplotypes vs *Helicobacter pylori* infection in an Indonesian population. *Asian Pac J Cancer Prev*, **13**, 1247-51.

World Applied Sciences Journal 32 (5): 747-751, 2014
ISSN 1818-4952

DOI: 10.5829/idosi.wasj.2014.32.05.8479

The Characteristics of *Helicobacter pylori* infection and Clinical Outcomes of Patient with Upper Gastrointestinal Bleeding Admitted at Hospital Universiti Sains Malaysia

[1]Hussein Ali Osman, [1]Habsah Hasan, [2]Rapeah Suppian, [3]Nor Aizal Che Hamzah, [4]Sharifah Emilia Tuan Sharif, [5]Noorizan Abdul Majid and [5]Bin-Alwi Zilfalil

[1]Department of Medical Microbiology and Parasitology, School of Medical Sciences, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia
[2]Department of Biomedical Science, School of Health Sciences, Universiti Sains Malaysia, Health Campus16150 Kubang Kerian, Kelantan, Malaysia
[3]Department of Medicine, School of Medical Sciences, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia
[4]Department of Pathology, School of Medical Sciences, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia
[5]Department of Paediatrics, School of Medical Sciences, Universiti Sains Malaysia, Health Campus16150 Kubang Kerian, Kelantan, Malaysia

Abstract: Upper gastrointestinal bleeding (UGIB) remains one of the most common clinical life threatening emergencies which are associated with a high morbidity and mortality. The main aim of this study was to determine the cause of *Helicobacter pylori* (*H. pylori*) infection and the use of non-steroidal anti-inflammatory drugs (NSAID) in upper gastrointestinal bleeding patients. A retrospective record review study was conducted among UGIB confirmed patients from January 2009 and December 2012 at Hospital Universiti Sains Malaysia. All patients who were admitted in hospital were recruited. Data collection included age, gender, *Helicobacter pylori* positivity, associated symptoms and Endoscopic findings. There were 46 patients with a mean age of 62 years. *H. pylori* was detected only in 2 (4.3%) both in Male among UGIB patients by Campylobacter-like organism (CLO) test. The prevalence of UGIB was higher in men than women 27(58.7%). The most common cause of UGIB was peptic ulcer (56.5%) and especially high amongst male patients (59.2%).The second common cause of UGIB was gastritis (19.6%). The majority of the patients are NSAID users 25 (54.3%). In conclusion, Peptic ulcer disease is the leading cause of UGIB and mainly common among males and *H. pylori* infection in upper gastrointestinal bleeding patients was low.

Key words: *Helicobacter pylori* · Campylobacter-Like Organism Test · Gastritis · Peptic Ulcer · Upper Gastrointestinal Bleeding

INTRODUCTION

Upper gastrointestinal bleeding (UGIB) is a common medical emergency that requires hospitalization leading to higher patient morbidity and medical care [1]. The overall mortality rate associated with UGIB is nearly 10-15% [2]. The incidence rates of UGIB reveal a large geographic variation ranging from 100 to 150 cases per 100 000 population, with regular reports of higher incidences among men and elderly people [3, 4].

The most common cause of UGIB is Peptic ulcer bleeding [PUB], accounting for 31%-67% of all cases, followed by erosive disease, variceal bleeding, oesophagitis, malignancies and Mallory-Weis tears

Corresponding Author: B.A. Zilfalil, Department of Paediatrics, School of Medical Sciences, Universiti Sains Malaysia. Tel: +6097676531, Fax: +6097658914.

[5]. In PUB patients, bleeding from duodenal ulcers is slightly more frequent than from gastric ulcers [6].

A study done in Malaysia among 128 UGIB patients found that Peptic ulcer is the main cause of UGIB [7]. *H. pylori* infection and Non-steroidal anti-inflammatory drugs (NSAID) use are independent risk factors for UGIB, especially PUB [8].

