HISTOLOGICAL EFFECTS OF AQUEOUS EXTRACT FROM *Aquilaria malaccensis* AND *Aquilaria subintegra* TOWARDS ICR MICE

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ABSTRACT

The study was conducted to investigate the acute toxicity of aqueous extract from *Aquilaria malaccensis* (AMAE) and *Aquilaria subintegra* (ASAE) leaves and their histological effects on several organ in ICR mice. Comparison between both extracts was included in order to determine which toxicity is more potent. A total of 156 ICR mice was used in this study consisted of 78 male and 78 female with the age of 12 weeks old. The mice were divided into 13 groups containing 12 mice (6 males and 6 females) in each group. 12 groups orally received one type of extract either AMAE or ASAE with only one type concentration. The concentrations used were 4000, 5000, 6000, 7000, 8000, 9000 mg/kg body weight. One group on the other hand act as the control and treated with distilled water. Extract administration was conducted only once on the first day. Mice body weight and organ weight were compared and analyzed using one-way ANOVA and evaluation of abnormalities in term of appearance and behavioral were conducted. After the 14th day, mice undergone euthanisation approved by UPSI ethics committee (reference number: 2021-0002-03) in order to conduct histological analysis on their organs which were liver, kidney and stomach. Evaluation on their cellular structure were also conducted. Results showed significant differences in both AMAE and ASAE male mice body weight. AMAE also showed higher significant difference when compared with ASAE using t-test. On top of that, all treated mice displayed drowsiness and their fur seems to be affected, primarily AMAE and ASAE female mice face fur which appeared to be balding. Faeces condition in some AMAE treatment groups also showed abnormalities. In terms of histological evaluation, mice liver, kidney and stomach tissues were also affected. Some liver's portal vein and sinusoids in AMAE treatment were filled with blood. All treated mice glomerulus expanded while some glomeruli burst in AMAE treatment. All treated mice mucosa length reduced and some gastric glands in AMAE treatment showed abnormal changes. Therefore, it can be stated that both *Aquilaria malaccensis* and *Aquilaria subintegra* is toxic due to the adverse effects it caused on mice appearance, behaviour and organ tissues. Furthermore, *Aquilaria malaccensis* showed more adverse reactions compared to *Aquilaria subintegra* indicating higher toxicity. This implicates that both *Aquilaria malaccensis* and *Aquilaria subintegra* can be toxic and unsafe if being use in a higher concentration.

KESAN HISTOLOGI EKSTRAK AKUEUS *Aquilaria malaccensis* DAN *Aquilaria subintegra* TERHADAP MENCIT ICR

ABSTRAK

Kajian ini dijalankan untuk menyiasat ketoksikan ekstrak akueus daripada daun *Aquilaria malaccensis* (AMAE) dan *Aquilaria subintegra* (ASAE) dan kesan histologi pada beberapa organ dalam mencit ICR. Perbandingan antara kedua-dua ekstrak juga dimasukkan untuk membandingkan kekuatan ketoksikan. Sebanyak 156 mencit ICR digunakan dalam kajian ini yang terdiri daripada 78 jantan dan 78 betina berumur 12 minggu. Mencit tersebut dibahagikan kepada 13 kumpulan yang mengandungi 12 tikus (6 jantan dan 6 betina) dalam setiap kumpulan. 12 kumpulan menerima satu jenis ekstrak sama ada AMAE atau ASAE dengan hanya satu jenis kepekatan. Kepekatan tersebut ialah 4000, 5000, 6000, 7000, 8000, 9000 mg/kg berat badan. Terdapat satu kumpulan yang menerima air suling dan dijadikan sebagai kumpulan kawalan. Pemberian ekstrak dijalankan hanya sekali pada hari pertama. Perbandingan berat badan dan berat organ telah dilakukan dengan menggunakan ANOVA satu hala dan segala kelainan dari segi fizikal dan tingkah laku juga telah dilakukan. Selepas hari ke-14, mencit-mencit tersebut menjalani eutanisasi yang telah diluluskan oleh jawatankuasa etika UPSI (nombor rujukan: 2021-0002-03) supaya dapat menjalankan analisis histologi pada organ mereka iaitu hati, buah pinggang dan perut. Penilaian struktur selular juga telah dilakukan untuk organ-organ tersebut. Keputusan menunjukkan perbezaan yang ketara dalam kedua-dua berat badan mencit jantan AMAE dan ASAE. AMAE juga telah menunjukkan perbezaan signifikan yang lebih tinggi ketika dibandingkan bersama ASAE apabila menggunakan ujian-t. Selain itu, semua mencit yang dirawat menunjukkan rasa mengantuk dan bulu mereka terjejas, terutamanya pada AMAE dan ASAE mencit betina yang kelihatan botak di muka. Keadaan najis dalam beberapa kumpulan rawatan AMAE juga menunjukkan kelainan. Dari segi penilaian histologi, tisu hati, buah pinggang dan perut turut terjejas. Beberapa vena portal hati dan sinusoid dalam rawatan AMAE dipenuhi dengan darah. Semua glomerulus mencit yang telah diberi ekstrak mengembang manakala beberapa glomeruli pecah dalam rawatan AMAE. Kepanjangan mukosa untuk semua mencit berkurang dan beberapa kelenjar gastrik dalam rawatan AMAE menunjukkan perubahan yang tidak normal. Oleh itu, boleh dinyatakan bahawa kedua-dua *Aquilaria malaccensis* dan *Aquilaria subintegra* adalah toksik disebabkan oleh kehadiran kesan buruk pada penampilan, tingkah laku dan tisu organ mencit. Tambahan pula, *Aquilaria malaccensis* menunjukkan lebih banyak tindak balas negatif berbanding *Aquilaria subintegra* menunjukkan ketoksikan yang lebih tinggi. Ini membuktikan bahawa kedua-dua *Aquilaria malaccensis* dan *Aquilaria subintegra* boleh menjadi toksik jika digunakan dalam kepekatan yang lebih tinggi.

