HISTOLOGICAL EFFECT OF HIGH DOSE *Aquilaria subintegra* LEAVES AQUEOUS EXTRACT ON SEVERAL ORGANS IN ICR MICE

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Abstract. The study was conducted to investigate the histological effects of high dose *Aquilaria subintegra* aqueous extract (ASAE) leaves on several organs in ICR mice. The purpose of this study is to determine the toxicity limit of ASAE. An acute toxicity study of ASAE was studied. 42 male and 42 female mice aged 12 weeks old were used. The mice were divided into seven groups, and each group contained twelve mice (six males and six females). All mice orally received the ASAE at a specific concentration of 4000, 5000, 6000, 7000, 8000, 9000 mg/kg body weight, and only one group was used as control which was treated with normal saline. The extract was administered only once on the first day. Male groups 4000, 5000, 7000, 8000 and 9000 mg/kg showed a significant difference in their body weight. There were also some signs of abnormality in mice motility and fur growth in all treated mice. Female mice face fur group 4000, 5000, 7000, and 9000 mg/kg appeared to be balding, and drowsiness was detected in all treated mice. Liver, kidney and stomach tissues were also affected. Liver's portal vein in male group 6000 mg/kg and female group 4000 mg/kg were seen filled with blood. All treated mice glomerulus located in kidney expanded and stomach mucosa length also reduced. Based on the data obtained, *Aquilaria subintegra*, if taken at a dosage of 4000 mg/kg, is toxic to the ICR mice in this study due to the harmful effect it caused on mice appearance, behaviour and organ tissues.

Keywords: Agarwood, Aquilaria, Tissues, Light Microscope, Toxicity

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Introduction

To date, medicinal plants have played an essential role in developing human health. It has been stated that some plants can be considered medicinal plants due to the rich source of beneficial ingredients it posseses [1]. The usage of plants in medicinal practices has been around for ages. These plants have been labelled as the forefront for most medical practices in most civilizations and cultures in history [2]. It has been proven that medical practices involving plants have been practised in ancient China, Egypt, and even during Greece civilization. For example, plants like fennel, ginseng, or garlic have been used by these ancient communities in their medicinal practices for the sole purpose of treating their health. In ancient Egypt, raw garlic was frequently used for asthmatics treatments and those suffering from bronchial-pulmonary disease [3]. Meanwhile, in ancient China, a decoction consisting of ginseng was used as the primary source for treating ulcers, haemorrhages, and the common cold [4]. The renowned Greek herbalist Dioscorides has stated that apparently fennel can be used as a remedy for stomach and bladder problems [5].

However, the rising popularity of medicinal plants has made researchers worried about the dangers it may hold. They have found that most people would use medicinal plants without any advice or consent from doctors or herbalists. This attitude can lead to some misfortune to the users if they are not adequately educated. The harmful side effects the medicinal plants may possess if it is being used without any limitation. Consumption of modern or traditional medicines without limitation can be dangerous to the consumer [6]. There have been multiple cases where people used medicinal plants, believing it would cure their ailments but instead worsen them to a whole new level. One example is the *Glycyrrhiza glabra* plant or also known as liquorice. For 3000 years, western civilizations have commonly used this plant's root for asthma, arthritis, stomach and duodenal ulcers treatments. However, new research has shown that if this liquorice plant is taken in high amounts, sodium and water retention and potassium depletion can occur, making it dangerous to people who already suffer from heart diseases and high blood pressure [7]. St. John's wort plant can also be dangerous if taken without any consideration. This plant has been used for dietary and depression treatment primarily throughout America and Europe. Nevertheless, new research has shown that this plant can cause a reduction of blood concentration at a high rate if taken simultaneously with Indinavir, a type of modern medicine [8]. Therefore, it is vital to know the limitations and toxicity of medicinal plants so that harmful side effects can be avoided.

In Asian countries, one of the popular plants used traditionally among folks is the agarwood. It has been used to treat diabetes. However, information regarding its toxicity is limited. The available ones only scratched the surface of *Aquilaria subintegra* toxicity. One example is the administration of *Aquilaria subintegra* aqueous extract in mice orally for 28 days, and results showed that at 2000 mg/kg concentration, abnormalities can be observed on mice liver and kidney tissues, indicating a toxic reaction inside the mice body [9].

Furthermore, the method for toxicity evaluation used in their research was only acute toxicity analysis. Most toxicity research usually performed another method of toxicity analysis called sub-acute toxicity analysis. This type of analysis method often provides a better and accurate result regarding substance toxicity [10]. Even though the research gave valuable insights regarding the harmful effect of *Aquilaria subintegra,* it does not provide accurate information regarding its toxicity. This lack of data can be avoided if future researchers include information regarding *Aquilaria subintegra* acute toxicity and its effects

on humans. If this is conducted, people will surely start to acknowledge the pros and cons of *Aquilaria subintegra*, which makes it easier to be used commercially.

Therefore, this study is essential in providing the scientific data of *Aquilaria subintegra*. Since there is limited scientific information regarding the systemic toxicity of single doses of *Aquilaria subintegra* aqueous extract (ASAE), conducting this research should be considered a priority. Vast parameters such as observation of abnormal behaviour, analysis of body weight and relative organ weight, mortality and lethal dose 50 (LD50) assessment, and histological evaluation were approached so that accurate information regarding *Aquilaria subintegra* toxicity can be acquired.

Materials and Methods

Plant Material

Aquilaria subintegra leaves were collected from Agarwood Al-Hilmi plantation in Slim River and Tanjung Malim, Perak, Malaysia. The species was identified by a taxonomist from Biology Department, Universiti Pendidikan Sultan Idris (UPSI).

Extraction Method

The collected leaves were washed, air-dried for 14 days and grind using an electrical grinder until fine powder were formed [11,12]. The powder were then underwent a maceration process where it was mixed with distilled water and left for 24 h at room temperature with some occasional stirring. 1000 g of the fine powder were mixed with 1000 mL of distilled water, making the ratio 1:1 [11,13]. The mixture was then filtered [14], and the filtrate obtained was dried inside an oven with a temperature of 55 ^{o}C for 48 h [15]. Lastly, the filtrate underwent a freeze-drying process for 72 h until brown crude extract formed [16]. The extract obtained was then stored inside a 20 ^{o}C freezer for further uses. The obtained extract were then mixed with distilled water and administered to mice according to their body weight. The extracts concentration used can be seen in Table 1.