Helicobacter pylori (*H. pylori*), a Gram-negative microaerophylic bacterium, is associated with gastrointestinal diseases such as chronic gastritis, gastric and duodenal ulcers and gastric cancer [9-11]. *H. pylori* infection can be diagnosed by invasive techniques requiring endoscopy and biopsy (histological examination, culture, Polymerase chain reaction (PCR) and rapid urease test) and by non-invasive tests (serology, urea breath test, detection of *H. pylori* antigen in stool specimen) [12, 13]. The prevalence of *H. pylori* infection in UGIB patients varies between 24.4% (Serbia) to 92.4% (Spain) [14, 15].

UGIB has high morbidity and mortality rate worldwide and to the best of our knowledge only one study addressed these issues in Malaysia [7] However, it did not study the contribution of *H. pylori* to the morbidity and mortality rate of UGIB in the local population. Therefore, the aim of this study is to find out the causes of UGIB and incidence of *H. pylori*, use of NSAID in upper gastrointestinal patients.

MATERIALS AND METHODS

This retrospective study was conducted at Hospital Universiti Sains Malaysia. The study involved 46 patients who were hospitalized for UGIB with clinical complaints, black tarry stool (melena),coffee-ground vomiting or hematemesis and who underwent endoscopy between January 2009 and December 2012. Cases with UGIB were identified from the records department for the period under study. Case files were then individually analysed to collect data according to the scopes of the current hypothesis.

Collected demographics data (age, gender, race and place of residence), clinical characteristics, cause of UGIB (peptic ulcer, gastritis, duodenitis,varices, erosions and others), *H. pylori* infection and predisposing factors (NSAID). In addition, endoscopy reports were individually examined to ascertain endoscopic findings and confirm the underlying aetiology of bleeding. Patients who had received treatment with antibiotics or proton pump inhibitors were excluded in order to avoid false negative *H. pylori* results.

Helicobacter pylori presence in UGIB patients was confirmed by the invasive method Campylobacter-like organism (CLO) test.

This study was approved by the Human Research Ethics Committee, Universiti Sains Malaysia (USM), Kubang Kerian, Kelantan, Malaysia.

Statistical Analysis: Statistical Package for Social Science (SPSS 20) was used to perform the analysis.Numerical variables are given as means and standard deviation (SD) while Categorical variables were expressed as frequency and percentages. Categorical data were analysed by Chi- square test and Fisher's exact test. The results were considered significant if the P value was less than 0.05.

RESULTS

A total of 46 patients with diagnoses of UGIB were admitted to Hospital Universiti Sains Malaysia between the year 2009 and 2012 and comprised of 27 (58.7) males and 19 (41.3) females. The mean age of patients was 62.3% (range 12 to 83 years) (Table 1). Our study shows that the incidence of UGIB is more common in males than in females.

The ethnic distribution were 41 (89.1%) Malay, 4 (8.7%) Chinese and 1(2.2%) Indian (Table 1). Malay represents the highest ethnic group in this study, probably because they are the highest inhabitant of this state (95%).

The most common presenting complaints were melena (22) and epigastric pain (16) followed by haemetemesis and coffee ground (Table 2). Most of the patients had more than one symptom.

The leading causes of upper gastrointestinal bleeding were Peptic ulcer 26 (56.5%), gastritis 9 (19.6%) and duodenitis 7 (15.2%). Other less common causes included gastric erosion, gastric polyp and oesophageal varices (Table 3).

Peptic ulcers were the most common cause of UGIB 26 (56.5%) and males being with highest percentage 16 (59.2%) as compared with females 10 (52.6%).

Helicobacter pylori infection was found in 2(4.3%) of upper gastrointestinal patients by Campylobacter-like organism (CLO) test (Table 1).

Table 1: Demographic characteristics of patient with upper gastrointestinal bleeding at Hospital Universiti Sains Malaysia n= (46)

Age (years; mean±SD)	62.37±17.86
Gender	
Male	27(58.7%)
Female	19(41.3%)
Race	
Malay	41(89.1%)
Chinese	4(8.7%)
Indian	1(2.2%)
H. pylori infection	2(4.3%)
Male	2(7.4%)
Female	0(0.0%)
Drug	
NSAID	25(54.3%)

NSAID: Non-steroidal anti-inflammatory drugs.