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LIST OF ABBREVIATION

AMAE	*Aquilaria malaccensis* Aqueous Extract
ANOVA	Analysis of variance
ASAE	*Aquilaria subintegra* Aqueous Extract
BC	Bowman's Capsule
BS	Bowman's Space
b.w.	Body weight
DCT	Distal Convoluted Tubule
G	Glomerulus
GG	Gastric Glands
HA	Hepatic Artery
ICR	Institute of Cancer Research
LD50	Lethal Dose 50
MD	Macula Densa
OECD	Organization of Economic Cooperation and Development
PCT	Proximal Convoluted Tubule
PV	Portal Vein

ROW	Relative Organ Weight
S	Sinusoids
s.d.	Standard Deviation
spp.	Species
SPSS	Statistical Packages for the Social Science
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

1.1 Background Study

Advancement in botany research has made society understand more about plant life's pros and cons. As the plant world's mysteries become uncovered, people understand its capabilities and implement its uses in their everyday lives. There are many examples of plant uses in human life. One of them is using it as an alternative medicine for treating any disease. These days, the term “Alternative Medicine” has become very common in western culture; it focuses on using plants for medicinal purposes (Bassam Abdul Rasool Hassan, 2012). In other words, alternative medicine is a type of treatment where plants that contain medicinal properties are used to treat any disease or sickness. This statement has reduced the usage of

conventional medicine in society. Conventional medicine can be described as medical knowledge practised by most medical officers in today's western culture (Dalen, 1998). Most people commonly use this kind of medicinal treatment in these modern times. Even though modern medicines are available in many countries, herbal medicines have often maintained popularity for historical and cultural reasons. That being the case, more and more people have started to be involved in this kind of medicinal treatment, thus increasing medicinal plants popularity.

Remember that this type of medical practice has been around since ancient times. Many ancient civilizations such as Egypt, China and even Greece treat their illness by using plants that contain beneficial medicinal properties. In ancient Egypt, raw garlic was routinely given to asthmatics and those suffering from bronchial-pulmonary complaints (El-Soud, 2010). Meanwhile, in ancient China, ginseng decoctions were the primary source for treating haemorrhages, ulcers and sores. It is even used to treat a person who catches a common cold (Jingcheng Dong, 2013). In ancient Greek times, the famous Greek herbalist Dioscorides stated that fennel was an excellent remedy for stomach and bladder troubles (Kerr, 2015). This situation shows the potential medicinal plants and proves the impact they had made in ancient civilization medicine.

Plants that possess medicinal properties are usually called medicinal plants. Bassam Abdul mentioned that these medicinal plants are considered a rich resource of ingredients used in drug development and synthesis (Bassam Abdul Rasool Hassan, 2012). To put it in Layman's terms, medicinal plants must have a high number of suitable contents and ingredients that can be used to develop a proper medicine for a specific disease. For example,

one research conducted mentioned antioxidants called vitamin A, C, E, and carotenoids in Aloe Vera leaf that help treat irritated and inflamed skin (Singh, 2015). This statement further strengthens the fact that medicinal plants must contain many suitable substances to be characterized as medicinal plants.

As mentioned before, medicinal plant is essential in medicinal practice. They have contributed to developing cures for many types of diseases. Not only that, they still play a significant role in the advancement of medicine on developing cures for currently unsolved illnesses and possible future illnesses. It is also said that medicinal plants have been used for thousands of years to flavour and conserve food, treat health disorders, and prevent diseases, including epidemics (Singh, 2015). Research conducted by Shakya (2016) concluded that the medical system would be dependent on herbal medicine in the next few decades due to a high percentage (80%) of the world population relying mainly on traditional medicine for primary healthcare. This situation further proves the importance of medicinal plants to our civilization and give to our future generations.

