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Acute Oral Toxicity and Brine Shrimp Lethality of *Pterocarpus indicus* Standardized Ethanol Extract

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Abstract: *Pterocarpus indicus* is widely used in Indonesia as a traditional medicine for treating various illness. A scientific study is needed to support the empirical study, an evaluation on the toxicity of extracts of this plant is crucial to support the therapeutic claims. In this research, the acute oral toxicity and brine shrimp lethality of a standardized ethanol extract of this plant was tested. Oral administration of ethanol extract at the highest dose of 18,000 mg/kg resulted in no mortalities or evidence of adverse effects, implying that *LD 50 >15. 000 mg/kg bb which shown that extract is non toxic.*. Normal behavioral pattern, clinical signs and histology of vital organs confirm this evidence. The *P. indicus ethanol* extracts also screened for toxicity against brine shrimp had 50% lethal concentration (LC50) values of more than 1.0 mg/mL (23.6 mg/ml), confirming that the extract was not toxic. Maximum mortalities occurred at 1000 mg/mL concentration while the least mortalities happened to be at 25 mg/mL concentration. The results of both tests confirm that *P. indicus standardized ethanol extract* is nontoxic.

Keywords: *Pterocarpus indicus; acute oral toxicity; BSLT.*

1. Introduction

Traditional and alternative medicine is extensively practiced in the prevention, diagnosis, and treatment of various illnesses. It has regained public attention over the past 20 years as this type of medicine is easily accessible in some regions[1]. Toxicity is an expression of being poisonous, indicating the state of adverse effects led by the interaction between toxicants and cells. This interaction may vary depending on the chemical properties of the toxicants and the cell membrane, as it may occur on the cell surface, within the cell body, or in the tissues beneath as well as at the extracellular matrix. The toxic effects may take place prior to the binding of the toxicants to the vital organs such as liver and kidneys. Hence, evaluation of toxic properties of a substance is crucial when considering for public health protection because exposure to chemicals can be hazardous and results to adverse effects on human being. In practice, the evaluation typically includes acute, sub-chronic, chronic, carcinogenic and reproductive effects[2].The genus *Pterocarpus*, a close relative of *Dalbergia*, belongs to the family Leguminosae-subfamily Papilionoideae (Fagaceae) and is the largest family of flowering plants[3]. It produces many beautiful, highly decorative timbers that are rank among the finest luxury timbers. Among them is *Pterocarpus indicus* Willd. which is locally known as narra in the Philippines, sonokembang or angsana

in Indonesia, pradoo or padauk in Thailand, rosewood in Papua New Guinea, nanara in Vanuatu, liki in Solomon Islands and sena or angsana in Malaysia, Brunei and Singapore. P. indicus in traditional treatment is used as rheumatoid arthritis, diabetic, menstrual pain[4]. In the present study of the ethanol extracts of *P.indicus* leaf, both the acute oral toxicity test in animal models[5] and the brine shrimp lethality test[6] were applied to determine its toxic properties. The acute oral toxicity testing was carried out for both sexes of animals under the Organization for Economic Cooperation and Development (OECD) guidelines[7].

2. Materials and Methods

2.1. Plant Material

Fresh samples of *P. Indicus* leaves was obtained from Province garden of Kawasan Puspiptek Serpong Banten, Indonesia in November 2012 and determination in Herbarium of Bogoriense Research Centre For Biology Indonesian Institutes of Science.

2.2. Preparation of the Crude Extracts

The leaves of P. indicus was washed thoroughly and rinsed with tap water and dried in oven at 50 °C for two to four days. The leave sample was grinding and sequentially extracted with ethanol 70 %.The extraction was carried out at room temperature for 24 hours and continue until the filtrate colorless. The filtrate from each extraction was combined and concentrated under vacuum by rotary evaporator

2.3. Acute Oral Toxicity Study

2.3.1. Target Organisms-Mice

The experiment was conducted on 100 healthy Swiss albino mice (males and females) weighing 25 to 35 g and aged 8 weeks, acquired from the Veterinary Faculty Intitute Pertanian Bogor. Those mice were distributed into five groups in each sex *i.e.*, 4 treated groups and one control groups.The experimental procedures relating to the animals were authorized Ethical commission health faculty University of Indonesia

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2.3.2. Toxicity Test

The mice used in the experiment were selected at random and marked on the tails for individual identification. Ten mice of the same sex were kept in a matte plastic cage, with dimensions of 17 × 27 × 14 cm. All of the cages were located in a room at temperature approximately 23 °C with constant humidity. The room is regulated with cycles of 12 h of light and 12 h of darkness. The mice were acclimated to the laboratory environment for a week earlier before starting the experiment. Drinking water and food were provided *ad libitum* through the experiment, except for the short fasting period where the drinking water was still in free access but no food supply was provided 12 h prior to treatment.