Table 2: Clinical characteristics of patient with upper gastrointestinal bleeding at Hospital Universiti Sains Malaysia n = (46)

Symptoms *	No
Melena	22
Epigastric pain	16
Hematemesis	9
Coffee ground	6
Vomiting	2
Abdominal pain	1
Lack of appetite	1

*All patients had one or more complaint;

Table 3: Distribution of upper gastrointestinal bleeding by cause in patients admitted to Hospital Universiti Sains Malaysia n= (46)

	Male		Female		Total	
Cause	Number of cases	%	Number of cases	%	Number of cases	%
Peptic ulcer	16	61.5	10	38.5	26	56.5
Gastritis	6	22.2	3	15.8	9	19.6
Duodenitis	4	14.8	3	15.8	7	15.2
Gastric erosion	0	0.0	2	10.5	2	4.3
Oesophageal varices	1	3.7	0	0.0	1	2.2
Gastric polyp	0	0.0	1	5.3	1	2.2

Most of the patients occasionally consumed NSAIDs, particularly for a fever or moderate pain. In the present study population, the majority of the patients are NSAID users 25 (54.3%) (Table1).

DISCUSSION

In the present study, there were more males infected with UGIB as compared with females in similar to other studies in which there was a distinct male preponderance [16, 17].

Upper gastrointestinal bleeding tends to occur mostly at an older age. The mean age of 62.3 years in our study is slightly younger than other studies. In a study in Italy and Canada the mean age of patients hospitalized for UGIB was 68 and 66 years respectively [17, 18]. While a recent study in Libya reported lower mean age of 51.75 years [19].

The most common cause of upper gastrointestinal bleeding was Peptic ulcer 26 (56.5%) of all the cases. This finding is in agreement with studies done in Libya [19], Iran [20] and Turkey [4]. Other causes of UGIB that are found in this study are gastritis, duodenitis, gastric erosion, gastric polyp and oesophageal varices. These findings are also similar with others [16, 19]. This study indicates that peptic ulcer disease and gastritis are more common in male than female.

Helicobacter pylori infection in UGIB patients concerned in this study was extremely low 4.3%, probably being the lowest reported worldwide. This is very low compared to other studies which reported higher prevalence in Italy 47.94% [21], Spain 92.4% [15] and Serbia 24.4% [14].

CLO test has been used in the diagnosis of *H. Pylori* in dyspeptic and UGIB patients. Study done by said *et al* found the sensitivity and specificity of CLO test in dyspeptic patients as 99.8% and 96.4% respectively [22]. CLO test has also been used for the diagnosis of *H. Pylori* in UGIB patients [23].

This is not surprising as a study of *H. pylori* prevalence in Malaysia have shown that ethnic Malays have a remarkably low prevalence of *H. pylori* infection [24]. Uyub *et al* later reported that even within Malays there is difference in the prevalence, their study showed that there is low prevalence of *H. pylori* among the Malays in Kelantan [25]. This findings was later validated by Raj *et al*. showing the lowest *H. pylori* prevalence reported in the world (7%) among the the Malays in Kelantan [26]. This low prevalence of *H. Pylori* could be due to a combination of genetic, host and environmental factors among the Malays in Kelantan. This may explain why the rate is also low among the upper gastrointestinal patients in our study.

NSAIDs are major causes of upper gastrointestinal bleeding worldwide with an an increasing mortality rate among users with UGI bleeding [27]. Peptic ulcers are encountered in 15-30% of NSAIDs users and 3-4.5% of NSAIDs users annually develop clinically upper gastrointestinal disease [28]. In the present study 54.3% are NSAID users. This finding is in agreement with other studies in Greece 42.7% [29] and Iran 75% [20], but different from a study in Libya which reported a lower rate 9.7% [19].