Medicinal plants have been playing an essential role in developing human culture. As a source of medicine, medicinal plants have always been at the forefront of virtually all cultures of civilizations (Dar, Shahnawaz, & Qazi, 2017). With its history dating back to ancient Greece and Egypt, many types of plants are used for medicinal uses. As mentioned before, ancient civilisations used plants such as fennel, ginseng or garlic as their means of medicine. As times advances on, medicinal plants offer different kinds of medicinal treatment in these modern times. For example, back then, fennel was used to treat stomach problems by soothing the digestive systems, but now it is also said that it can help in

increasing milk production in nursing mothers due to its estrogenic activity present in its seeds (Wesam Kooti, Maryam Moradi, Sara Ali-Akbari, Majid Asadi-Samani, & Damoon Ashtary-Larky, 2015). Besides, ginseng was used to heal haemorrhages, ulcers, and sores, but now much research has stated that it can cure central nervous system (CNS) disorder and neurodegenerative disorder. Furthermore, garlic also possesses multiple medicinal values besides having the ability to treat asthma, and it can also treat a digestive disorder by stimulating peristalsis or movement of intestines (Balch, 2010) and can even break up cholesterol in blood vessels, therefore, preventing any hardening of arteries which lead to high blood pressure and heart attack (Duke, Telatnik, & Duke, 2007).

Agarwood (also known as gaharu or aloeswood) is another famous example of a medicinal plant due to its unique medicinal properties. Agarwood comes from the *Aquilaria* species plant in the Thymelaceaceae family. The wood formed when the heartwood was infected with mould. It will form a fragrant dark resinous wood that can also be used in incense and perfumes. Besides having those capabilities of medicinal property, it has been used for religious purposes as well. For example, the wood will be made into sculptures, beads, and boxes used in religious ceremonies (Nor Azah et al., 2008). It also involves aromatic preparation due to its fragrant characteristic. The agarwood was involved in incense production, and the incense was used in homes, religious ceremonies, rituals and meditation and it is also said that many European perfumers highly prized the incense in the mid-90s (Barden, Awang, Mulliken, & Song, 2000). This situation shows what agarwood is capable of and how important it is to society besides being used to treat diseases. *Aquilaria* planters say that agarwood production can be a green ‘gold mine’ in the future (Mamat, Yacob, Fui, & Rdam, 2010).

Even with its various uses, agarwood still showed its potential in medicinal practice. Countless research has been conducted in finding hidden agarwood potential in the medicinal aspect. One research stated that the leaves in agarwood could reduce one's blood sugar level, thus preventing one from becoming diabetic (Liyana Zulkifle, Adila Omar, Tajuddin, & Shaari, 2013). Another research mentioned the same thing. It is stated that agarwood can increase glucose uptake up to 20% in diabetic rats, thus helping the rats lower their blood sugar level (Said & Kamaluddin, 2016). Both of these research further proven the potential of agarwood in treating diabetic patients. Besides, another research mention agarwood anti-cancer properties. Based on research conducted in 2013, anti-cancer activity was observed when a product from agarwood resin distillate was treated on breast cancer cells. It also shows no effect when treated on normal cells (Phirdaous Abbas et al., 2013). Another research also conducted a similar experiment but used agarwood oil instead of agarwood resin, and it concluded that agarwood possessed anti-cancer properties (Hashim, Phirdaous, & Azura, 2014). This situation proved the potential of agarwood in medicinal treatment.

Agarwood's potential in treating illness still wavers due to its limit when used as a medicine. Like any other medicine, overuse may lead to various health problems resulting in fatal injuries. Even in the hands of a professional pharmacist or herbalist, any kind of medicine may be fatal if it is not used with caution, even if it is conventional medicine or medicinal plants. This situation is mainly related to the toxicity it may contain. It is said by the father of modern toxicology, Philippus Theophrastus Aureolus Bombastus von Hohenheim or also known as Paracelsus, that "All substances are poisons; there is none that is not a poison. The right dose differentiates a poison and a remedy". This situation shows what medicinal plants can become if used with no precaution. Therefore, every treatment

involving medicinal plants should be conducted with enough information and evidence from past research or professionals to avoid harm.

The usage of herbal medicine has increased significantly over the past two decades, but information regarding medicinal plant data is still insufficient due to the diversity of medicinal plants and herbal medicines scattered throughout the globe (World Health Organization, 1999). That is why more research in this field should be invested so that enough information regarding the usage, toxicity and safety of herbal medicine or medicinal plant can be obtained for future use. There are research that focuses on an overview of toxicity in medicinal plants. It tells about various kinds of medicinal plants and the dangers it may come if it is used without any precaution. For example, ginseng has been popular in Chinese culture, and it is the fourth most widely used medicinal plant in its culture. This plant can treat hypertension, diabetes, and depression, but lately, ginseng has been stated to cause mastalgia and make blood thinner, making blood clotting much more difficult (Becker, 1996). Another example of a medicinal plant is the aloe vera, and it is said to be the seventh most widely used herb for centuries. It is mainly used for treating minor bruises, but recent studies have stated that prolonged usage of this plant in latex form may lead to potassium deficiency due to the presence of anthraquinone glycosides (George, 2011). That is why more research should be done in this field so that the dangers can be avoided and precaution steps can be appropriately introduced.