The acute oral toxicity of *P.indicus* ethanol extracts was evaluated in mice according to the procedures outlined by the Organization for Economic Co-operation and Development (OECD)[8,11]. A single dose of 18000; 9000; 4500; 2250 mg/kg bw respectively of extracts was administered to both ten male mice and ten female mice per group through the oral route. The extracts were suspended in a distilled water). Body weight of the mice was determined before experiment and the dose was calculated in reference to the body weight as the volume of the extracts solution given to the mice is 1 ml/ mice. Another ten male mice and ten female mice are given distilled water and were regarded as the control groups. Food was provided to the mice approximately an hour after treatment. The mice were observed in detail for any indications of toxicity effect within the first an hour before and after the treatment period, and daily further for a period of 14 days. Surviving animals were weighed and visual observations for mortality, behavioral pattern, changes in physical appearance, injury, pain and signs of illness were conducted daily during the period. All data analyzed use anova one way.

2.4 Histological Analysis

2.4.1. Organs and Body Weight Statistical Analysis

Finishing the 14 day period, all the mice were sacrificed. Vital organs such as heart, kidneys, liver ,lung and spleen were isolate. All of the individual organs were weighed and their features were compared between both

treated and control groups. Statistical analysis to assess the significant difference between both groups was conducted by running a one way anova.

2.4.2. Histopathology of Heart and kidney

Each individual organ were fixed in 10% buffered formalin, routinely processed and embedded in paraffin wax. Paraffin sections (5 μm) were cut on glass slides and stained with haematoxylin and eosin. The slides were examined under a light microscope and the magnified images of the tissues structure were captured for further study[11].

2.5. Brine Shrimp Lethality Test

2.5.1. Hatching Shrimp

Brine shrimp eggs, *Artemia salina* were hatched in a vessel containing seawater. The vessel was kept under an inflorescent bulb and facilitated with good aeration for 48 h at room temperature. After hatching, nauplii released from the egg shells were collected at the bright side of the vessel (near the light source) by using micropipette.

2.5.2. Brine Shrimp Test

The bioactivity of the extracts was monitored by the brine shrimp lethality test[7] to predict the cytotoxic activity in the compound. The extracts was dissolved in methanol and diluted with sea water. The variation of concentrations from 1000 to 25 mg/mL., and the dead larvae from each wells were counted after 24 h. Based on the percentage of the mortality, the concentration of 50% lethality (LC50) to the nauplii was determined by using the graph of mean percentage mortality *versus* the log of concentration[6].

2.5.3. Statistical Analysis

The mean results of mortality percentage of the brine shrimp *versus* the log of concentrations were plotted using the Microsoft Excel spreadsheet application, which also formulated the regression equations. These equations were later used to calculate LC50 values for the samples tested with consideration of value greater than 1.0 mg/mL, suggesting that the extract is non toxic

3. Results and Discussion

3.1. Lethality and Behavioral Analysis

The lethality and toxicity effect of the ethanol extracts of *P.indicus* on the appearance and behavioral pattern mice showed that there were no changes behavioral before and after giving *P.indicus* extract (data not shown). There were no deaths among the animals during the observation period and no significant changes in general appearance or behavioral pattern were noted either. Moreover, all the organs either of the control or the test groups were in good shape and condition.

3.2. Organs and Body Weight Statistical Analysis

The body weight as well as the weights of the vital organs of the animals were calculated and recorded in figure 1-4, There were no significant differences in the changes of each organ weight and body weight in both male and female mice ($P > 0.05$).