As this study is a single-centre research where one ethnic group is predominant, our result cannot be generalized. Furthermore, as this study is retrospective, some data are not well documented equally for all patients and this has hindered our study as we were not able to include all the risk factors and co-morbid conditions. Despite the limitations, the results of this study are important as it outlines the characteristics of patients with upper gastrointestinal bleeding for the first time in this region.

CONCLUSIONS

Peptic ulcer disease remains the primary cause of upper gastrointestinal bleeding and commonly in males. The prevalence of *H. pylori* infection is low in upper gastrointestinal patients.

ACKNOWLEDGEMENT

The authors of this study wish to thank the endoscopy and the records division staff for their support. This study was supported by the Research University Grant number 1001 / PPSP / 812108 from USM. We would like to thank the Islamic Development Bank for giving the first author scholarship.

REFERENCES

1. Ozkan, S., P. Durukan, V. Senol, A. Vardar, E. Torun and I. Ikizceli, 2011. Upper gastrointestinal system hemorrhage in the emergency department. Bratisl Lek Listy, 112(12): 706-710.
2. Katschinski, B., R. Logan, J. Davies, G. Faulkner, J. Pearson and M. Langman, 1994. Prognostic factors in upper gastrointestinal bleeding. Dig. Dis. Sci., 39(4): 706-712.
3. Theocharis, G., K. Thomopoulos, G. Sakellaropoulos, E. Katsakoulis and V. Nikolopoulou, 2008. Changing trends in the epidemiology and clinical outcome of acute upper gastrointestinal bleeding in a defined geographical area in Greece. J. Clin Gastroenterol, 42(2): 128-133.
4. Mustafa, B., O. Serhat, S. Esin, O. Mustafa, A. Gökhan, K. Cemil and S. Tamer, 2012. Evaluation of Risk Factors and Clinical Characteristics of Elderly Patients with Acute Upper Gastrointestinal Hemorrhage. Tr J. Emerg Med, 12(4): 157-162.
5. Holster, I. and E. Kuipers, 2012. Management of acute nonvariceal upper gastrointestinal bleeding: current policies and future perspectives. World J. Gastroenterol, 18(11): 1202-1207.
6. Paspatis, G., E. Matrella, A. Kapsoritakis, C. Leontithis, N. Papanikolaou, G. Chlouverakis and E. Kouroumalis, 2000. An epidemiological study of acute upper gastrointestinal bleeding in Crete, Greece. Eur J. Gastroenterol Hepatol., 12(11): 1215-1220.
7. Lakhwani, M., A. Ismail, C. Barras and W. Tan, 2000. Upper gastrointestinal bleeding in Kuala Lumpur Hospital, Malaysia. Med J. Malaysia, 5(4): 498-505.
8. Van Leerdam, M., 2008. Epidemiology of acute upper gastrointestinal bleeding. Best Pract Res Clin Gastroenterol, 22(2): 209-224.
9. Suzuki, R., R. Cola, L. Cola, C. Ferrari, F. Ellinger, A. Therezo, L. Silva, A. Eterovic and M. Speranca, 2012. Different risk factors influence peptic ulcer disease development in a Brazilian population. World J. Gastroenterol, 18(38): 5404-5411.
10. Devrajani, B., S. Zaman and S. Shah, 2011. Helicobacter pylori: A Cause of Vitamin B12 Deficiency (A Hospital Based Multidisciplinary Study). WASJ, 12(9): 1378-1381.
11. Salehi, Z., F. Akhshabi and E. Talachian, 2011. Prevalence of vacA and cagA Genotypes of Helicobacter pylori in Iranian Children with Peptic Ulcer Disease, WASJ, 12(6): 840-844.
12. Vaira D., L. Gatta, C. Ricci and M. Miglioli, 2002. Review article: diagnosis of Helicobacter pylori infection. Aliment Pharmacol Ther, 16(Suppl1): 16-23.
13. Mégraud, F. and P. Lehours, 2007. Helicobacter pylori detection and antimicrobial susceptibility testing. Clin Microbiol Rev., 20(2): 280-322.
14. Sokic-Milutinovic, A., M. Krstic, D. Popovic, N. Mijalkovic, S. Djuranovic and D. Culafic, 2007. Role of Helicobacter pylori infection and use of NSAIDs in the etiopathogenesis of upper gastrointestinal bleeding. Acta Chir Iugosl, 54(1): 51-62.
15. Gisbert, J., L. Gonzalez, A. de Pedro, M. Valbuena, B. Prieto, L. Llorca, R. Briz, S. Khorrami, R. Garcia-Gravalos and J. Palares, 2001. Helicobacter pylori and bleeding duodenal ulcer: prevalence of the infection and role of non-steroidal anti-inflammatory drugs. Scand J. Gastroenterol, 36(7): 717-724.
16. Kaliamurthy, M., M. Lee, M. Mills and T. Murphy, 2011. Upper gastrointestinal bleeding: a Jamaican perspective. West Indian Med J., 60(3): 289-292.
17. Romagnuolo, J., A. Barkun, R. Enns, D. Armstrong and J. Gregor, 2007. Simple clinical predictors may obviate urgent endoscopy in selected patients with nonvariceal upper gastrointestinal tract bleeding. Arch Intern Med, 167(3): 265-270.