When it comes to the toxicity present in agarwood, there has not been much research conducted in this area. Only a couple has been done, but it still lacks the information needed to ensure agarwood safety when using it in any medicinal practice. One research stated that

hazardous content is present in *Aquilaria* species (agarwood tree) leaf extracts which are phenolic compounds that may be dangerous to humans (Aimi Zafirah et al., 2018). Another research mentioned the same thing, but the difference is that it uses rats as its subject. It says *Aquilaria* leaves extract can become toxic to rats liver and kidneys if taken slightly higher than the normal amount (Redzuan Razak et al., 2018). Based on these two research, *Aquilaria* species leaves can become toxic if used in a higher dose. That is why it is essential to know about the toxicity of medicinal plants so that any harm or injuries can be avoided. Therefore, precautionary steps must be taken when considering using it in medicine. Also, more information about the toxicity and safety of using agarwood (*Aquilaria* species) in medicinal practice can be obtained if more research regarding this topic is conducted.

With half a million plants around the globe, medicinal plants indeed have a fantastic future ahead. Sadly, a significant portion of them still has not been determined whether they have suitable medicinal properties in medicinal practice. Therefore, research regarding this field should be conducted because it may hold the possibility to cure current unresolved illnesses and future illnesses that may come. All information regarding the medicinal plant should be considered when conducting the research. The properties, chemical contents, toxicity and possible side effects should be recorded. Without it, people will never understand the dangers it may hold and indeed, they will use it without any considerations and limitations. This situation may lead to harmful results and cause problems to the user and society. That is why research in this field should be conducted so that people will know its pros and cons.

1.2 Problem Statement

Currently, research in medicinal plants has grown significantly high in the last few decades (World Health Organization, 1999). As technology becomes more advance and convenient, people start to initiate proper experiments on various types of plants to investigate their potential in medicinal practice. Some of the results obtained from those research were decent enough to identify medicinal properties in certain plants, but they still lack the information needed to safely initiate them in any medicinal practice due to the lack of information on toxicity level. One example of a frequently used plant in medicinal practice is the St. John's wort plant. This plant is commonly used throughout Europe and America to treat patients who suffer from depression or even dietary supplements. Research has stated that St. John's wort cause can cause harmful side effects if taken with a drug called Indinavir for infection treatment. It can reduce blood concentration at a high rate (International Agency for Research on Cancer, 2002). Without this information, the usage of this plant may lead to various kinds of problems for its users and society. With the help from the research above, precaution steps were prepared, and the information obtained about the dangers of the plant can help avoid any future problems that may come.

Another example of a medicinal plant with little information regarding the toxicity and its dangers is the Glycyrrhiza glabra plant, also known as liquorice. This plant has played a role in medicinal practice for over 3000 years from a western perspective. Its root can treat various types of ailments such as stomach and duodenal ulcers, asthma, and arthritis (George, 2011). However, new information regarding this plant healing properties has surfaced in a recent study that shows the side effects that may come from the presence of glycyrrhizinic

acid. This active ingredient can cause sodium and water retention and potassium depletion to its user when it is taken in large quantities. It can be dangerous to people who already suffer from blood pressure or heart diseases. Besides, hypertension and oedema were also potential side effects if the plant is used excessively (George, 2011). Even though the liquorice plant has been used for many years, there is still lots of new information concerning the positive and negative effects that researchers of this single plant have discovered. It takes roughly 3000 years for researchers to understand this plant's toxicity fully and include a safety regulation on using it. That shows how unaware society is regarding the dangers of practicing alternative medicine using medicinal plants because it took them such a long time to notice it.

Agarwood is just the same when it comes to being used as a medicine among people today. As mentioned before, agarwood comes from the *Aquilaria* species, and it can be made from many types of its species. Such species are the *Aquilaria malaccensis*, *Aquilaria subintegra*, *Aquilaria crassna* and *Aquilaria sinensis* and all of them has many medicinal uses that have given lots of benefits when it is being used. It has become quite a famous plant in recent times due to the credit given by its users today. Even with its outstanding reviews, surely enough, there are still problems that come up when using the plant without any precaution whatsoever. A study from Switzerland stated that cultivated and natural agarwood incense smoke from *Aquilaria malaccensis* caused adverse health effects and showed cytotoxic (toxic to living cells) and mutagenic (ability to cause permanent change to organism's gene) properties when tested with salmonella reversion test (Zhou, Yang, & Wang, 2016). These characteristics are said to be harmful and very dangerous to humans as they can cause serious health problems and fatalities not only to the user but also to their future children. With the chances to cause health problems still present, agarwood's position

as a medicinal plant starts to falter even if it has a low probability of causing any health problems to its users.