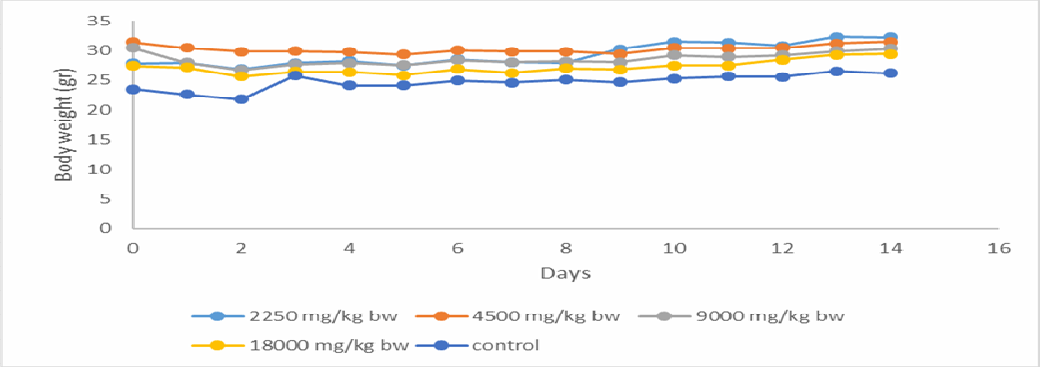


Figure 1. Body weight of female mice in 14 days observation

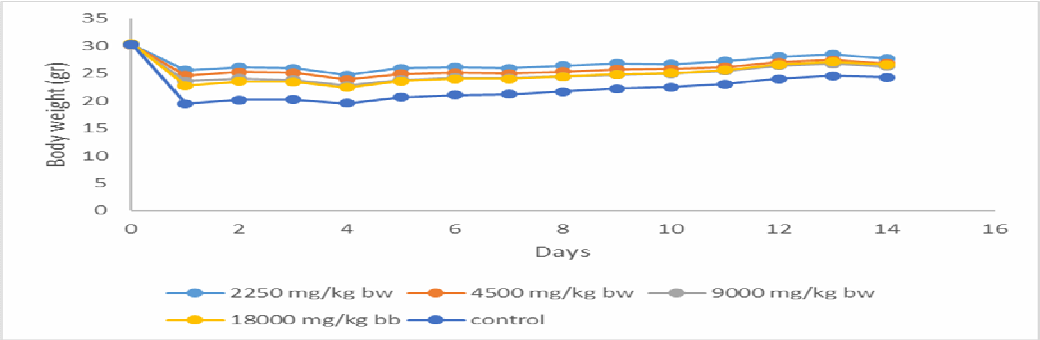


Figure 2. Body weight of male mice in 14 days observation

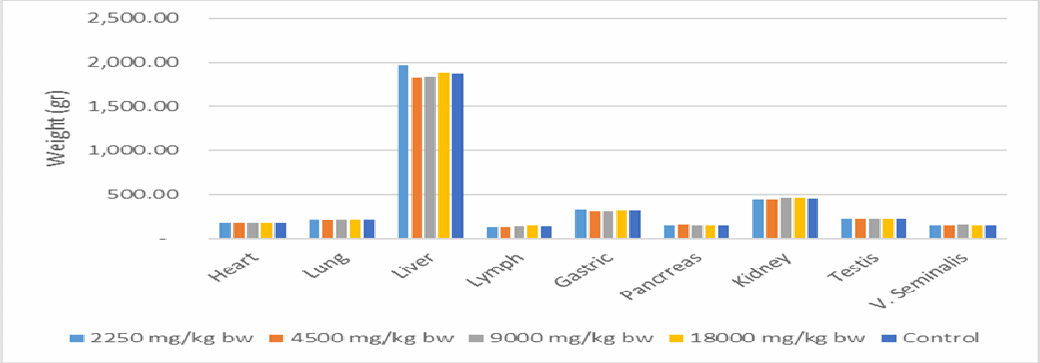


Figure 3. Organ weight of male mice after 14 days observation

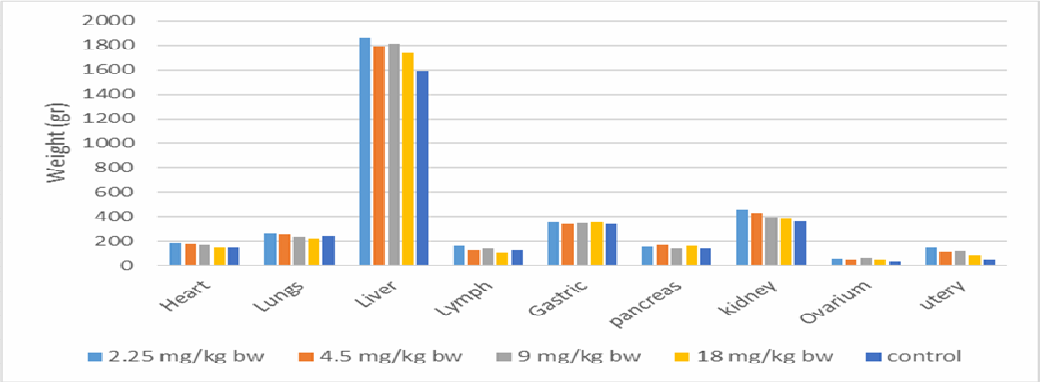
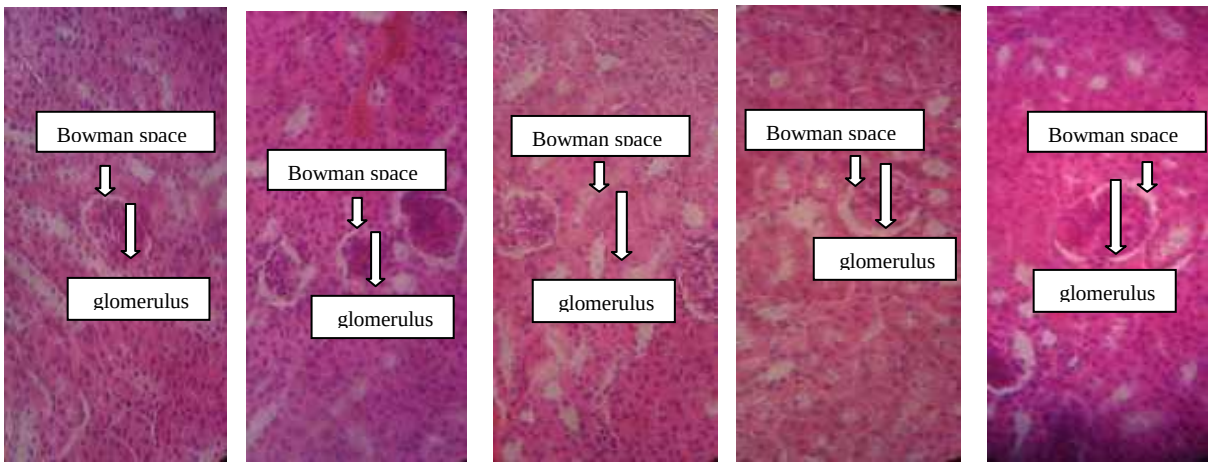


Figure 4. Organ weight of male mice after 14 days observation

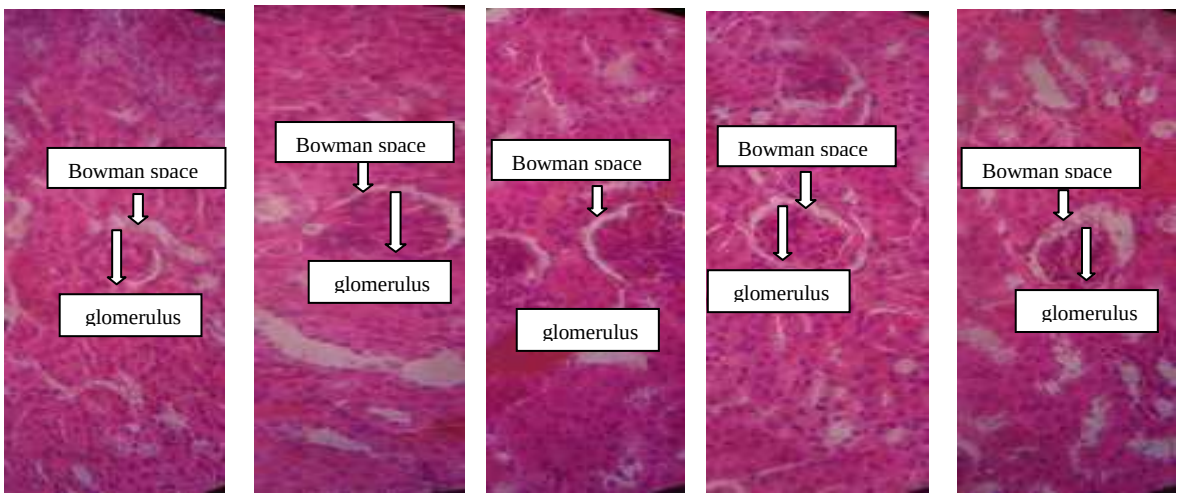
3.3. Histopathology Analysis of Heart and Kidneys

The microscopic structures of the kidney depicted through Figure 5 and 6 show unnoticeable differences between the control (group 5) and test group (group 1,2,3,4). There were also no cell degradation or any unfavorable effects observed when viewed under the light microscope using multiple magnification power. Also measured the space between capsula bowman and glomerulus (figure 7 and 8); diameter of capsula bowman for kidney parameter and also centralis vein diameter for liver parameter.



