

The Effect of Barley (*Hordeum Vulgare L.*)  
Water Extract as A Chemolytic Agents in  
Cholelithiasis (Gallbladder Stone) -  
An In-vitro Study

By

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Dissertation submitted in partial fulfillment of the requirements for the  
degree of Master of Medicine (General Surgery)



UNIVERSITI SAINS MALAYSIA

2017

## ACKNOWLEDGEMENTS

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

All praise to Allah the Lord of universe whom in His infinite blessing and help get me through my research dissertation. Special thanks and I am really indebted to many people who have supported me in preparing and executing this project. This dissertation is a prerequisite in getting Master of Medicine (Surgery) in school of medical sciences in Universiti Sains Malaysia (USM)

My special thanks to my supervisor Ass. Prof. Dr Andee Dzulkarnaen bin Zakaria, General Surgeon and Medical lecturer in the Department of Surgery School of Medical Science for the unconditional support and relentless effort in my project and training despite his limited time.

Other thanks to my co supervisor Dr Noor Zuhartini Bt Md Muslim, Forensic Lecturer PPSK, USM who is behind my success during laboratory expertise and experimental period. She was supportive and full of ideas to facilitate me in finishing this project. She also one of the main figure in analyzed the stone samples. I also owe gratitude to the following people, Cik Mardhihah from Statistics department for the important contribution on data analysis. My appreciation goes to Mr Baharuddin Zakaria and Mr Rosliza Haron for the immense assistance for the biochemical and FTIR analysis.

I would like to pay gratitude to Ass. Prof. Dr Zaidi bin Zakaria, Head of Surgical Department, HUSM in helping me to complete this project. To all my lecturers, colleagues and all staffs of Department of Surgery who were involved in the research.

Finally, my special thanks and appreciation I would like to express to my beloved wife, Wan Nur Aisyah; my children; Insyirah, Khaleed and Ammar for their kind support and understanding during my research and study in general. The same thanks to my parents, Hj Sulaiman Mohd and Hajjah Fatimah Chek, brothers and sisters for their continuous support.

May Allah reward all those involved in this research in one way or the other. Ameen.

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## ABBREVIATIONS

HUSM	Hospital Universiti Sains Malaysia
PPSK	Pusat Pengajian Sains Kesihatan
FTIR	Fourier Transform Infrared Spectroscopy
TSPMS	Thin-section petrographic microscopic study
ANOVA	Analysis of variance
UDCA	Ursodeoxyeholie acid
CDCA	Chenodeoxycholic acid
ERCP	Endoscopy Retrograde Cholangiopancreatogram
USG	Ultrasonography
Na <sup>+</sup>	Natrium
Mg <sup>2+</sup>	Magnesium
Al <sup>3+</sup>	Aluminium
Ca <sup>2+</sup>	Calcium
Cl <sup>-</sup>	Chloride
PO <sub>4</sub> <sup>3-</sup>	Phosphate
Mn <sup>2+</sup>	Manganese
Fe <sup>2+</sup>	Ferrous
Cu <sup>+</sup>	Cuprum
NO <sup>3-</sup>	Nitrate
Zn <sup>+</sup>	Zinc



## ABSTRAK

### PENGENALAN

Penyakit batu karang pundi hempedu biasanya dihidapi oleh manusia semenjak dahulu lagi, bermulanya zaman awal tamadun manusia. Direkodkan pada awal tamadun lama India dan Mesir purba, perawatan untuk penyakit batu karang pundi hempedu dengan menyahtoksinkan pundi hempedu dan hati menggunakan campuran minyak herba. Kepentingan dalam perawatan dan kajian amat penting untuk mengetahui sifat semulajadi setiap jenis batu pundi hempedu. Dengan mengetahui jenis-jenis batu tersebut, memudahkan perawat dalam mengenal pasti risiko-risiko terjadinya pembentukan batu dan memformulasi cara untuk mengurangkan keadaan tersebut.

Walaupun rawatan untuk penyakit ini telah lama dikenal pasti, iaitu melalui cara pembedahan, samada melalui pembedahan terbuka ataupun menggunakan alatan kamera untuk membuang pundi hempedu, terdapat juga sebahagian perawat menggunakan cara mendebu dan menghancurkan batu pundi hempedu tanpa dokumentasi keberkesanan cara tersebut. Ujikaji ini dilaksanakan dengan cara 'in vitro' untuk mengenalpasti keberkesanan ekstrak air barli dalam proses 'kemolisis' menggunakan kepekatan yang berbeza.