The situation for *Aquilaria subintegra* is somewhat different when compared to *Aquilaria malaccensis*. The plant has some beneficial properties because it has also been used to treat some illnesses. Research and statements about the harmful aspect of this plant were challenging to find, and a lot of the research found were about its potential as a cure for Alzheimer's disease. One research stated that Acetylcholinesterase inhibitor from leaves crude extracts of *Aquilaria subintegra* was used to treat Alzheimer's disease on male and female mice, and it has been concluded that due to the presence of kaempferol inside the leaves and stem, the process was a success (Bahrani, Mohamad, Paydar, & Rothan, 2014). Even with its ability to treat Alzheimer's Disease, the researcher did not mention the toxicity of the plant and the specific amount required for it to be safely used in any authorised medicinal practice. Therefore, much research still needs to be conducted to ensure the toxicity limit of *Aquilaria subintegra* can be determined in order for it to be safely used with the prescribed amount and to avoid any health hazard that may come to its users soon.

There are many ways in identifying toxicities in either drug, medicine or medicinal plants. One of the conventional ways is by studying its effects through histological perspective within test subjects through in vivo or in vitro study. This method is usually referred to as toxicologic pathology, where potentially noxious substances or chemicals such as drugs, medicine or even medicinal plants undergo tests to identify their adverse effects on the body (Haschek, Rousseaux, & Wallig, 2010). Usually, tissues samples from organs such as kidneys or liver are observed, and the toxicity is determined histologically. Any changes

within the morphological tissue structures indicate that the substance administered may be toxic. If there are significant changes, the substances are highly toxic, but they are considered low toxic if few changes. It is one of the efficient ways to detect any harmful effects at a microscopic level, and it has been considered an essential part of toxicity studies. Preferably, when assessing toxicologic potency in certain substances, it should factor in the pathologic lesion in the tested animal, and complete examination of each cell of the animal sample is required (Bregman, Adler, Morton, Regan, & Yano, 2003). One example that related toxicity with histology was a research conducted on female Swiss albino mice where 2000 mg/kg of aqueous leaf extract of *Eclipta Alba* was administered orally to determine the acute toxicity of the plants, and results shows that it had caused alterations in the mice liver (Singh, Sinha, & Singh, 2013). By reviewing the research, the toxicity of any substances can be determined histologically due to the presence of acute toxicity role in the toxicity study.

Finding the toxicity present in any substances such as medicines, drugs, or even medicinal plants will usually require information in specific fields that can help label it as a potentially dangerous and poisonous. The majority of toxicity research conducted will include several tests that will include the information leading to the acute and sub-acute toxicity of the substances. Both toxicities are similar in some areas, but certain aspects make them different in every way. Determining the adverse effects of any drug or medicine when it is taken in single or multiple doses by any mammalian species within 24 hours is considered an acute toxicity study (Faqi, 2017), while sub-acute toxicity study is the evaluation of drugs and medicine possibility to cause any adverse effects to the subject following its treatment ranging from 2 to 4 weeks (Colerangle, 2017). Both can provide the data needed to determine the level and dangers of any toxicity in the material. This study will provide the information only for the acute toxicity of the plants and eventually help

determine its toxicity making it easier for future researchers to conduct a similar study given that the information has already existed.

One way to understand acute and sub-acute toxicity properly is by studying their role in past research. Both items have provided important information regarding the toxicity of newly discovered medicinal plants, thus enabling society to take precautionary steps in manipulating plants properties. For example, a toxicological study was conducted on *Ocimum sanctum* Linn leaves effects haematological, biochemical, and histopathological perspectives (Gautam & Goel, 2014). This research has provided the toxicological evidence of the plant by evaluating the acute and sub-acute effects on mice while using different extracts concentrations through oral administration. It was concluded that no toxicities were present due to the absence of any adverse effects observed. Thus, increasing the probability of safe usage among humans. Another research with a similar methodology was also conducted that focused on the acute and sub-acute toxicity of *Acrocomia aculeata* in rats (Traesel et al., 2014). The results are similar to the previously mentioned research, where no toxicities were present due to no adverse effects shown by the rats. By looking at both studies, it can be justified that acute and sub-acute toxicity are helpful in any toxicological research because it is one of the quickest ways to evaluate the toxicities present in any medicinal substances (Kuete, 2014), thus helping societies quickly identify its harmful or safe potency for usage. Even though there are no acute and sub-acute toxicities detected in those research, further studies are still required in the future as the toxicity of the plants may be present when using different extract concentrations. If the toxicity of the agarwood is discovered, it can help in future studies as well.

The most common evaluation method for acute and sub-acute toxicity studies usually involves observing the adverse effect on treated mice. Constantly observing clinical signs such as body weight changes can assist in understanding the adverse effects of substances (Chapman et al., 2013). This statement indicates that changes in body weight can be a sign of adverse effects. Organ weight is also considered essential in animals' pathological and physiological studies (Ogbuehi, Ebong, & Obianime, 2015). These two statements are often related because changes in body weight and relative organ weight are considered crucial in distinguishing the possibility of organ damages caused by toxic substances (Abid & Mahmood, 2018). Besides weight evaluation, toxic reactions resulting from drugs or substances in vital organs are revealed by observing the clinical signs, symptoms and abnormalities it caused (Subramanion Jothy et al., 2011). Abnormality in behaviour and physical changes such as tremors, convulsions, salivation, diarrhoea, lethargy, sleep, and coma are the usual indicators in most toxicological studies (Subramanian, Sankaramourthy, & Gunasekaran, 2018). To simply put it, adverse effects usually involve changes in their body weight, organ weight, physical and behavioural abnormality and tissue damage.