Experimental Animals

Institute of Cancer Research (ICR) mice were used as test subjects. ICR mice have been one of the most widely used rodents for decades in pharmacology, oncology, and toxicology research due to its easy breeding process. Healthy 42 male and 42 female ICR mice aged 12 to 14 weeks old were used in this experimental research [17]. All ICR mice underwent acclimatisation for one week after they were acquired [18]. The experimental procedures conducted on mice followed the proper research ethics and cared approved by the University Pendidikan Sultan Idris research ethics committee (reference number: 2021-0002-03). Mice were grouped into seven groups (six males and six females in each group) where 1 group acted as a control group while the other six were treated with different concentrations of ASAE. Mice were kept inside polypropylene cage according to their treatment group and gender to avoid any reproduction. Standard animal housing conditions were applied to all mice living conditions where controlled lighting (12 h dark-light cycles) and temperature (25 ± 2 ^{o}C) were maintained. Food and water were given *ad libitum*.

Acute Oral Toxicity Study

For this procedure, only a single dose of ASAE with different concentrations (Table 1) was used on each treated mice[19,20]. This study aimed to examine the toxicity effect of a single dose ASAE on ICR mice using different concentrations. The mice were treated orally using a ball-tipped stainless steel feeding needle attached to a syringe. After extract administration, changes in mice appearance and behaviour were periodically observed for the first 24 h. Afterward , daily observations were conducted for 14 days [21]. Also, any changes detected on mice body weight, organ weight, and mortality throughout the experiment were recorded. All mice were euthanised humanely using diethyl ether on the 14th day to withdraw several organs in order to execute the histological evaluation.

Table 1: Crude extract administration concentration

Categories	Groups	Extract's concentration (mg/kg body weight)
Control	CON	Distilled Water
Aquilaria subintegra	ASAE1	4000
Aqueous Extract (ASAE)	ASAE2	5000
	ASAE3	6000
	ASAE4	7000
	ASAE5	8000
	ASAE6	9000

n=12 in each group, six male and six female

Body Weight

Most acute toxicity study involves the evaluation of body weight. This type of toxicity evaluation focused on the changes in treated animals' body weight [22]. In this study, all mice body weights were weighed on day 0, 7 and 14 [23,24]. The obtained data were analysed using one-way ANOVA method followed by Tukey's test for multiple comparisons between control and treated groups. Any significant changes ($p < 0.05$) detected while comparing were considered as substantial [23].

Relative Organ Weight (ROW)

On the last day of the experiment, all mice were euthanised using diethyl ether and dissected to retrieve their liver, kidney and stomach organs. The organs were then rinsed with normal saline, dried and immediately weighted [25,26]. The organs were then undergone a fixation process so that they can be used for histological purposes. All data regarding organ weight were calculated using a formula acquired from the previous study [27]. Similar to body weight evaluation, a one-way ANOVA method followed by Tukey's test was conducted to find significant differences between control and treated groups.

Statistical Data Analysis

All of the results obtained were calculated and expressed in mean ± standard deviation (SD). Data analysis was conducted using one-way ANOVA method followed by Tukey's test for multiple comparisons between control group and treated groups. Any

significant changes detected while comparing were considered as *p* value where *p* was less than 0.05 ($p < 0.05$) meaning that the changes that occurred were substantial.

Lethal Dose 50 (LD50)

The evaluation of LD50 involves the mortality rate of the treated mice population [28]. During the experimental process, any mortality that occurred were recorded to determine the LD50 of ASAE. If the death rate is equal to or more than 50 % of the mice population, then the concentration of ASAE used is dangerous to be administered to humans [29].

Abnormality In Appearance and Behaviour

Assessing the abnormality in mice appearance and behaviour is an effective way to evaluate a substance's toxicity [30]. In this study, changes in mice appearance and behaviour were observed and recorded daily for 14 days. Changes in appearance include their skin, fur, eyes and bodily fluid secretion of bodily fluid while behavioural include breathing condition, drowsiness and overall locomotor activity [21,23]. The data acquired were analysed and compared to the control group so that ASAE toxicity can be further understood.

Histological Slide Preparation

After all of the mice were euthanised, their liver, kidney and stomach organs were dissected, rinsed with normal saline, dried, weighted and fixed inside 70 % alcohol [25,26]. The reason why 70 % alcohol was used is because of its high capabilities to preserve the native structure of the organs. On top of that, these three organs were selected due to their ability to filter dangerous substances inside mice body. The organs then undergo the standard process of tissue preparation and fixation was done using paraffin wax in the mold. As soon as the paraffin hardened, the mold was placed on a microtome and immediately sectioned. Tissues sections with 4 μm to 5 μm thickness were produced and were stained with hematoxylin and eosin (H&E) [31,32]. All of the procedures conducted were based on previous research with some slight modifications [33]. Any significant changes when compared were recorded.

Results and Discussion

All mice survived throughout the experimental period. Therefore, no mortality was recorded among the mice population. However, the mice absolute body weight differs between male and female. Tables 2 and 3 tabulated all of the mean±SD for both male and female mice, respectively. Male mice showed significant differences ($p<0.05$) compared to the control group, while female mice displayed no significant difference. Five groups on days 7 and 14 showed significant differences, which were 4000, 5000, 7000, 8000 and 9000 mg/kg. For relative organ weight, no significant differences were detected on both the male and female mice treated group compared to the control group (Tables 4 and 5).

It has been said that changes in body weight may help to reveal the substance's toxicity [22]. While evaluating the mice body weight, most of the male mice group showed a significant changes in their body weight compared with the control and this may have been due to the stress they endured during the experimental procedure. Stress can sometimes lead to a change in food intake resulting to either an increase or decrease in body weight [34][35].

This can indicate that the extract used may have caused an adverse reaction inside the mice body. This situation can also be seen in other plant research as well. When *Nephelium lappaceum*, also known as rambutan honey was administered orally to male and female mice, significant increase in male mice mean body weight can be seen [36].