Group 1 Group 2 Group 3 Group 4 Group 5

Figure 5. Histological examination of female kidney



Group 1 Group 4 Group 3 Group 2 Group 5

Figure 6. Histological examination of male kidney

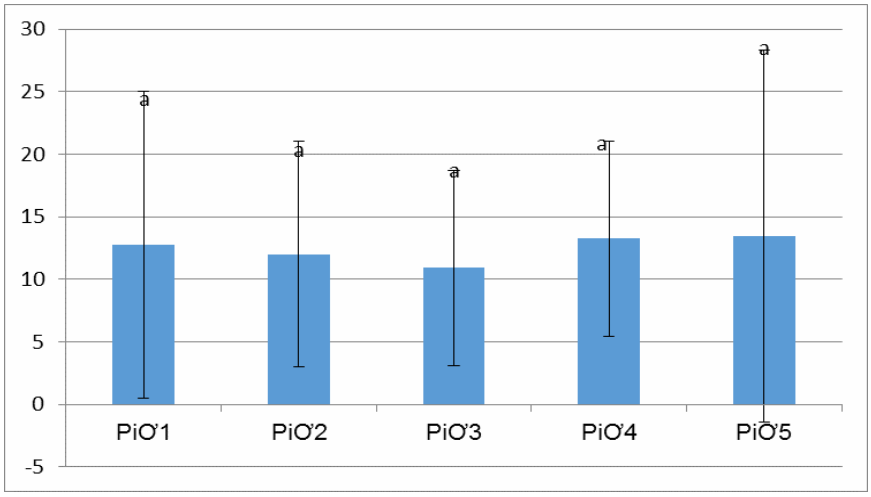


Figure 7. Average of space between capsula bowman and glomerulus in male kidney (a= p>0.05)

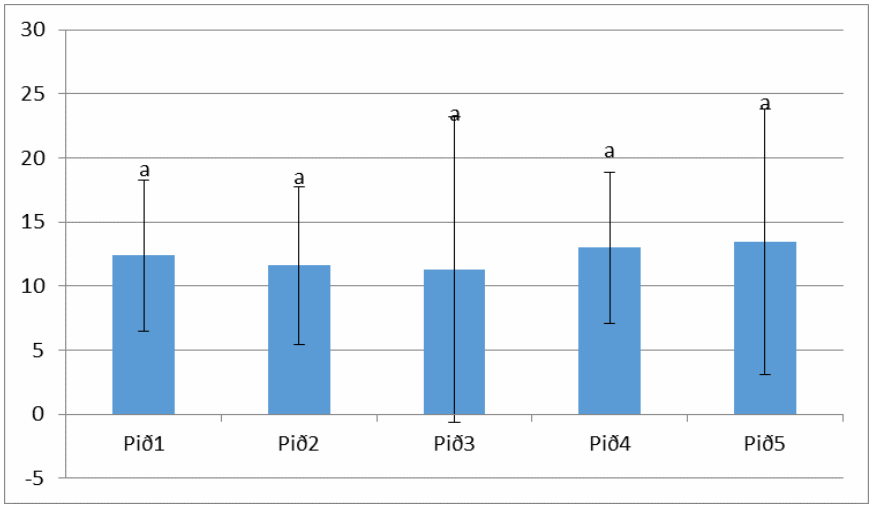


Figure 8. Average of space between capsula bowman and glomerulus in female kidney (a= p>0.05)

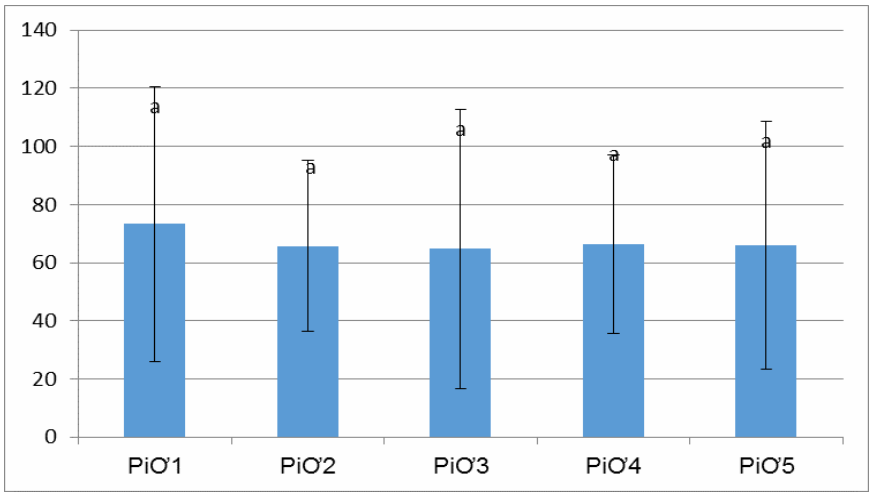


Figure 9. Average of bowman diameter in male kidney (a= p>0.05)