One of the primary objectives in performing acute toxicity tests is to determine the substances' Lethal Dose 50 (LD50). The basic understanding for LD50 is quite simple as it is the statistical estimation of a value that represents the number of toxicants in mg/kg of body weight that kills 50% of the test animal population, as mentioned by the World Health Organization (OMS, 2009). To further simplify the concept, the toxicity of a substance is determined and calculated when 50% of the tested animal population, usually mice, died from the administrated substances, such as drugs, medicines, or even medicinal plants. LD50 is usually related to acute and sub-acute toxicity studies as all play significant roles in any toxicological research. Usually, when acute or sub-acute toxicity study is conducted, LD50

is used to determine the toxicity of the substances by measuring the concentration used. One research about the toxicity of *Allium sativum* on rabbits discussed the acute toxicity and LD50 as it was important for the researcher to determine the toxicity of the garlic (Mikail, 2018). Another research also discussed acute toxicity, sub-acute toxicity, and LD50 when Wistar rats were exposed to *Crataegus Aronia Syn*. *Azarolus* (Shatoor, 2011). This situation shows how vital LD50 is in any toxicological research, as it has been labelled as one of the keys to defining any substance's toxicity. Simply put, it was mainly designed for the biological normalisation of hazardous drugs. (Kuete, 2014).

Although multiple facts from past research have been given to society, many people still use medicinal plants without knowing the consequences that may come. People still think that using medicinal plants rather than using conventional medicine is somewhat safer and healthier due to the natural properties present within the plant. This statement can be confirmed by observing people's perspectives on using medicinal plants. Malaysian cancer patients outlook toward alternative medicine is somewhat on a positive side because it is common among them to practice complementary and alternative medicine or known as CAM, and among those practices is the use of medicinal plants in treating their illness (Maryam Farooqui et al., 2016). In their mind, CAM can improve their quality and life and help them cope with emotional distress. Moreover, it is found that Malaysian women tend to use herbal medicine to help ease their pregnancy process, and a majority of them use it without any supervision (Law Kim Sooi & Soon Lean Keng, 2013). This type of practice should not be performed by anyone, especially by pregnant women, as it is hazardous and cause unknown adverse health effects that could be fatal. If steps are not taken to prevent unsupervised behaviour, a negative impact will mark today’s society.

To further strengthen the statement mentioned, look at the incidents over the past few decades made by people who use medicinal plants without any precaution and awareness of its dangers. There have been many incidents due to the misuse of medicinal plants, and some of them are pretty surprising. For example, a man took 5.4 mg yohimbine three times daily in 4 days to treat his erectile dysfunction (Sandler & Aronson, 1993). He had stopped taking medicine because it had made the problem much worse than before, and he also became increasingly ill. Not only that, he was then diagnosed with renal failure and a lupus-like syndrome. Ultimately, he made a miraculous recovery. This situation happened due to the man lack of knowledge and awareness. This problem can easily be avoided if the man seeks out professional advice from a medical officer. Besides, a report by Food and Drug Administration (FDA) has confirmed that 15 deaths were reported in 1990 on the accounted of using an ephedra-based drug, making it necessary to conduct a legal restraint on the dose and duration of the drug. At first, this was thought to be a wake-up call to society on the dangers of medicinal plants. However, the number of deaths increased to roughly 20 to 30 deaths. On top of that, there were 800 reports of adverse reactions mentioned by its consumers in 2003 (Inamul Haq, 2004). This situation happened due to the social misconduct performed by ignorant society that has led to this kind of problem.

Due to insufficient data regarding agarwood toxicity, this research will mainly focus on uncovering its anonymous data. Agarwood has been chosen simply due to its increasing popularity and hidden medicinal potential. Many researchers now are researching agarwood, hoping to uncover its medicinal properties. As mentioned before, *Aquilaria subintergra,* which is an agarwood producing tree, has the potential to cure Alzheimer's disease (Bahrani et al., 2014), and *Aquilaria malaccensis* has the potential to cure breast cancer (Phirdaous Abbas, Yumi Zuhanis Has-Yun Hashim, & Hamzah Mohd Salleh, 2018) and increase sperm

motility among male mice (Nur Hidayat Che Musa et al., 2019). This situation increased its popularity, and people started to use agarwood as their alternative medicinal treatment. That is why this research will use agarwood as a sample. This research will also use histology as its evaluation method to ensure detailed results are obtained. Past research has mentioned that histological evaluation has shown excellent results when studying toxicity. For example, (Redzuan Razak et al., 2018) have used histological evaluation when evaluating the toxicity of agarwood toxicity, and the results he obtained are very detailed and quite understandable.