Table 2: Male mice absolute body weight for 14 days after *Aquilaria subintegra* aqueous extract (ASAE) treatment

***Aquilaria subintegra* Dosage Concentration (mg/kg)**	**Body weight in gram (g) mean±standard deviation**		
	Day 1	**Day 7**	**Day 14**
Male Control	40.27±2.48	42.43±2.26	43.60±2.06
Male 4000	32.60±1.98	33.14±3.80*	32.09±5.23*
Male 5000	35.26±3.53	34.21±5.43*	34.26±6.31*
Male 6000	35.08±1.78	36.84±3.98	37.82±3.41
Male 7000	35.36±1.12	35.63±2.04*	36.21±2.49*
Male 8000	37.01±2.64	35.70±2.95*	35.99±2.75*
Male 9000	35.78±0.30	34.19±2.87*	32.81±3.86*

n = 6 in each group
*indicate $p<0.05$, which showed a significant difference

Table 3: Female mice absolute body weight for 14 days after *Aquilaria subintegra* aqueous extract (ASAE) treatment

***Aquilaria subintegra* Dosage Concentration (mg/kg)**	**Body weight in gram (g) mean±standard deviation**		
	Day 1	**Day 7**	**Day 14**
Female Control	30.98±4.20	32.07±3.08	31.22±2.82
Female 4000	31.60±1.76	30.16±2.07	29.97±2.45
Female 5000	31.34±0.75	29.37±2.58	30.44±2.05
Female 6000	32.39±0.40	30.22±1.48	29.66±1.69
Female 7000	33.01±0.94	31.44±2.04	31.58±1.71
Female 8000	33.24±1.79	30.42±2.49	31.44±1.80
Female 9000	33.63±2.17	31.48±3.24	31.66±2.58

n=6 in each group
*indicate $p<0.05$, which showed a significant difference

Table 4: Male mice relative organ weight 14 days after *Aquilaria subintegra* aqueous extract (ASAE) treatment

***Aquilaria subintegra* Dosage Concentration (mg/kg)**	**Relative organ weight in gram (g) mean±standard deviation**		
	Liver	**Kidney**	**Stomach**
Male Control	4.54±0.64	0.78±0.09	0.73±0.07
Male 4000	4.92±1.22	0.84±0.07	0.91±0.19
Male 5000	5.34±0.98	0.81±0.02	0.74±0.15
Male 6000	5.01±0.63	0.83±0.08	0.88±0.08
Male 7000	5.36±1.31	0.80±0.04	0.72±0.12
Male 8000	5.02±0.55	0.83±0.05	0.88±0.08
Male 9000	5.64±1.13	0.85±0.15	0.96±0.14

n=6 in each group

*indicate $p<0.05$, which showed a significant difference

Table 5: Female mice relative organ weight 14 days after *Aquilaria subintegra* aqueous extract (ASAE) treatment

***Aquilaria subintegra* Dosage Concentration (mg/kg)**	**Relative organ weight in gram (g) mean±standard deviation**		
	Liver	**Kidney**	**Stomach**
Female Control	3.92±0.49	0.64±0.03	1.10±0.16
Female 4000	4.17±0.38	0.61±0.08	0.88±0.12
Female 5000	4.68±1.13	0.63±0.06	0.99±0.27
Female 6000	4.52±0.56	0.60±0.07	0.88±0.13
Female 7000	4.66±0.34	0.65±0.07	1.07±0.15
Female 8000	4.63±0.31	0.59±0.07	1.03±0.06
Female 9000	4.07±0.24	0.61±0.06	0.85±0.11

n=6 in each group

*indicate $p<0.05$, which showed a significant difference

Abnormality in mice physical appearance and behaviour are displayed in Tables 6 and 7. Observable abnormalities that were detected regarding mice physical appearance were their fur growth. All treated mice showed uneven fur when compared to the control. In addition, balding and a reduction of fur growth were also detected on treated female mice. The skin on their face started showing, and their fur seemed to be significantly reduced. Figure 1 shows all of the affected female mice. In terms of behavioural abnormality, all treated mice displayed drowsiness after they were fed with ASAE. for 24 h. Furthermore, a decrease in their overall activity was also detected. When compared to the control group, all treated mice displayed a more passive behaviour. This occurred for seven days straight, but on day 8, their daily activity started to normalise again.

The toxicity of a substance can be exposed simply by examining the clinical signs, symptoms and abnormalities it produced [30]. Based on this research, the abnormalities in

appearance that were detected were changes in mice fur and sleep condition. After the extract were administered, all treated mice showed abnormal fur growth. On top of that, baldness was also detected in some female mice face similar to alopecia areata. Both of this can be related with colchicine and cucurbitacin present inside ASAE. *Gloriosa superba* which also contain colchicine have been reported to cause a reduction in hair growth in an individual after consumption [37]. This predicament also occurred in other research as well [37,38]. Based on previous research, it can be stated that ASAE used in this research may have caused an adverse reaction on mice fur growth due to the abnormal fur growth and balding observed.

All treated mice showed sedative effect after being treated with ASAE. This adverse reaction can be seen in other research and it have been speculated that the phytoconstituents present within the plant may have been the cause of the effect [39]. Another research also discussed similar reaction as well. Aqueous extract from *Aquilaria malaccensis* leaves which is another type of *Aquilaria* plant also showed drowsiness and sedative effects in treated mice [23]. Therefore, the sedative and drowsiness effect caused by ASAE can be considered as an adverse reaction due of its capabilitiy to alter sleep conditions making it hazardous to be used in high dosage.

Table 6: Signs of abnormality on male mice after *Aquilaria subintegra* aqueous extract (ASAE) treatment

Abnormal signs	**Yes/No**						
	***Aquilaria subintegra* Dosage Concentration (mg/kg)**						
	Control	**4000**	**5000**	**6000**	**7000**	**8000**	**9000**
Changes in skin	No	No	No	No	No	Yes	Yes
Changes in body fur	No	Yes	Yes	Yes	Yes	Yes	Yes
Changes in face fur	No	No	No	No	No	No	No
Changes in eyes	No	No	No	No	No	No	No
Breathing condition	No	No	No	No	No	No	No
Salivation	No	No	No	No	No	No	No
Changes in faeces	No	No	No	No	No	No	No
Sleepy condition	No	Yes	Yes	Yes	Yes	Yes	Yes

n=6 in each group

Table 7: Signs of abnormality on female mice after *Aquilaria subintegra* aqueous extract (ASAE) treatment