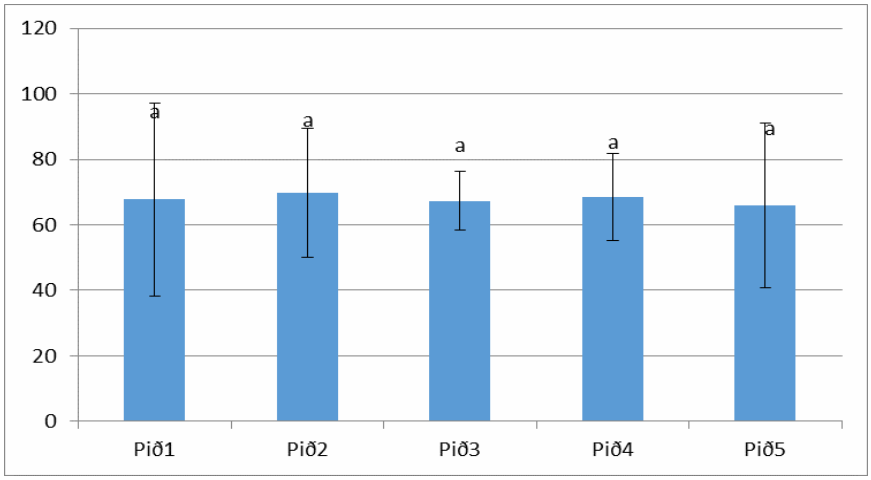
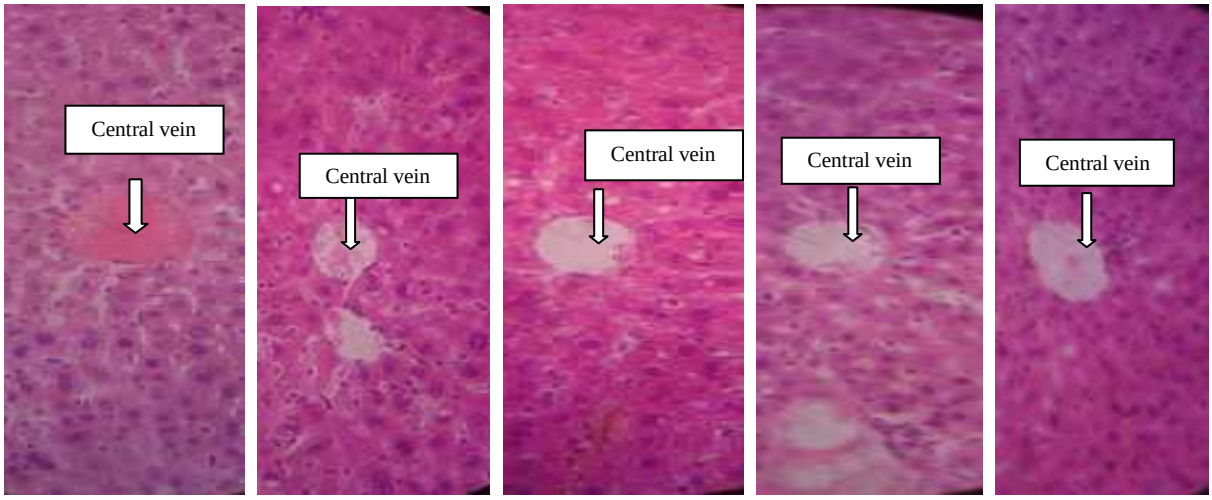
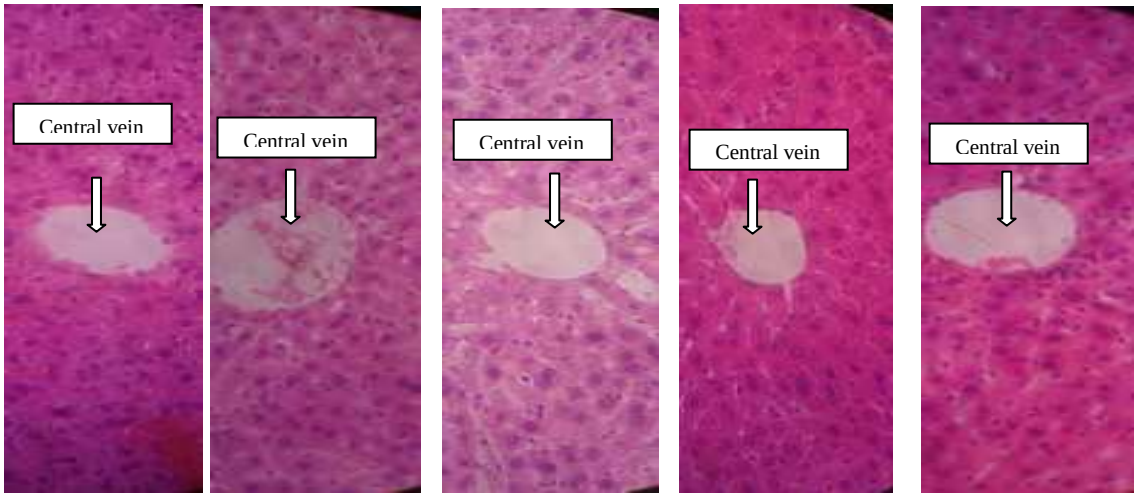