### OBJEKTIF

Objektif ujikaji ini dijalankan adalah untuk mengenalpasti komposisi atau jenis batu hempedu berdasarkan ujian dan formulasi oleh FTIR. Selain itu, ia juga dijalankan untuk mengetahui kepekatan ekstrak air barli yang optimum dalam proses 'kemolisis'. Yang terakhir ia dilakukan untuk menilai komposisi manakah bahan kimia batu hempedu yang mempunyai kesan terbanyak dalam proses uji kaji ini.

Di harap, dengan keputusan ujikaji yang dijalankan ini, perawatan terbaru boleh disarankan dan dilaksanakan dalam membantu pesakit yang menghidapi penyakit batu hempedu.

## KAEDAH

Kajian sampel batu hempedu dikumpul daripada pesakit bermula pada tahun 2011 sehingga 2015 selepas pembedahan terbuka ataupun menggunakan alatan kamera. Batu-batu hempedu ini dibahagikan mengikut 3 jenis utama (kolesterol, pigmentasi, campuran) menggunakan alatan 'Fourier Infra-Red Spectrometry' (FTIR). Setiap sampel batu hempedu ini telah direndam di dalam ekstrak *Hordeum Vulgare L.* (barli) pada tiga kepekatan yang berbeza 3mg/ml, 5mg/ml dan 10mg/ml pada pH 6 dengan suhu sekitar 37°C. Larutan Saline digunakan sebagai bahan kawalan uji kaji.

Kajian ini dijalankan selama tempoh 4 minggu dan pengurangan berat batu hempedu dicatatkan setiap minggu. Batu-batu ini telah direndam di dalam bekas steril bebas kebocoran dengan bahan ujian dan bahan kawalan. Larutan ujukaji ditukar setiap hari pada kadar 6ml/100mg pada suhu tetap 37°C.

Pada akhir ujian dilaksanakan selepas 4 minggu, peratusan berat terakhir telah dikira dengan menggunakan formula seperti di bawah:

% berat batu hempedu yang berkurang =  $\frac{\text{berat awal} - \text{berat akhir}}{\text{berat awal}} \times 100$

Min pengurangan berat batu hempedu minimum bagi setiap jenis batu ditentukan, peratus pengurangan ditentukan dan data telah dianalisis dengan menggunakan ujian Repeated Measure ANOVA, dengan pembetulan oleh Greenhouse-Geisser disebabkan terdapat ralat pada Mauchly's Test of Sphericity. Dengan P value < 0.005, ujian Bonferroni Post Hoc dijalankan untuk mengenalpasti nilai tepat perbezaan ini.

## KEPUTUSAN

Sejumlah 30 sampel ujikaji di jalankan, di akhir ujian selepas 4 minggu, peratusan (%) pengurangan berat batu hempedu dicatatkan. Kumpulan batu hempedu kolesterol didapati mengalami kesan kemolisis yang ketara oleh ekstrak barli, ini berdasarkan peratusan pengurangan berat.

Namun, berdasarkan kepekatan ekstrak barli, hanya kepekatan 3mg/ml yang mempunyai perbezaan ketara dalam pengurangan berat dengan P value < 0.005. Larutan kontrol tetap, kepekatan ekstrak barli 5mg/ml dan 10mg/ml tidak memperolehi perbezaan yang ketara selepas ujikaji dengan nilai P value > 0.005.

Ujian FTIR pula, daripada 30 sampel batu hempedu, 22 sampel adalah batu hempedu kolesterol dan 8 sampel lagi adalah batu hempedu jenis campuran. 7 (31.8%) daripada 22 sampel batu hempedu kolesterol didapati mengalami pengurangan jaringan karbon, dan bagi batu hempedu jenis campuran, terdapat 6 (75%) daripada 8 sampel batu hempedu yang mengalami perubahan yang sama.

## KESIMPULAN

Kesan disolusi oleh ekstrak barli dengan kepekatan yang berbeza berdasarkan ujikaji ini hanya diperolehi dengan kepekatan 3mg/ml, dan ini boleh disimpulkan, ekstrak barli mempunyai nilai pencegahan dalam merawat dan mengelakkan pembentukan batu hempedu berdasarkan proses kemolisis. Suyugia, kajian lanjut ekstrak barli ini diharap dapat membawa kepada penemuan yang dapat mengelakkan terjadinya batu hempedu, digunakan sebagai rawatan alternatif untuk pesakit batu hempedu yang tiada gejala serius ataupun untuk pesakit yang memilih untuk tidak melakukan rawatan pembedahan.