Abnormal signs	Yes/No						
	Aquilaria subintegra Dosage Concentration (mg/kg)						
	Control	4000	5000	6000	7000	8000	9000
Changes in skin	No	No	No	No	No	Yes	Yes
Changes in body fur	No	Yes	Yes	Yes	Yes	Yes	Yes
Changes in face fur	No	Yes	No	Yes	No	Yes	Yes
Changes in eyes	No	No	No	No	No	No	No
Breathing condition	No	No	No	No	No	No	No
Salivation	No	No	No	No	No	No	No
Changes in faeces	No	No	No	No	No	No	No
Sleepy condition	No	Yes	Yes	Yes	Yes	Yes	Yes

n=6 in each group

Figure 1: (A) Photograph of female mice from control group, (B) Photograph of female mice with 4000 mg/kg ASAE treatment showing their bald spot, (C) Photograph of female mice with 6000 mg/kg ASAE treatment showing their bald spot, (D) Photograph of female mice with 8000 mg/kg ASAE treatment showing their bald spot and (E) Photograph of female mice with 9000 mg/kg ASAE treatment showing their bald spot.

As mentioned before, histological observation and evaluation only focussed on a specific cellular structure. In Figures 2 and 3, photomicrographs of mice liver section can be seen for the treated mice. Based on those images, changes in mice portal vein and sinusoids can be observed (mice group 6000 mg/kg and female group 4000 mg/kg), which showed blood-filled portal vein. In Figures 4 and 5, photomicrographs of the treated mice kidney sections show abnormal changes in the glomerulus. Both male and female mice showed the glomerulus were expanding. For stomach histological changes, it can be observed in Figures 6 and 7. All treated mice showed a reduction in their mucosa length indicating abnormal changes in their stomach tissues.

In mice liver histology, two groups showed blood-filled portal vein indicating thrombosis or interruption of blood flow inside mice liver organ. As we all know, liver plays a role in the filtering and excretion of any foreign substances. These foreign substances (ASAE) tend to damage liver cell [41]. On top of that, Thrombosis, thrombus or also known as blood clot can occurred in the lumen of portal inside the liver organ [42]. Necrosis on liver has also been reported before [43].

Expanded glomerulus can be seen in all treated mice groups. Expansion of glomerulus can indicate glomerulosclerosis or endocapillary proliferative glomerulonephritis. Moreover, the cause for this expansion of glomerulus may be due to the content in the blood that interrupts its flow inside the kidney. It has been stated that expansion of the glomerulus can be linked to glomerulosclerosis where chronic histological pattern of injury can be seen inside the treatment [44]. Based on those results, it is safe to say that ASAE at a dosage of 4000 mg/kg can be hazardous due to the adverse effect it caused on mice kidney organs.

The length of the stomach mucosa layer for all treated groups seems to be shorter when compared to the control group indicating an adverse reaction where the mucosa is eroded, similar to acute gastritis [47,48]. With shorter mucosa in all treated groups, making it dangerous for ASAE to be consumed at 4000 mg/kg.