18. Marmo, R., M. Koch, L. Cipolletta, L. Capurso, E. Grossi, R. Cestari, *et al.*, 2010. Predicting mortality in non-variceal upper gastrointestinal bleeders: validation of the Italian PNED Score and Prospective Comparison with the Rockall Score. Am J. Gastroenterol, 105(6): 1284-1291.
19. Elghuel, A., 2011. The characteristics of adults with upper gastrointestinal bleeding admitted to Tripoli Medical Center: a retrospective case-series analysis. Libyan J. Med, pp: 6.
20. Kaviani, M., M. Pirastehfar, A. Azari and M. Saberifiroozi, 2010. Etiology and outcome of patients with upper gastrointestinal bleeding: a study from South of Iran. Saudi J. Gastroenterol., 16(4): 253-259.
21. Pilotto, A., G. Leandro, F. Di Mario, M. Franceschi, L. Bozzola and G. Valerio, 1997. Role of Helicobacter pylori infection on upper gastrointestinal bleeding in the elderly: a case-control study. Dig Dis. Sci., 42(3): 586-591.
22. Said, R., P. Cheah, S. Chin and K. Goh K, 2004. Evaluation of a new biopsy urease test Proton Dry, for the diagnosis of Helicobacter pylori infection. Eur J Gastroenterol Hepatol Eur J. Gastroenterol Hepatol., 16(2): 195-199.
23. Castro Fernández, M., D. Sánchez Muñoz, E. García Díaz, M. Galán Jurado and C. Rodríguez Alonso, 2004. Diagnosis of Helicobacter pylori infection using urease rapid test in patients with bleeding duodenal ulcer: influence of endoscopic signs and simultaneous corporal and antral biopsies. Rev Esp Enferm Dig, 96(9): 599-605.
24. Goh, K. and N. Parasakthi, 2001. The racial cohort phenomenon: seroepidemiology of Helicobacter pylori infection in a multiracial South-East Asian country.Eur J. Gastroenterol Hepatol, 13(2): 177-183.
25. Uyub, A., S. Raj, R. Visvanathan, M. Nazim, S. Aiyar, A. Anuar and M. Mansur, 1994. Helicobacter pylori infection in North-Eastern Peninsular Malaysia; evidence for an unusually low prevalence. Scand J. Gastroenterol, 29(3): 209-213.
26. Raj, S., Y. Lee, K. Choo, A. Noorizan, A. Zulkifli, M. Radzi and S. Ang, 2008. Further observations in an area with an exceptionally low prevalence of Helicobacter pylori infection.Trans R. Soc Trop Med. Hyg, 102(11): 1163-1164.
27. Straube, S., M. Tramer, R. Moore, S. Derry and H. McQuay, 2009. Mortality with upper gastrointestinal bleeding and perforation: effects of time and NSAID use. BMC Gastroenterol, 9: 41.
28. Peura, D. and L. Goldkind, 2005. Balancing the gastrointestinal benefits and risks of nonselective NSAIDs. Arthritis Res Ther, 7: S7-13.
29. Tsesmeli, N., P. Kotsaftis, C. Savopoulos, A. Hatzitolios, G. Kaiafa, A. Kounanis and D. Karamitsos, 2007. Incidence and etiology of acute non-malignant upper gastrointestinal bleeding in northern Greece. J. Gastroenterol Hepatol., 22(7): 1009-1013.