## ABSTARCT

### Introduction:

Background: Cholelithiasis is a common disease since the early age of civilization, documented in history in ancient India and Egyptian few methods of treatment for the cholelithiasis, including detoxification of the body via gallbladder and liver by using specially prepared herbal oils. Fundamentally, clinical and research practices would have analyzed the nature of the gallstone. Knowing the type of stone is a precondition in helping medical personnel to detect the risk factors of gallstone formation as well as to formulate the methods to minimize the occurrence.

Even though they are well established treatment modalities in treating this disease, either by open or laparoscopic method of cholecystectomy, nevertheless chemolysis therapy of gallstone still practice in curtain part of the globe without any documented outcome. In this study, an in vitro experiment was conducted on extract of *Hordeum Vulgare L.* (Barley) to assess its effectiveness as a chemolytic agents in Cholelithiasis (Gallbladder Stone) under different concentration.

### Objectives:

One of the objectives of this study is to determine the composition or types of gallbladder stones based on the test result and calculation by FTIR. On top of that, we also try to identify the optimum level of concentration of *Hordeum Vulgare L.* extract that has the best chemolytic effect on different types of gallbladder stone. Apart from that, the result of this study could also show us the stone type that has the maximum impact from *Hordeum Vulgare L.* extract. Our last objective is to distinguish the most

affected chemical component of gallstone by the *Hordeum Vulgare L.* extract at the end of this test. Hopefully, after studying the result and outcomes of this research, new complimentary treatment strategies may be recommended and implemented to help patients with gallstones disease.

#### Methodology:

Test samples were collected from the patients within the period of 2011-2015. Stone removal was either by open or laparoscopic surgery, and these stones were divided by 3 types (Cholesterol, Pigmented, Mixed) based on identification using Fourier Infra-Red Spectrometry (FTIR). Each sample were immersed in the water extract of *Hordeum Vulgare L.* at three different concentration, 3mg/mL, 5mg/mL and 10mg/mL at pH of 6 at temperature control of 37°C. A normal saline is used as experimental control.

This study was conducted for a total period of 4 weeks with weekly weight reduction measurement. The gallstones were submersed in a leak-proof sterile container with the extract test solution and control solution. The test solutions were changed daily at 6ml/100mg per stone under controlled and constant temperature of 37°C.

After 4 weeks of tests, the final weight measurements were recorded for each stone based on the formula below:

$$\% \text{ stone weight loss} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

The means of the minimum stone weight reductions for each type of stones are determined. The percentage of gallstone weight reduction was established and the data were analyzed using repeated measure ANOVA, with the compliments of Greenhouse-Geisser correction because of Mauchly's Test of Sphericity indicated that the assumption of sphericity had been violated. To identify differences between groups at p

value  $< 0.005$ , Bonferroni post hoc test was proceeded to discover the specific means differed.

#### Results:

A total of 30 samples was included in this study. At the end of the fourth week, the percentage of gallstone weight reduction was measured. The cholesterol-type of gallstones were the most affected by the barley water extract based on percentage of weight reduction.

However based from the concentration of the extract, only 3mg/ml had significant impact in weight reduction with p value  $< 0.005$ . The control solution, 5mg/ml and 10mg/ml made no significant difference with p value  $> 0.005$ .

From FTIR test, 22 samples of the gallstones were cholesterol-type, and the remaining 8 samples were mixed type. Seven (7) (31.8%) out of twenty two (22) cholesterol types of stone had reduced carbon chain, whereas amongst the mixed-type of gallstone, 6 (75.0%) out of 8 samples had the same effect after this study conducted.

#### Conclusion:

Dissolution effects of barley extract with different concentration shown in this study by the concentration of 3mg/ml, thus it's may have a prophylactic value in term of preventing the formation of gallstone by the present of chemolysis reaction, with cholesterol type of gallstone significantly affected. Therefore, usage of barley water extract should merit further study in helping us to find a way for the usage of barley as a prophylaxis for the occurrences of gallstone, asymptomatic cholelithiasis or in patients who are not keen for any surgical intervention.

### 1.0.0 INTRODUCTION

Cholelithiasis is the presence of solid concretions in the gallbladder. Back in 3500 years ago, cholelithiasis is known to affect the human society. Autopsy done in ancient entity of Egypt and Chinese found to have gallstones (Stinton et al., 2010). For example, early civilization of Ancient Egypt and India, few modalities of treatment for gallstones disease mainly from natural source for example oils from plants and grains. Cholelithiasis is a major surgical problem with high morbidity, high cost of treatment and sometimes can cause mortality if in severe form.