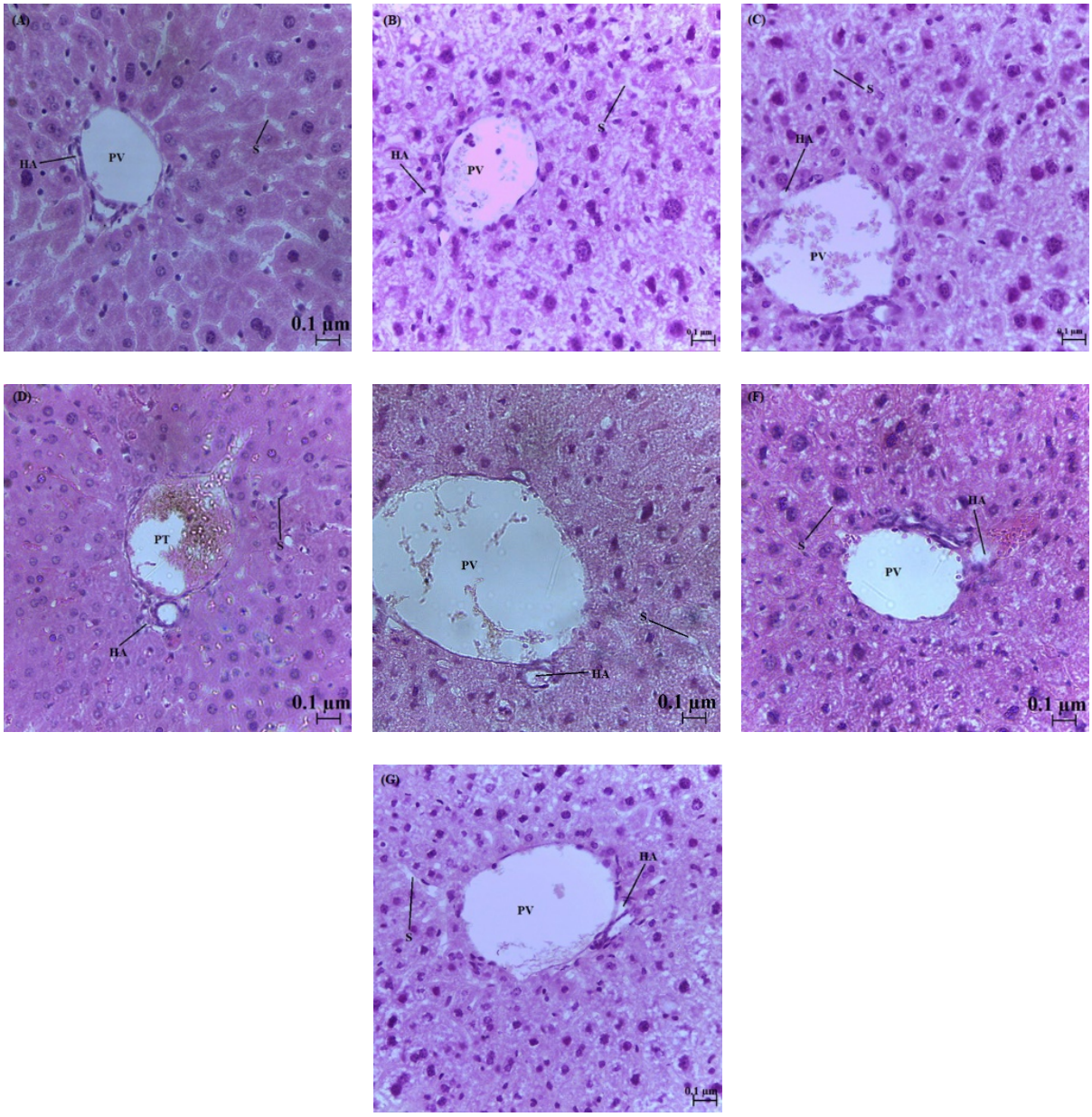

Figure 2: Photomicrograph of male mice liver section from (A) control group showing normal portal vein, hepatic artery and sinusoids, (B) 4000 mg/kg ASAE treatment showing no abnormality, (C) 5000 mg/kg ASAE treatment showing no abnormality, (D) 6000 mg/kg ASAE treatment showing portal vein filled with blood, (E) 7000 mg/kg ASAE treatment showing no abnormality, (F) 8000 mg/kg ASAE treatment showing no abnormality and (G) 9000 mg/kg ASAE treatment showing no abnormality. PV = Portal vein, HA = Hepatic artery, S = Sinusoids. (H&E stain, X40).

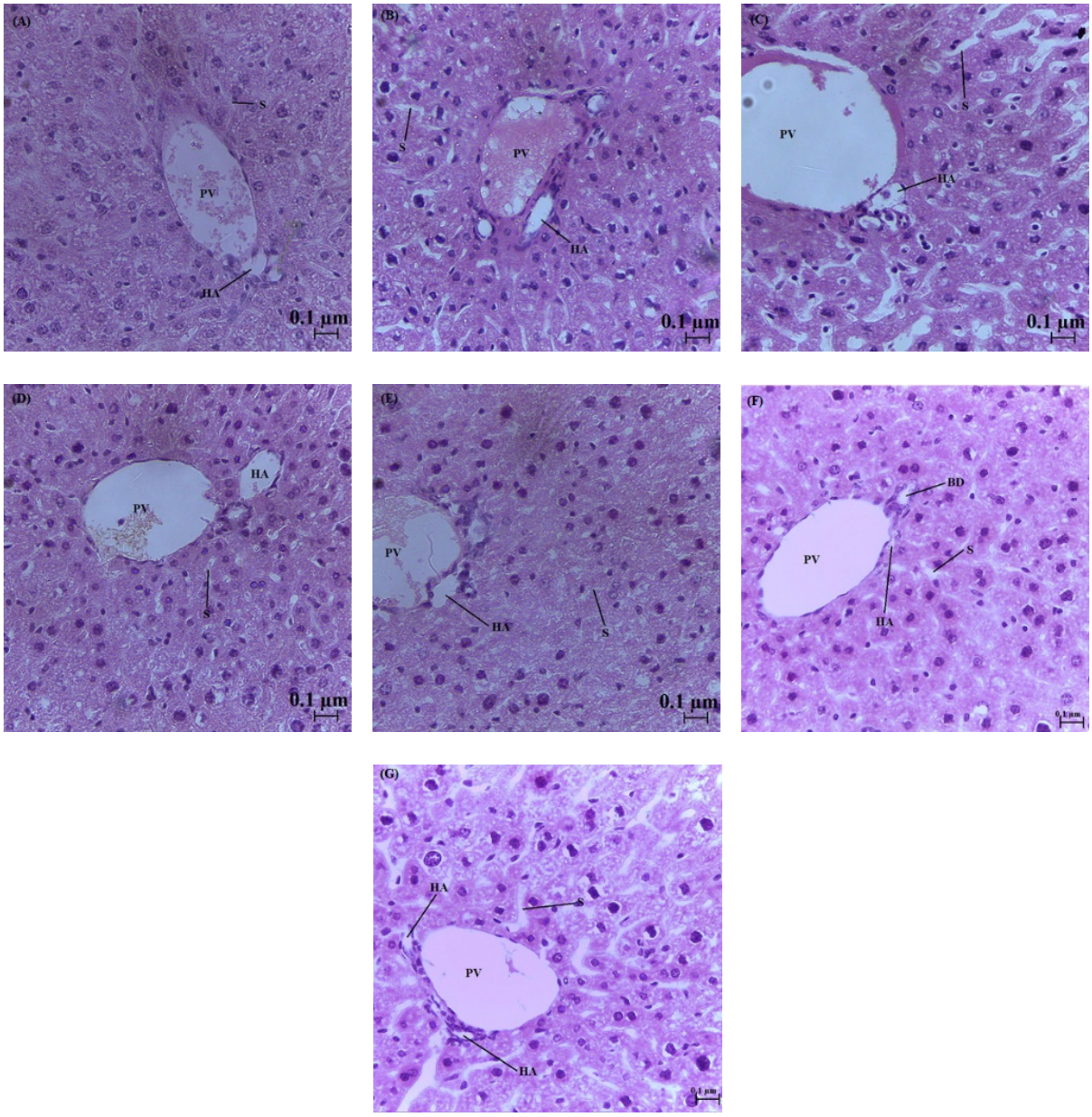

Figure 3: Photomicrograph of female mice liver section from (A) control group showing normal portal vein, hepatic artery and sinusoids, (B) 4000 mg/kg ASAE treatment showing portal vein filled with blood, (C) 5000 mg/kg ASAE treatment showing no abnormality, (D) 6000 mg/kg ASAE treatment showing no abnormality, (E) 7000 mg/kg ASAE treatment showing no abnormality, (F) 8000 mg/kg ASAE treatment showing no abnormality and (G) 9000 mg/kg ASAE treatment showing no abnormality. PV = Portal vein, HA = Hepatic artery, S = Sinusoids. (H&E stain, X40).