Figure 10. Average of bowman diameter in female kidney (a= p>0.05)



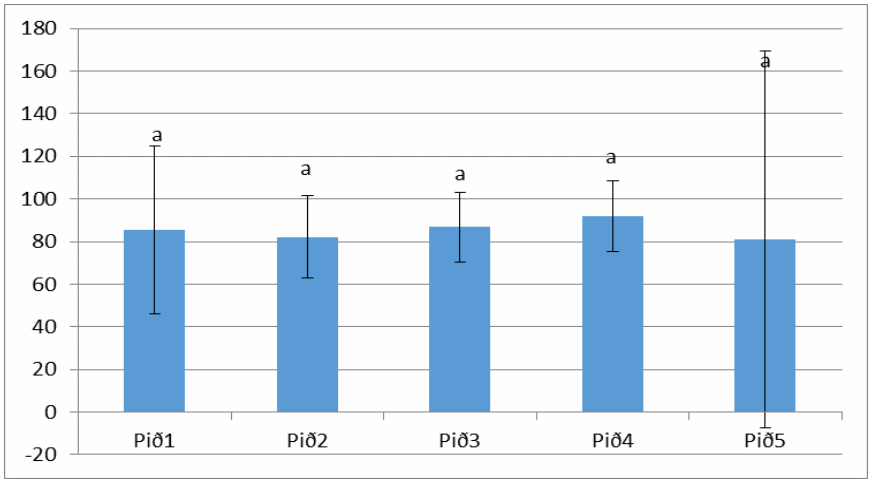
Group 1 Group 2 Group 3 Group 4 Group 5

Figures 11 Central vein of liver (female)

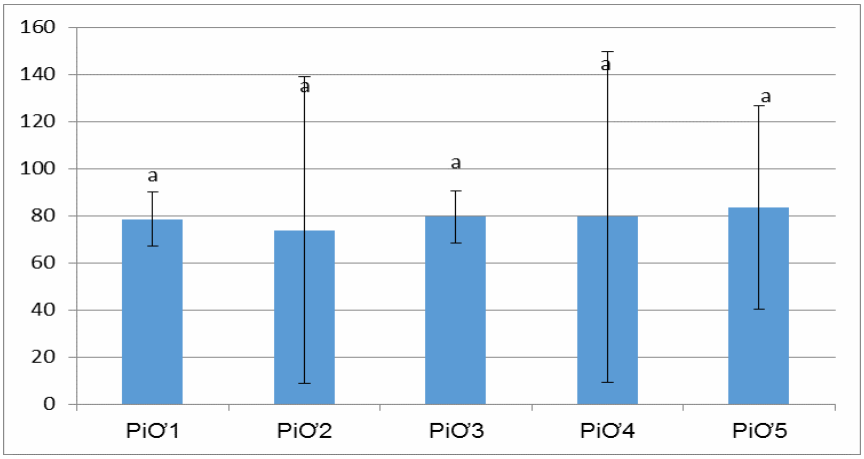


Group 1 Group 2 Group 3 Group 4 Group 5

Figures 12. Central vein of liver (male)