Gallstones are hard, pebble-like deposits that form inside the gallbladder. There are various sizes of gallstones, may be as small as a grain of sand or as large as a golf ball.

Gallstones formed and retain in the gallbladder but may exit into the bile ducts (choledocholithiasis). As a result, it may cause either cystic or bile duct obstruction symptoms.

### 1.1.0 INCIDENCE AND PREVALENCE OF GALLSTONE

Cholelithiasis is encountered in medical practice worldwide. Documented in postmortem studies in adult found 12% of men and 24% of women had gallstones. Its merge as one of the main problems in healthcare mainly in the developed world countries and impose a major significant economic burden on healthcare (Chowdhury and Lobo, 2011).

The prevalence of gallstones varies depend on the nation and populations, in North hemisphere, mainly in European countries the prevalence ranging from 6-22%, occur 1 in 200 populations incident per year. In America, 20-25 million had gallstones, which occupy almost 10-15% adult population. As a result from this data, the US government had to spend approximately USD 6.2 billion for the costs annually (Stinton et al., 2010). Furthermore, up to 30% of patients may present with symptoms, which end up by endoscopic or open surgical interventions. Approximated 50 000 cholecystectomy performed in United Kingdom itself, where else 700 000 cholecystectomy being performed yearly in US (Chowdhury and Lobo, 2011). Besides that, the prevalence generally increase with age, about 2% to 20% between age of 20 years to 40 years and its significantly increase over 30% at age over 70 years (Shiina et al., 2011).

In American continence, obesity is one of the main factors that increase prevalence and frequency of gallstone incident in Brazil. One study conducted at Child and Adolescent Obesity Outpatient clinic in 2011 with the numbers of 66 obese patient, clinically obese with suggestive BMI, supported with laboratory parameters and radiological imaging (ultrasonography) , cholelithiasis recorded in 6.1% obese patient, hepatic steatosis incident was documented in 21.2% patient (Nunes et al., 2014). Improper dietary intake for example high amount of animal based fat, with additional unhealthy lifestyle, indicate dietary factors not only act as protective factors, but as significant factors that associated with gallstone disease (Kranz et al., 2001).

General surgeon most commonly treats the gallstone disease and its complications. Approximated 30 to 40 million residents in United States have gallstone disease, with prevalence rate of 1 in 10 populations. Even though 80% of the patients are asymptomatic, its might complicate into few sequelae, for example annually 0.3%



will developed acute cholecystitis, 0.2% for obstructive jaundice, and biliary pancreatitis between 0.1 to 1.5% (Victor Zaydfudim MD et al., 2014). In Brazil, gallstone in general population age more than 20 years old is around 9.3%, plays significant prevalent and economical cost in health sectors (Souza et al., 2010).

Prevalence of gallstone in Malaysia is not properly concluded, but consensus for management of gallstone disease and its complications had been formularized as a guideline to make a decision based on individual condition and availability of manpower and resources at hand (Ministry Of Health et al., 1997).

From The Malaysian Journal of Analytical Sciences (2008), reported significant increment in number of patients who had been diagnosed with cholelithiasis in the South Asia region, for example in Pakistan, even though the stone composition is heterogeneous, its might be necessary to help and decide whether to treat the disease surgically or pharmacologically (Nassem A. Channa, 2008).

### 1.2.0 LITERATURE REVIEW

Gallstones represent a significant burden on healthcare systems throughout the world. In the UK, around 10-15% of the adult population develop gallstones, with approximately 70,000 cholecystectomies being performed in England annually. Incidence rates vary worldwide from 0% to 10% reported in Africa to 70% in certain populations such as Pima Indians, reflecting a combination of dietary, environmental and genetic factors (Gallagher and Parks, 2014).

Gallstones remain asymptomatic in up to 80% of carriers, however, it is estimated that within 5 years, 10-20% became symptomatic (Friedman, 1993) .