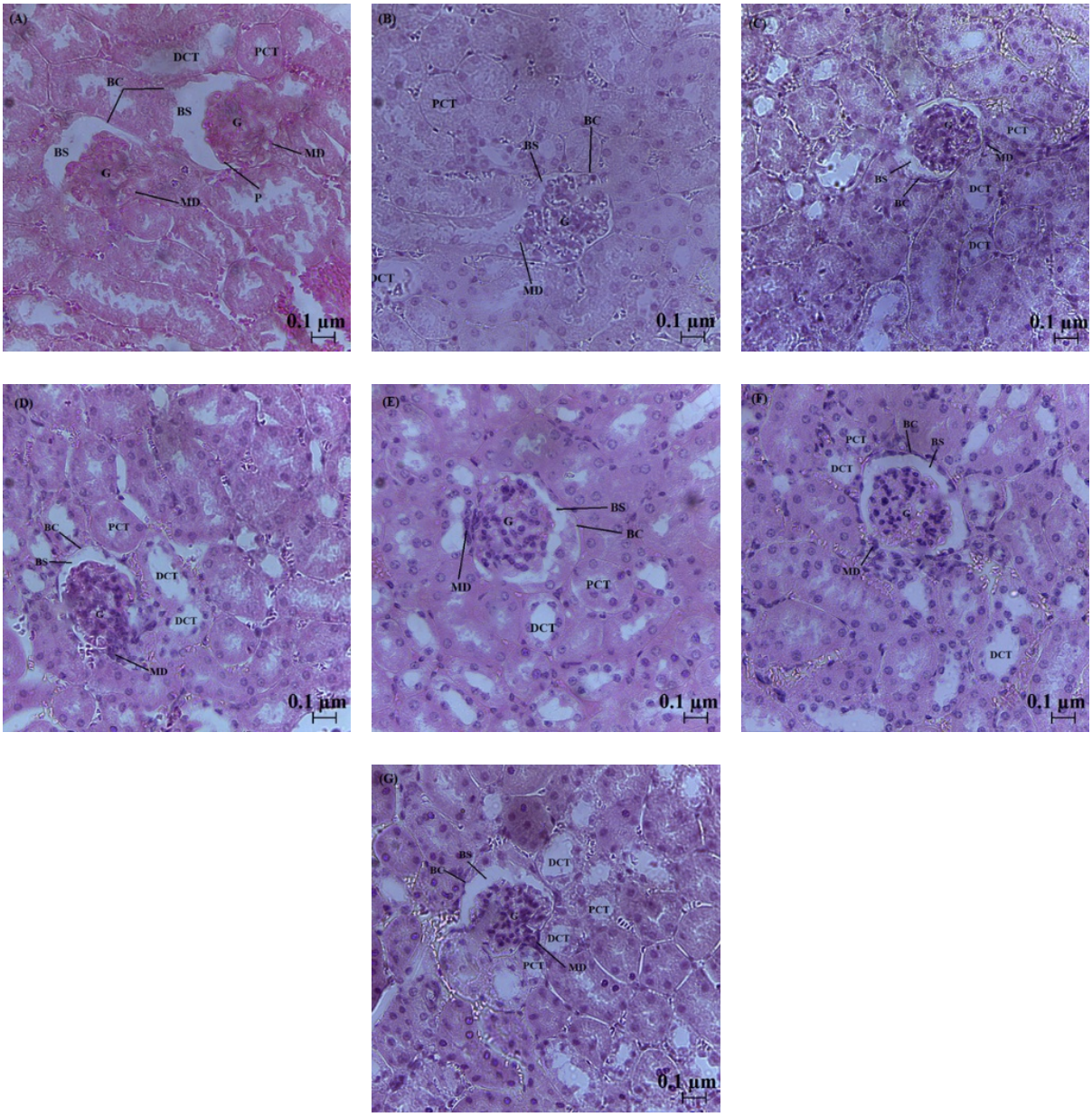

Figure 4: Photomicrograph of male mice kidney section from (A) control group showing normal glomerulus, proximal and distal convoluted tubule, (B) 4000 mg/kg ASAE treatment showing an expanded glomerulus, (C) 5000 mg/kg ASAE treatment showing an expanded glomerulus, (D) 6000 mg/kg ASAE treatment showing an expanded glomerulus, (E) 7000 mg/kg ASAE treatment showing an expanded glomerulus, (F) 8000 mg/kg ASAE treatment showing an expanded glomerulus and (G) 9000 mg/kg ASAE treatment showing an expanded glomerulus. G = Glomerulus, BC = Bowman's capsule, BS = Bowman's space, MD = Macula densa, PCT = Proximal convoluted tubule, DCT = Distal convoluted tubule. (H&E stain, X40).