As the popularity of *Aquilaria* increases, people start to use it in their daily lives, making it crucial to acquire detailed information regarding its dangers. That is why this research will focus on finding the evidence needed to ensure safe usage of the *Aquilaria* plant. This research provides crucial information concerning the *Aquilaria* plant, specifically *Aquilaria malaccensis* and *Aquilaria subintegra* toxicities. As mentioned before, information regarding both *Aquilaria* plants toxicities is quite scarce. The available ones only discuss the plant's toxicities at a specific limitation. For instance, Aimi Zafirah (2018) mentioned that *Aquilaria malaccensis* toxicities can be seen if used at 4537 mg/kg or higher in aqueous extract form. This statement was confirmed when the researcher used the extract on mice (Aimi Zafirah et al., 2018). Furthermore, it has been stated by Nur Hidayat Che Musa that drowsiness and sedative effect was seen in mice after it was treated with *Aquilaria malaccensis* and *Aquilaria subintegra* (Nur Hidayat Che Musa et al., 2018, 2019). Therefore, it is essential to investigate the toxicities of *Aquilaria malaccensis* and *Aquilaria subintegra* because of its limited information. If this information is obtained, the usage of agarwood products produced from the *Aquilaria* tree improves. On top of that, uncovering its toxicities can surely help improve the perception and usage of *Aquilaria* plants not only to society but also throughout the world.

Harmful situations can easily be avoided if enough knowledge and information about the dangers of medicinal plants and their proper use are given to society. Research regarding this issue should frequently be conducted to initiate the given statement. It is to ensure the information concerning this problem are true and proven with enough evidence to support it. Surely it will help society understands them better and become more aware of the pros and cons of medicinal plants, making them used with enough care and precaution to give more advantages rather than disadvantages and prevent any harm that may come to them.

1.3 Research Objectives

This research aims to investigate the histological effects of aqueous extract from two agarwood species on severed organs in ICR mice. Specifically, the study objectives are:

1. To determine the acute toxicity and lethal dose 50 (LD50) of both agarwood extracts.
2. To examine the histological effects of agarwood extract towards the kidney, liver and stomach of ICR mice.
3. To compare the distinction between two different species of agarwood leaves extracts in terms of acute toxicity level and histological changes on kidney, liver and stomach of ICR mice.

1.4 Research Questions

This study will be carried out based on the questions below:

1. What are the acute toxicity and lethal dose 50 (LD50) of both agarwood extracts?
2. Does the agarwood extract have any histological effect on the liver, kidney and stomach of ICR mice?
3. Do both species have any distinction in terms of acute toxicity level and histological changes on kidney and liver of ICR mice?

1.5 Research Significance

A 5000 years old Sumerian clay slab from Nagpur is said to be the oldest written proof of medicinal plants used to prepare drugs in early human civilization. It contained 250 types of plant that was used to prepare drugs in 12 recipes (Kelly, 2009). As time goes on, humans start to understand plants' capabilities and discover their medicinal properties. Now, as we arrived at a more advanced time in human civilization, the quality and quantity of medicinal plants have grown exponentially due to the discoveries made. With half a million medicinal plants scattered around the globe, its future is somewhat promising. Most of their medical activities still have not been investigated. Studying their medical properties could decide the future of medical treatment, thus advancing the future of human medicine (Singh, 2015). Medicinal plant has helped improve society's way of life by treating their illness, curing fatal diseases, and prolonging their quality of life. This situation shows how important medicinal

plants are for humankind development as it plays an essential role since the dawn of the first civilization, and it still holds much potential for future medicine as well.

Even though medicinal plants have helped improve the quality of life for humankind, some have caused problems rather than solved them. As mentioned before, some cases have been reported on the adverse effects caused by medicinal plants, and some of those have served humankind as early as first Egypt, Greek and Chinese civilisations. Due to the problems that arise from those plants, much research has been conducted to ensure the problems that it may give can be prevented by looking at its chemical content and determining its toxicity. Those research have given the scientific community a great deal of new information regarding the use of medicinal plants more appropriately and safely, making them pave the way for a better medicinal practice. Although many researchers have lent a hand in solving this issue, more data is needed to be gathered to strengthen further the security of using medicinal plants as there are nearly half a million types spread throughout the world. Advancement in research methodology that consists of better screening methods of plants and other natural sources can lead to the development and characterization of new natural drugs (Shakya, 2016).