#### 1.2.1 Pathogenesis of Gallstone:

Misbalance of equilibrium between cholesterol, lecithin (phosphatidylcholine) and bile salt are considered the major event for formation of gallstone. With the super saturation of bile salt and cholesterol are the main factors for formation of cholesterol gallstone. Its happen with the cholesterol crystallization and super saturation occur with lots of cholesterol or less solubilizing bile salt and lecithin molecules are produce to complete micellar solubilization of cholesterol (Van Erpecum, 2011, Gallagher and Parks, 2014, Friedman, 1993, Ko and Lee, 2005, Ko and Lee, 2004)

Pigmented gallstone is less frequent than cholesterol gallstone. They are being divided as 'black' and 'brown' pigmented gallstone. Prerequisite factor for formation 'black' pigmented stone is hyperbilirubinemia (hyper secretion of bilirubin conjugates in biliary system), mainly by the process of hemolysis, ineffective erythropoiesis, abnormal pathology of bilirubin cycle of unconjugated bilirubin and liver cirrhosis (Vitek and Carey, 2012, Cariati, 2013). The 'brown' pigmented stone on the other hand developed secondary to stasis and anaerobic bacterial infection in the biliary system and cholesterol much more present in the proportion of 'brown' pigmented stone compared to 'black' pigmented stone (Vitek and Carey, 2012, Friedman, 1993).

Alcohol on the other side induced liver impairment called liver cirrhosis, which predisposed the formation of pigmented gallstone (Friedman, 1993).

### 1.2.2 Anatomic distribution of gallstones:

The proportions of gallbladder, common bile duct, and intrahepatic duct stones among total gallstone patients were 64.0%, 21.9% and 14.1%, respectively (Shim, 2000).

Based on their composition, gallstones can be divided into the following types:

1) Cholesterol stones: vary from light yellow to dark green or brown. They must be at least 80% cholesterol by weight and are radiolucent (Gallagher and Parks, 2014).

2) Pigment stones: black pigment stones are small and dark. They comprise bilirubin and calcium salts that are found in bile and have cholesterol content of less than 20%. Over 50% of these calculi are radiopaque, and are typically seen in patients with cirrhosis or increased red cell destruction. Brown pigment stones arise in the gallbladder and bile ducts in association with biliary infection or bile stasis, and are common in Asia, with reported prevalence rates as high as 20% in some parts of China (Gallagher and Parks, 2014).

3) Mixed stones: typically contain 20-80% cholesterol. Other common constituents are calcium carbonate, palmitate phosphate, bilirubin and other bile pigments. Because of their calcium content, they are often radiographically visible (Gallagher and Parks, 2014).

In south Asia continent, for example in Pakistan, there are increasing number of

patients who being diagnosed with cholelithiasis and end up with removal of gallbladder stone. Documented about 67.9% had pure cholesterol stone, 4.6% pure calcium carbonate and others 20% are mixed form of stone. This may help practitioners and surgeon to identify the main cause of gallstone and will guide whether to treat cholelithiasis patients therapeutically or surgically (Nassem A. Channa, 2008). Besides cholesterol gallstone, others element make up the process of gallstone stone, mainly the trace element are  $\text{Na}^+$ ,  $\text{Mg}^{2+}$ ,  $\text{Al}^{3+}$ ,  $\text{Cl}^-$ ,  $\text{PO}_4^{3-}$ ,  $\text{Mn}^{2+}$ ,  $\text{Fe}^{2+}$ ,  $\text{Cu}^+$ ,  $\text{NO}_3^-$  and  $\text{Zn}^+$ . This may differ mainly due to dietary intake for example of present of significant amount of  $\text{Fe}^{2+}$  in gallstone in South Indian pigmented stone due to regular intake of tamarind in their daily population foods (Rautray et al., 2007).

Cholesterol gallstone disease is multifactorial. Cholesterol absorption and insulin resistance may play a pivotal role in gallstone development (Miquel et al., 2009). Others risk factor that promote the formation of gallstone are age, sex, total parenteral nutrition, hemolysis, congenital heart disease, liver cirrhosis, post gastrectomy and pregnancy (Shiina et al., 2011). There is numerous investigation done, to detect the main factors contributing the formation of cholesterol gallstone, the result was inconclusive. Predisposing factors for example alcohol intake, high intake of fat and simple sugars, dietary protein and fibers had found related to increase incident of gallstone disease. Recently with the high intake of carbohydrates in daily meal are related to subsequent cholecystectomy (Ko and Lee, 2005). In children age group, cholesterol type of gallstone present in overweight adolescent girls with strong family history of gallstones (Stringer et al., 2007).

In the Western world, approximately 70% of gallstone carriers exhibit cholesterol gallbladder stones (cholesterol content > 50%), and 30% black pigment gallbladder stones. In East Asia, there is traditionally a high prevalence of brown

pigment stones residing in the bile ducts, and causing potentially devastating cholangitis (Van Erpecum, 2011). 50% of Hispanic women with gallstone had increase plasma cholesterol level compared with Hispanic women without gallstone. With the synthesis of bile acids and cholesterol induced the formation of gallstone disease in Hispanic women by 22% increment (Gälman et al., 2004).