Figures 13. Average of centralis vein diameter (female)



Figures 14. Average of centralis vein diameter (male)

3.4. Brine Shrimp Lethality Test

Brine shrimp lethality results of the standardized ethanol extracts of *P. indicus* are shown in figures 15 LC50 values calculated are 23. 6 ppm. The extracts show positive results, indicating that the samples are biologically active. Crude extracts resulting in LC50 values of less than 1 mg/mL are considered as significantly active which suggests that the *P. indicus ethanol extract* showed the values 23.6 ppm have a very low toxicity. Plotting of mortality percentage *versus* log of concentration for test (Figures 15) demonstrates an approximate linear correlation. Furthermore, there is a direct proportional relation between the concentration of the extracts and the degree of lethality. This is shown by the fact the maximum mortalities occurred at a concentration of 1000 mg/mL whilst a concentration of 25 mg/mL only caused very minor mortalities.

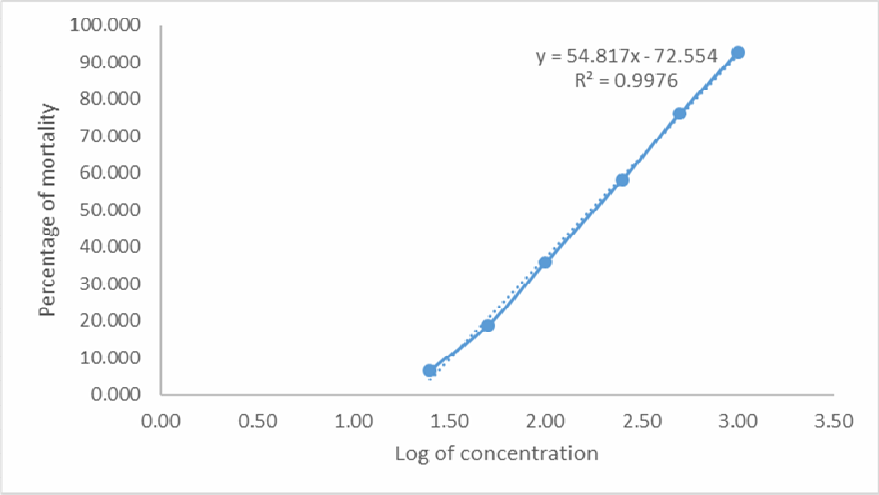


Figure 15. Concentration vs Percentage of mortality in BSLT

Investigation of acute toxicity is the first step in the toxicological analysis of any medicinal plant. Oral acute toxicity testing in mice could be used to evaluate natural remedies for different pharmacological activities, taking into account the basic premise that pharmacology is simply toxicology at a lower dose[8]. A toxic substance might elicit interesting pharmacological effects at a lower non-toxic dose. Toxicity results from animals will be crucial in definitively judging the safety of *P.indicus* extracts if they are found to have sufficient potential for development into pharmacological products[9]. Although there is a problem regarding extrapolating animals data to humans, a study has shown that mice give better prediction for human acute lethal dose compared to rats . All the procedures were performed based on the appropriate OECD guideline [7,10].Because it is generally known that *P.indicus* leaf is edible to animals, hence it is preliminarily assumed to be not toxic. Therefore in this up limit of dose in this study, a very high doses level of 18,000 mg/kg of crude extracts were administered orally to the tested mice (OECD)[8,10].

From the current testing, no mortalities were reported as well as no adverse effects were observed on the tested mice throughout the 14 day test period. All of the mice gained weight and displayed no significant changes in behavior. The body weight of the mice showed as increase, this indicates that the administration of the crude extract does not affect the growth of the animals. Thus, in this test *P.indicus* extract does not cause acute toxicity effects and an LD50 value greater than 5,000 mg/kg. Therefore, according to the chemical labeling and classification of acute systemic toxicity recommended by OECD, the crude extract of *E. guineensis* was assigned class 5 status (LD50 > 5000 mg/kg) which was the lowest toxicity class. Based on the histopathology analysis, all of the tissues of organs presented good structures with no cellular lesions being observed. There were no significant differences when comparing both the slides of the organs from tested animals and the controls, which suggesting that the crude extracts did not interact with the target cells or change the biological systems of the animals[11]. The study on brine shrimp lethalty test also shown that *P.indicus* showed low toxicity on shrimp.

Conclusion

Our results suggest that *P.indicus* ethanol extract does not cause any apparent *in vivo* toxicity in Brine shrimp and also in an animal model.

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