Medicinal plants may hold the key for future drug development, and studying them should be necessary. An example of a medicinal plant that requires proper research studies is the *Aquilaria* species. It is widely known for its aromatic scent when crafted into agarwood. Many proposed that the plant contained a few medicinal properties that can help many types of incurable diseases such as Alzheimer's and even cancers (Bahrani et al., 2014; Phirdaous Abbas et al., 2018). Nevertheless, humankind still needs to conduct more research

regarding this plant's medicinal properties because there is not enough information to be used appropriately in any medicinal practice. For instance, with the potential of *Aquilaria subintegra* as a cure for Alzheimer's Disease, a more detailed mechanistic investigation is needed to detect any side effects that may come when using it for treatment (Bahrani et al., 2014). Another example that similarly discusses the same issue is the *Aquilaria crassna* and the possibility of killing the cancer cell. A detailed study on the crude extract of *Aquilaria crassna* could introduce the discovery of promising chemotherapeutic agents to cure digestive system cancer cells (Saad Sabbar Dahham et al., 2014). This research mentions the plant's potential as a cure for the complicated disease, but it also lacks information on its toxicity, additional chemical content, concentration, and the amount required to be used in any medicinal practice.

Therefore, this study is essential for future medicine development because it can contribute to the advancement of Malaysia medical practice and other countries. By reviewing past research, most of them have stated that the lack of information on medicinal plant can lead to harmful consequences to its user and there are prove of that happened (Aimi Zafirah et al., 2018). Due to the *Aquilaria* plant, the primary production is in South East Asia; many people in those countries use this plant as their medicine and essential oils (Fauzi Elias, Husni Ibrahim, & Wan Rusmawati Wan Mahamod, 2017). That is why this research is essential not only to the scientific community but also to society as well as they will understand better the dangers of *Aquilaria* species plants and take precautions when using them for any treatment. On top of that, products extracted from the *Aquilaria* plants can also be manufactured safely as the information gained here will surely helped in understanding the limitation of the plant. Needless to say, this study can give a lot more advantages to many parties as it can lead to a healthier society.

The methodology for this study is simple, but its results can offer various benefits to the medical field. Moreover, it can also help form the proper protocol of using *Aquilaria* as a fully fledge medicine. This research will eventually allow society to use *Aquilaria* with enough safety and confidence in their daily lives. This study will also encourage the scientific community to conduct more studies regarding the toxicity of various medicinal plants, therefore clearing up the haze on the mysteries of medicinal plants. Therefore, it hastened the advancement of future medicine and drug development, making diseases much easier to fend off. Death rates around the globe can also decrease due to humankind becoming much healthier than before, allowing for better community development for future generations. Extensive research is critically important in controlling the quality of raw drugs and their formulation to help further improve modern medicine from its source as a medicinal plant, and a practical plan should also be developed to preserve those resources. (Fatemeh Jamshidi-Kia, Zahra Lorigooini, & Hossein Amini-Khoei, 2018).

1.6 Scope and Limitation

Only two types of Aquilaria species were used in this study: *Aquilaria malaccensis* and *Aquilaria subintegra*. Both were obtained from Al-Hilmi plantation in Behrang in Perak, Malaysia. The histological effect was exclusive on ICR mice liver, kidney and stomach due to the organ's capabilities on detecting toxic substances. On top of that, the doses used in this research were administered accordingly to mice body weight. This research can help in ensuring safe usage for human application. Results from this study are only credible for all

materials, situations and parameters mentioned above. Therefore, it does not mean that all *Aquilaria* species in Malaysia and other parts of the world will have the same properties.

1.7 Research Design

This study focused on the toxicity level of *Aquilaria malaccensis* and *Aquilaria subintegra* plants. The toxicity level will be verified by studying the effects it caused on ICR mice's kidney, liver and stomach organs from a histological perspective. It can be determined by observing the treated sample at a microscopic level, detecting changes, and comparing it with the control sample. Any differences in the structures and organelles between both samples shall be recorded. The procedures for this study will mainly focus on three sections that are the treatment process, the sectioning process and the staining process. All of those procedures were crafted based on past research methodologies from (Nur Hidayat Che Musa et al., 2018) and (Slaoui & Fiette, 2011) with some slight modifications so that good and quality samples can be obtained.

The first procedure is the treatment process where the ICR mice will be treated with an aqueous extract of *Aquilaria malaccensis,* and *Aquilaria subintegra* leaves acquired from Agarwood Al-Hilmi plantation in Behrang, Perak, Malaysia. The aqueous extract will be obtained by maceration process, and the mice have been treated with this extract orally with different concentrations. After roughly two weeks, the mice will undergo dissection for the dissection of liver and kidney organs. The organs were then paraffinized with wax to undergo a sectioning process using a rotary microtome. Using this method, a quality sectioned sample

for observation will be produced. After obtaining the sectioned sample, it will be stained with Hematoxylin and Eosin (H&E). The stain will help detect any changes in the sample by observing it under a 10X magnification microscope. By comparing the control sample with different concentrations of the treated sample from a cellular perspective, the toxicity level of *Aquilaria malaccensis* and *Aquilaria subintegra* can be determined.

The study will give a better understanding of the toxicity of agarwood as it has the potential to give good quality results based on the methodology crafted. Besides, various types of medicinal plants toxicity can also be determined if this study is used as their reference for their methodology.