Souza et al done one study in Brazil with the total of 22 males and 48 female patients with gallstones disease, 75% of the patient found to have cholesterol types of gallstone, and the rest 25% are the combination of bile and mixed types of stone. In general, the prevalence of types of stone almost similar in developed countries which have good health support with improvement of socio-economic status (Souza et al., 2010).

Recent study conducted on chronic renal failure patient who had chronic uremia is related to increased incident of gallstone disease about 22% as most similar to patient who had been diagnose with primary hyperparathyroidism (30%), majority of them had been discovered to have calcium phosphate gallstones. To establish the relationship of chronic renal failure and primary hyperparathyroidism with the formation of gallstone, further studies need to conducted (Cariati, 2013).

Stringer et al recruited in children, types of gallbladder stone differ from the adult population. Study done consist of total 63 children in North of England, documented about 30 (48%) are black pigmented stone, another 13 (21%) of children had cholesterol type of stone, 15 (24%) had calcium carbonate stones, 3 (5%) and 2 (3%) had protein dominant stone and brown pigmented stone respectively (Stringer et al., 2007). This may be due to geographical location, lifestyles and diet intake, which is different in other countries.

On the other hand, reported by Lie et al, based on thin-section petrographic microscopic study (TSPMS) done in Taiwan with total amount of 100 samples, about 35% patient affected with pure-cholesterol gallstone, 25% had black stone, 17% had mixed types of stone and 12 % had combination stone. This method using TSPMS is acceptably accurate, which can be done to chemically analyzed types of gallstone (Liu et al., 2002).

When the cystic duct obstructions occur either chronic or intermittent, its will lead to dysfunction of hydrogen secretion by gallbladder wall. It will multiply the amount calcium ion entering the gallbladder lumen, mixed with abundant amount of carbonate anion, increase the amount saturation index (SI\_CaCO<sub>3</sub>) from 1 to 22.4, makes the calcium carbonate to aggregate and form gallstone (Yu et al., 2013).

#### 1.3.0 Clinical presentation of Gallstone disease:

Gallstones maybe present as symptomatic or accidentally found in asymptomatic patients. These symptoms may arise as a result of stone locates either in the gallbladder, in the common bile duct or both.

Majority of the gallstone patient presented with abdominal discomfort and indigestion. Usually patient presented with biliary colic, precipitated by the contraction of gallbladder towards the closed orifice. Usually patient described as acute sharp pain and increase rapidly towards the peak, then constant for minutes to hours, before subsiding within hours (Friedman, 1993).

In the case of inflammation, its developed due to long standing obstruction of gallbladder. Patients come with right hypochondriac pain and signs of inflammation, mainly pyrexia. On physical findings, usually significant and positive Murphy's sign

may help the physician in diagnosed this disease (Gallagher and Parks, 2014, Friedman, 1993).

#### 1.4.0 Treatment of Gallstone: Surgical and non-surgical

Treatment for gallstone disease: pain control, medical chemolysis or drugs that can cause lowering the cholesterol level, before opted patient for cholecystectomy. The gold standard treatment of gallbladder stone is cholecystectomy either open or laparoscopic. In a hospital with laparoscopic expertise, readmission rate and rate of morbidity following elective laparoscopic cholecystectomy is low (Sanjay et al., 2011). Information regarding medical chemolysis in gallstone disease are not well developed compared to urinary tract stone.

Intake of protein and fat form animal origin, significantly associated with the gallstone disease, highly recommended that low intake of those types of foods mainly by the person who are at risk of developing of gallstones should be advice in daily meal (Kranz et al., 2001).

Based on study in 1912, medical treatment for gallstone mainly good lifestyles, adequate rest, avoidance of excessive meal and usage of Calomel (mercurous chloride) drugs that was believed to stimulate the liver and the gall bladder (Mayer, 1912). Based on safety regulation once the compounds of this drug had been discovered, by the



1960s, introduction of newer drugs and concerns about heavy metal poisoning led to the discontinuation of this drug.