NATIONAL CONFERENCE ON ENVIRONMENT & HEALTH 2008

"SUSTAINABLE ENVIRONMENT - BASIC FOR QUALITY LIFESTYLE"

Proceeding

29 - 30 October 2008
Grand Riverview Hotel, Kota Bharu, Kelantan
Malaysia

Editors:
Haliza Abdul Rahman
Hasmah Abdullah
Nik Norliza Nik Hassan
Norhaniza Abdul Khairi
Rapeah Suppian
Sakinah Harith

Organised by:
School of Health Sciences
Universiti Sains Malaysia
Health Campus
Kubang Kerian
Kelantan
MALAYSIA

59

THERAPEUTIC VALUES OF *CENTELLA ASIATICA* IN HUMAN HEALTH

Nor Zaharaini MG., Hasmah A. & Rapeah S.

ABSTRACT

Centella asiatica (CA) is a local plant and commonly distributed in most Asian region. This plant has many common names according to its origin; "Pegaga" in Malaysia, "Babassa", "Orilaittamara", "Vallarai", or "Vallari" in Sri Lanka, "Tapak Kuda in Indonesia and Brahmamanduki in India. CA is widely used for curative purposes and as food ingredients in many countries around the world. In this study we will review articles published since 1995 onwards and discussing the therapeutic values and potential of CA on human health. Related articles published in MEDLINE, Combined Health Information Database (CHID), Cochrane Database of Systemic Reviews, Sciences Direct and PubMed were cited. The review containing phytochemicals study of CA and its bioactivity to cure various range of health problem. Major active compounds in CA are asiaticoside, medicassoside and medicossic acid. These compounds can be used as anti-inflammatory, anti-microbial activities, helpful in reducing stress as well as offer a remarkable healing potential. More recently, the researchers are trying to identify more active compounds in CA with potential use as therapeutic agents against various diseases. In conclusion, there would be an advantage for the modern pharmaceutical to apply this traditional herb and manipulates it into modern practice. However, the toxicological and pharmacological aspects of this plant should also be studied in advanced to ensure the safety and efficacy of its uses.

Keywords: Active compounds, *Centella asiatica*, human health, therapeutic agents

INTRODUCTION

Centella asiatica (CA) has been used in Shennong, China since 2000 years ago, and one of the key herbs in ayurvedic medicine (Noraida, 2005). This plant contains flower, stem, leaf, fruit and root (Figure 1). In 100g fresh weight of CA contains 52 kcal energy, 88 g moisture, 3 g protein, 2.7 g fat, 1.92 g fiber, 2.54 g ash, and 3.81 g carbohydrates (Odhav et al., 2007).

Figure 1: *Centella asiatica* a) Flower, b) Whole plant, c) Stem base with young leaf, flowers and fruits

Its nutritional value has attracted a great deal of research to elucidate its medicinal property in human. Furthermore, the therapeutic values of CA have evident to curb various health problems such as skin disease, promoting burn wound healing, dehydration, mental illness as well as improve self rated mood (Meulenbeld & Wujastyk, 2001; Kimura et al., 2008). There are more than 70 phytochemicals exist in CA (Quan et al., 2007) identified by various methods including Thin Layer Chromatography (TLC) (Harborne, 1998) and High Performance Liquid Chromatography (HPLC). Some of the compounds are listed in Table 1. Among these, asiaticoside is the most promising compound that has been studied for many years to determine its therapeutic values. Figure 2 shows the result of detection of asiaticoside in CA by TLC method.