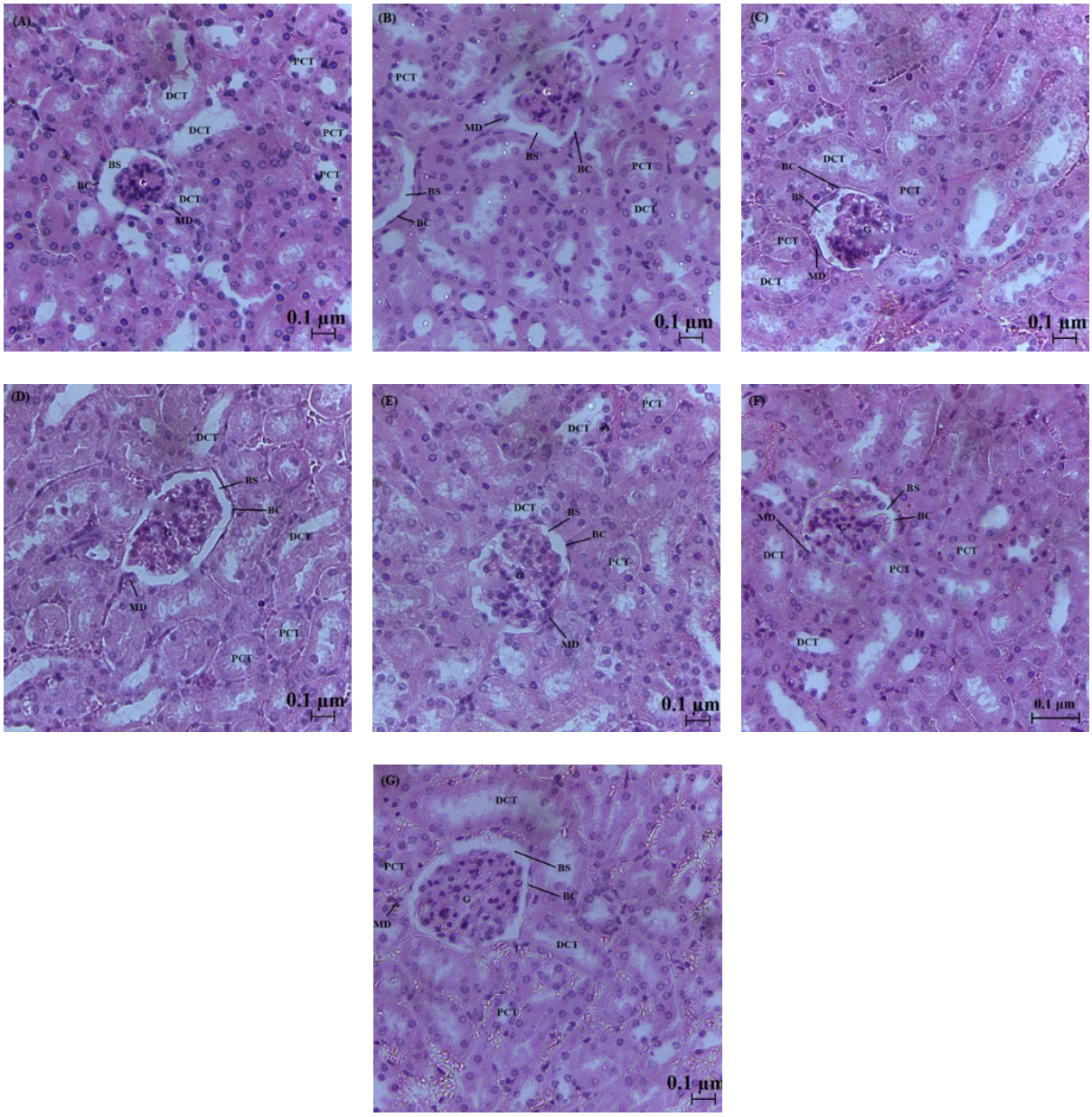

Figure 5: Photomicrograph of female mice kidney section from (A) control group showing normal glomerulus, proximal and distal convoluted tubule, (B) 4000 mg/kg ASAE treatment showing an expanded glomerulus, (C) 5000 mg/kg ASAE treatment showing an expanded glomerulus, (D) 6000 mg/kg ASAE treatment showing an expanded glomerulus, (E) 7000 mg/kg ASAE treatment showing an expanded glomerulus, (F) 8000 mg/kg ASAE treatment showing an expanded glomerulus and (G) 9000 mg/kg ASAE treatment showing an expanded glomerulus, G = Glomerulus, BC = Bowman's capsule, BS = Bowman's space, MD = Macula densa, PCT = Proximal convoluted tubule, DCT = Distal convoluted tubule. (H&E stain, X40).

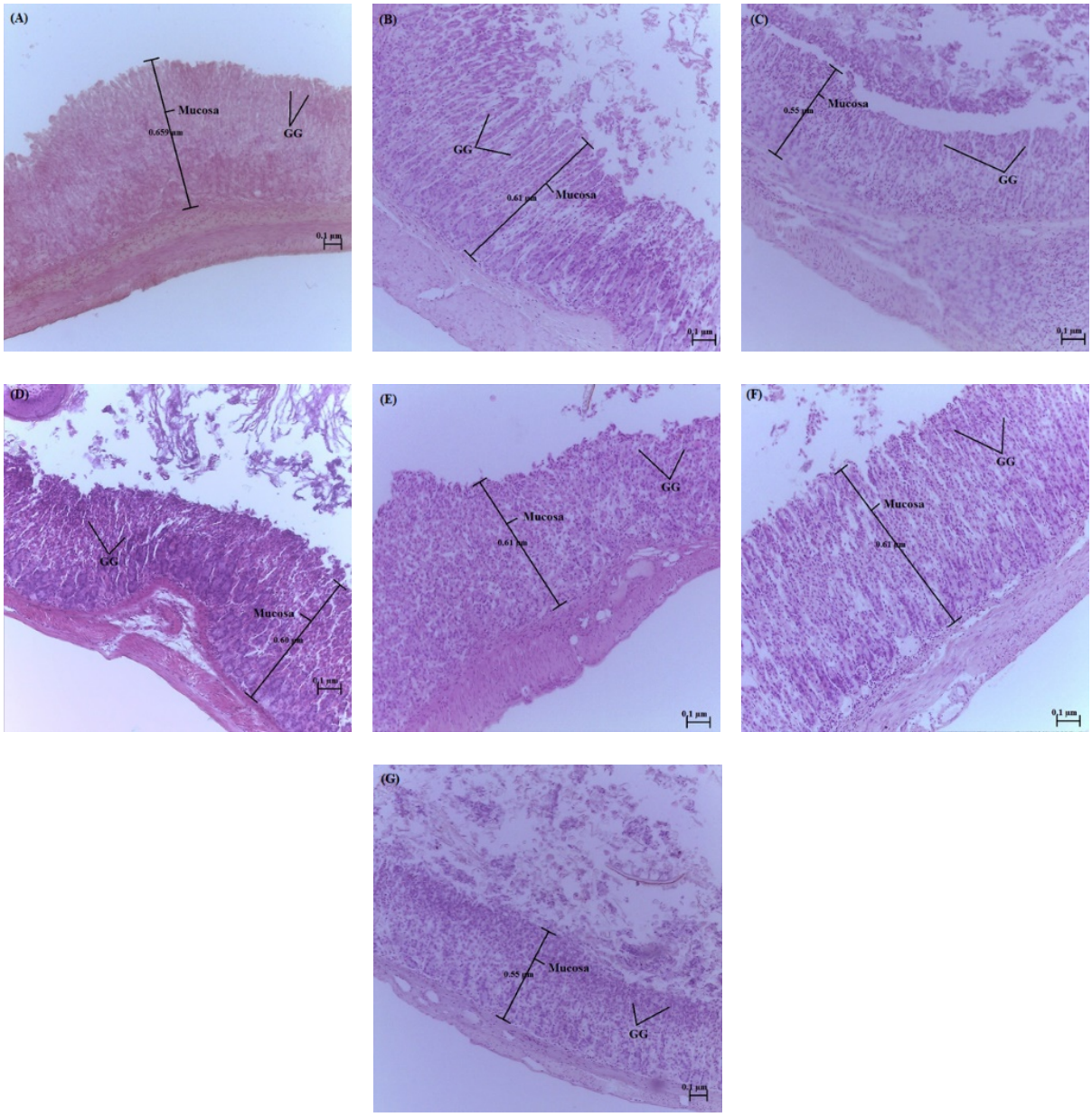

Figure 6: Photomicrograph of male mice stomach section from (A) control group showing normal gastric glands and length of mucosa, (B) 4000 mg/kg ASAE treatment showing reduced mucosa length, (C) 5000 mg/kg ASAE treatment showing reduced mucosa length, (D) 6000 mg/kg ASAE treatment showing reduced mucosa length, (E) 7000 mg/kg ASAE treatment showing reduced mucosa length, (F) 8000 mg/kg ASAE treatment showing reduced mucosa length and (G) 9000 mg/kg ASAE treatment showing reduced mucosa length. GG = Gastric glands. (H&E stain, X40).