Kelly et al reported there is three ways that gallstone can be spontaneously evacuated from the gallbladder: 1) formation of fistula between gallbladder and duodenum 2) gallstone dissolution and 3) by the movement of gallstone that move from gallbladder into the usual biliary system. It was confirmed by imaging cholecystogram and during cholecystectomy surgery. When the stone was discovered in feces with no evidence of fistula formation, its conclude that the gallstone can be passed out from the gallbladder to the intestine via common bile duct (Kelly and Schlueter, 1966).

There are few drugs that available for gallstone dissolution either pharmacologically or mechanically depending on the size, types and location of stone. Drug for example chenodeoxycholic acid (CDCA) and ursodeoxycholic acid (UDCA), have been shown to enhance the cholesterol solubilizing capacity of human bile. Others drug that can have same effect including glycerophosphate, Zanchol, ioglycamide, and the proprietary essential oil preparation Rowachol (Bell, 1979). In United State, usage of oral bile salt for cholesterol gallstone dissolution for example chenodeoxycholate taken by numbers of patients, ideally the dissolution process with significant weight reduction ranges 6 months to 4 years and the significant decrease in size within 9 months of treatment. Topical infusion therapy also documented been used, with the help of T-tube in case of retained stone in the biliary tract (Pitt et al., 1989).

Drug from CDCA group for example Clofibrate extensively use to treat hyperlipidemia. It acts on the liver to enhance biliary secretion of cholesterol and suppress the secretion of bile acids. As a consequence it renders the patient's bile "lithogeny," and this predisposes the patient to cholesterol gallstones (Bell, 1979).

On the other hand, Bell et al stated that Ursodeoxycholic acid (UDCA), especially when excreted into patients bile as the glycine conjugate, is greatly inferior to Chenodeoxycholic acid (CDCA) as a cholesterol solvent (Bell, 1981). Short-time intake of UDCA in gallstone disease patient increase the gallbladder bile flow, this believed to decrease risk of biliary pain and its complications (Guarino et al., 2003).

#### 1.5.0 Complications of Gallstone disease

Untreated gallstone disease may end up with few complications. Study by Friedman et al highlight that the main complication of inflammatory condition, which may lead to focal necrosis, gangrene, empyema and sometimes perforation. In certain cases, patient may be presented with jaundice in a condition of biliary obstruction by the gallstone, usually obstruction at common bile duct area. This may worsen patient condition when the obstruction leads to acute infection and liver damage (Friedman, 1993).

### 2.1.0 Barley

Barley (*Hordeum Vulgare L.*) is a versatile and cultivate grain that widely use as the main source of foods, drinks and also as a traditional herb. Barley belongs to a grass family (*Poaceae family*), which play in important role to population as a cereal grain. The scientific term of *Hordeum Vulgare L* has another synonyms name for example *Hordeum aegiceras Nees ex Royle*, *Hordeum distichon L.*, *Hordeum hexastichon L.*, and *Hordeum irregulare Aberg & Wiebe*. In Malaysia, it's known as 'Barli', but in another vernacular name such as al-Sheir (Arabic), Orge (France), Jelai (Indonesia) and Arpa (Turkish). In Malaysia, barley is used widely as beverages either homemade or from foods industries.

Act as one of the first cultivated grains in human civilization and its widely grown in all over the world, mainly in 4 climates countries. It can grow to a height of 1 meter tall. Barley is light white in color, appears as wheat berries, its contains significant amount of maltose, one of the main form of sugar, which act as the sweetener.

Early human civilization, for example in northern part of the continent, ancient

Vikings fermented barley to produce beer and other alcoholic drinks, as a preparation for long months of winter season. Besides that, barley also been used animal foods, and as an ingredients of health care products. It is used in soups and stews, vinegar, and barley bread in different part of the world. Barley grains are commonly made into malts, as form of grain before being used in different form products.

Mention in Holy Quran (Surah Yusuf 12:43) -The king (of Egypt) said: "I do see (in a vision) seven fat kine, whom seven lean ones devour, and seven green ears of corn, and seven (others) withered. O ye chiefs! Expound to me my vision if it be that ye can interpret visions." (Ali, 2011). Few Islamic scholars described 'green ear of corn' as barley (al-Sheir) (Khafagi et al., 2006).

Prophet Muhammad (PBUH) liked barley very much. He used its bread, talbina and sattu (powdered barley).(Al Jauziyah, 2003, Marwat et al., 2012). In Prophetic medicine also used Talbina (powdered form of barley), added with honey and milk to improved heart conditions. It's also as a good diuretic, beneficial to reduce cough and inflammation of stomach. Barley widely used in China as an herbal medication, believed to improve gastrointestinal digestion, abdominal bloatedness and anorexia.

### 2.2.0 Contents of Barley

Chemical composition of barley vary either in covered and naked types, but mainly their contents are protein, amylose, amylopectin and  $\beta$ -glucan (Oscarsson et al., 1996). Goupy et al reported that barley have been quantified and identified with antioxidants components for example polyphenols, carotenoids (lutein and zeaxanthin) and tocopherols ( $\alpha$ ,  $\delta$  and  $\gamma$ ) (Goupy et al., 1999).

Tiwari et al performed a meta-analysis on epidemiologic studies to assess the relation between b-glucan consumption from oats and from barley on blood cholesterol

level, triglyceride/triacylglycerol (TGL/TAG) level, and blood glucose level (BGL) in humans. Consumption of 3 g/d of oat or barley  $\beta$ -glucan is sufficient to decrease blood cholesterol, whereas the effect on blood glucose level (BGL) is still inconclusive, which need further study (Tiwari and Cummins, 2011). Another analysis by Harland et al, on the other hands documented intake of barley and oats reduce blood cholesterol level, and usage of 3g of soluble fiber from oats and barley reduced cholesterol by 0.13mmol/L. About 0.27mmol/L decrement of low density lipoprotein documented with the same analysis using same amount of soluble oats and barley fiber (Harland, 2014).

The molecular features of  $\beta$ -glucans and arabinoxylans are important determinants of their physical properties, such as water solubility, viscosity, and gelation properties as well as of their physiological functions in the gastro-intestinal tract. Barley  $\beta$ -glucans have been associated with lowering plasma cholesterol, reducing glycemic index, and reducing risk of colon cancer (Izydorczyk and Dexter, 2008). Besides that,  $\beta$ -glucans from barley kernels is important in diet as a preventive steps to prevent diabetes mellitus, obesity and stroke (López-Perea et al., 2012).

Lupton et al performed a study in US show a good result in reducing blood cholesterol level (LDL, HDL) after intake of barley bran flour or barley oil in 78 individuals with hypercholesterolemia (Lupton et al., 1994).

### 2.3.0 RATIONALE FOR THE STUDY

This study is aimed to evaluate the potential of *Hordeum Vulgare L.* extract as a chemolytic agent in cholelithiasis and compare it in different extract concentration.

2.0.0 STUDY PROTOCOL

2.1.0 DOCUMENT SUBMITTED FOR ETHICAL APPROVAL

PROPOSED PROJECT OF STUDY

FOR

MASTER OF MEDICINE (GENERAL SURGERY)

THE EFFECT OF BARLEY (HORDEUM VULGARE L.) WATER EXTRACT AS A

CHEMOLYTIC AGENTS IN CHOLELITHIASIS (GALLBLADDER STONE)-

AN INVITRO STUDY

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## 1. INTRODUCTION

Cholelithiasis is the presence of solid concretions in the gallbladder. Gallstones form and retain in the gallbladder but may exit into the bile ducts (choledocholithiasis). As a result, it may cause either cystic or bile duct obstruction symptoms.

Gallstones are hard, pebble-like deposits that form inside the gallbladder. There are various sizes of gallstones, may be as small as a grain of sand or as large as a golf ball.

## 2. LITERATURE REVIEW

Gallstones represent a significant burden on healthcare systems throughout the world. In the UK, around 10-15% of the adult population develop gallstones, with approximately 70,000 cholecystectomies being performed in England annually.

Incidence rates vary worldwide from 0% to 10% reported in Africa to 70% in certain populations such as Pima Indians, reflecting a combination of dietary, environmental and genetic factors (Gallagher and Parks, 2014).

Gallstones remain asymptomatic in up to 80% of carriers, however, it is estimated that within 5 years, 10-20% became symptomatic (Friedman, 1993).

Anatomic distribution of gallstones:

The proportions of gallbladder, common bile duct, and intrahepatic duct stones among total gallstone patients were 64.0%, 21.9% and 14.1%, respectively (Shim, 2000).

Based on their composition, gallstones can be divided into the following types:

1) Cholesterol stones: vary from light yellow to dark green or brown. They must be at least 80% cholesterol by weight and are radiolucent.

2) Pigment stones: black pigment stones are small and dark. They comprise bilirubin and calcium salts that are found in bile and have cholesterol content of less than 20%. Over 50% of these calculi are radiopaque, and are typically seen in patients with cirrhosis or increased red cell destruction. Brown pigment stones arise in the gallbladder and bile ducts in association with biliary infection or bile stasis, and are common in