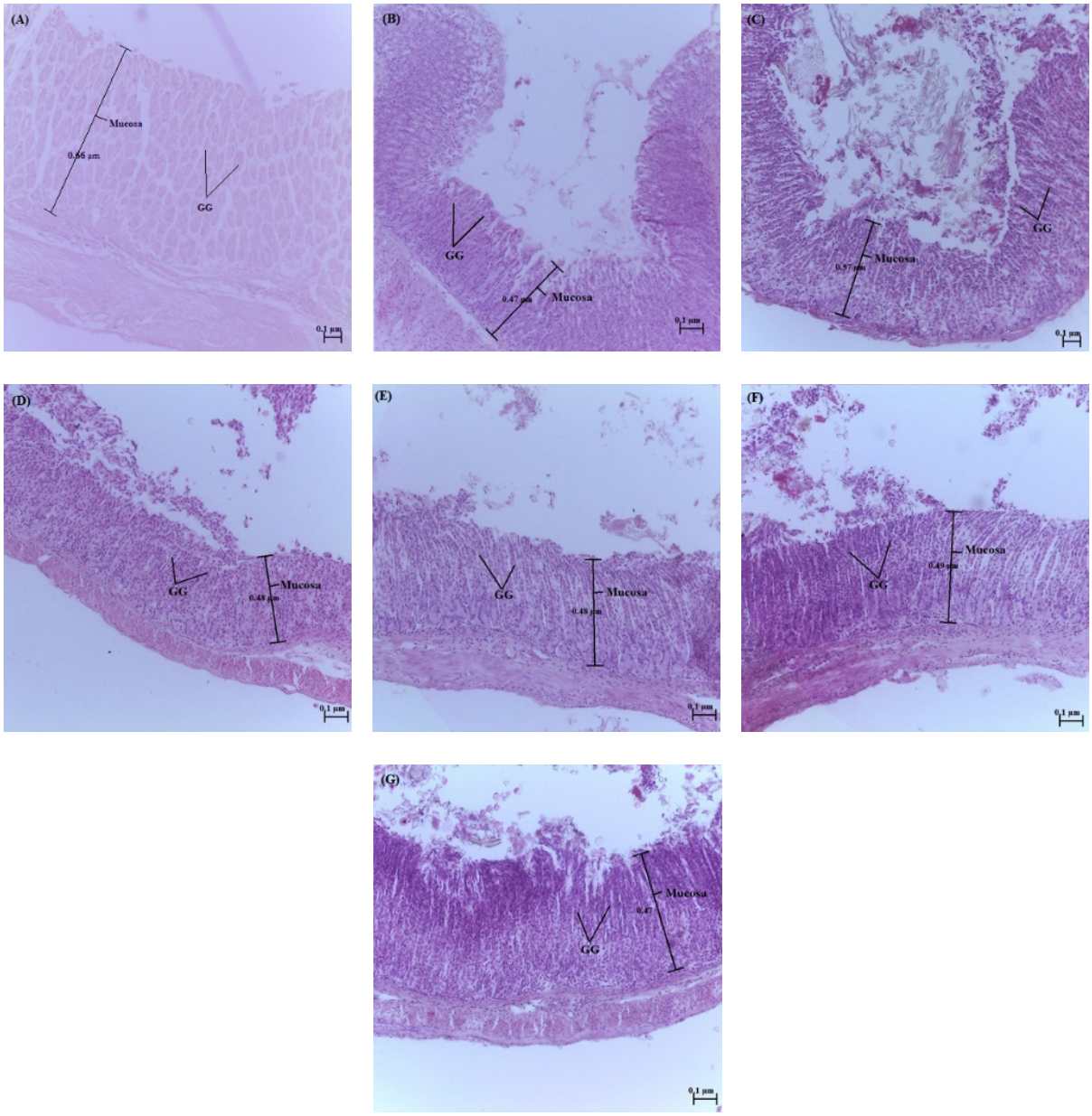

Figure 7: Photomicrograph of female mice stomach section from (A) control group showing normal gastric glands and length of mucosa, (B) 4000 mg/kg ASAE treatment showing reduced mucosa length, (C) 5000 mg/kg ASAE treatment showing reduced mucosa length, (D) 6000 mg/kg ASAE treatment showing reduced mucosa length, (E) 7000 mg/kg ASAE treatment showing reduced mucosa length, (F) 8000 mg/kg ASAE treatment showing reduced mucosa length and (G) 9000 mg/kg ASAE treatment showing reduced mucosa length. GG = Gastric glands. (H&E stain, X40).

Conclusions

In conclusion, this study revealed that *Aquilaria subintegra* aqueous extract (ASAE) can be toxic if taken at a dosage or concentration higher than 4000 mg/kg. The extract has caused many changes that were harmful to mice. The data obtained here has further proved why ASAE should not be used in high concentrations. All of this data can eventually help future researchers to understand *Aquilaria subintegra* better and ultimately conduct various

research that can help improve its uses, enabling it to be safely used commercially in society. It is recommended for future researchers to conduct studies on the sub-acute toxicity of ASAE while maintaining the concentration used in this research. This method can provide a much clearer understanding of this extract due to the repeated dose administered to mice.

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Author Contributions

All authors contributed toward data analysis, drafting and critically revising the paper and agree to be accountable for all aspects of the work.

Disclosure of Conflict of Interest

The authors have no disclosures to declare

Compliance with Ethical Standards

The work is compliant with ethical standards

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