Histopathological Analysis of the Hepetoprotective Property of *Ipomea Aquatica* (Water Spinach) Methanolic Extract against Acetamenophen-Induced Liver Damage Sprague Dawley Rats

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ABSTRACT

Acetaminophen also known as Paracetamol is an over-the-counter drug that is widely used as analgesic and antipyretic. It is generally considered safe when taken in the recommended dose (650 mg - 1000 mg every four (4) to six (6) hours). However, when taken in relatively large amount or overdosed, it tends to lead to hepatotoxicity and acute liver failure. Ipomea aquatica shows hepatoprotective property due to its certain effective component that exerts antioxidant capacities in vivo. In this study, the researchers investigated the hepatoprotective effect of I. aquatica methanolic extract against acetaminophen- induced liver damage in Sprague dawley rats. Results confirm that many of the slides showed no apparent lesion. The only lesion observed was the presence of generalized cloudy swelling. This kind of lesion was prevented in most of the liver given with water spinach extract. The dose of 1 g/kg had the same effect as the 2 g/kg extract. Based on the data obtained, the study concludes that the kangkong extract had apparently prevented the formation of lesion known as cloudy swelling or cytoplasmic granulation.

Keywords: Acetaminophen, Hepatotoxicity, Ipomeaaquatica, Hepatoprotective, Cytoplasmic granulation

INTRODUCTION

Acetaminophen also known as Paracetamol is an over-the-counter drug that is widely used as analgesic and antipyretic. It is a very safe drug when taken according to the recommended dose (650-1000mg every four (4) to six (6) hours) however, if taken in excessive amount it will be oxidized to a toxic metabolite N-acetyl-p-benzoquinone-imine (NAPQI) which leads to liver damage or hepatotoxicity. Drug-induced hepatotoxicity is one of the major causes of liver failure. Liver is the major site for detoxification and metabolism of drugs. Worldwide estimations show that 844 million people have liver disease and based from the report of World Health Organization, in the year 2017, the mortality rate attributed to liver disease in the Philippines reached 1.36% of total deaths.

Ipomoea aquatica or water spinach belongs to the Convolvulaceae family. It is used traditionally for the treatment of jaundice and liver diseases. The antioxidant property of water spinach is said to be due by the phytochemical, Flavonoid.

As the modern medicine fails to provide cure to certain diseases in a completely harmless and chemically untouched way, the research for natural ways and product in providing treatment for diseases still prevails. In some of the researches done, the hepatoprotective property of *I. aquatica* has been tested on the thioacetamide liver damage in rats (Alkiyumi, Abdullah, Alrashdi, Salama, Abdelwahab, and Hadi, 2012) and the protective effect of the Red-stemmed type of *I. aquatica*Forsk against CCl_4- induced oxidative liver damage in rats (Hirai, Ishibuchi, Watabe, Makita, Kishida, Takagaki, Kurauchi and Egashira, 2011); it has been proven that the plant has a significant property in protecting the liver from these said chemicals.

Review of literature

Acetaminophen (APAP) toxicity precipitates from either over dosage or drug abuse. Acute overdose is the excessive utilization of toxic amount of the drug within eight (8) hours, while chronic overdose is the result of consumption of repeated doses at or above the recommended limit. Unintentional overdose precipitates from consumption of multiple products containing acetaminophen. Acetaminophen overdose could result in multiple interrelated biochemical reactions is the liver, such as formation of reactive oxygen species, oxidation of proteins, lipid peroxidation and DNA fragmentation.

Acetyl-Para-Aminophenol (APAP) is metabolized by phase II conjugating enzymes, mainly by UDP-glucuronosyltransferase(UGT) and sulfotransferase (SULT), converting it to non-toxic metabolite which are excreted in the urine. Only 2% is excreted unchanged in the urine. And approximately 5–9% is metabolized mainly by CYP 2E1 into toxic metabolite which is known to be N-acetyl-p-benzoquinone imine (NAPQI). In general, NAPQI is detoxified by conjugating with Glutathione (GSH). However, excessive NAPQI depletes GSH following APAP overdose which results in mitochondrial oxidative stress and dysfunction, ultimately hepatocytes necrosis. (Yan, Huo, Yin, and Hu,(2018).

Kangkong is also known as water spinach, river spinach, water morning glory, water convolvulus. It flourishes naturally in water which requires little care which commonly found in the banks of streams, ponds, ditches, lakes and marshes that usually floats or creeps along the shorelines but it can also climb over emergent vegetation, some also thrives in the water.

The health benefits that you can get in *kangkong* or water spinach, is that it reduces cholesterol, helps in the treatment of jaundice, useful in treating anemia, in indigestion and constipation, and can act as an anti-diabetic, protection against heart diseases, prevention against cancer, and is beneficial for eyes, which can also boosts the body's immunity, and has an anti-ageing property.

This study aimed to determine the hepatoprotective property of *Ipomea aquatica* (water spinach) ethanolic extract against drug-induced liver damage and it specifically aims to seek information such and answer the following questions: 1) to determine the presence of flavonoids in the plant extract, 2) comparison of the hepatosomatic index of rats among different treatment groups and 3) evaluate hepatoprotective activity of *I. aquatica* methanolic extract against acetaminophen-induced liver damage by histopathological analysis.

MATERIALS AND METHODS

The researchers used the experimental research design. This was utilized to analyze the hepatoprotective property of *Ipomea aquatica* against acetaminophen-induced liver damage in Sprague-dawley rats. According to Castillo (2002), experimental method is defined as a research design wherein the cause and effect relationship of a treatment on a variable is determined. Moreover, a qualitative approach will be applied in the experiment as subjects for the study will purposively and carefully be chosen and the study does not seek to measure numerical data but to test the hepatoprotective property of the *I. aquatica* extract.

The research was carried out using Sprague Dawley rats (120g–200g). The animals were caged in 25 ± 3 °C temperature, 50–60% humidity, and under a 12 h light-dark cycle for a week before the experiment. The animals were provided with five (5) days acclimatization. The animals were maintained at standard housing conditions and had free access to standard diet and water during the experiment. The experiment was performed in accordance to the rules and regulations of the Conduct of Scientific Procedures using Animals.

Seventy-five (75) Sprague Dawley rats were randomly divided based on the weight of the rats into five (5) groups according to the five (5) treatment plan, each group is composed of five (5) rats. The first (1st) group served as the positive control which will only receive the toxic dose of acetaminophen (500mg/kg), the second (2nd) group served as the negative control which will only be receiving corn oil, the

third (3rd) group received both acetaminophen (500mg/kg) and silymarin (50mg/kg), the fourth (4th) both receivedg acetaminophen(500mg/kg) and *Ipomea aquatica* extract (2ml/kg (1g/ml)), the fifth (5th) or last group received both acetaminophen (500mg/kg) and *I. aquatica* extract (2ml/kg (2g/ml)). All the treatment plans were replicated three (3) times. The administration of drugs and extracts was done every day for thirty (30) days.

The plant material *Ipomea aquatica* was acquired from Barangay Lawa Calamba City, Laguna. The plant species was authenticated by Forest Products Research and Development Institute under Department of Science and Technology at the University of the Philippines Los Baños.

The plant material used was first wash with tap water to remove soil and dirt then with distilled water for 1 minute each stem. It was then air dried for five (5) days only to avoid the formation of molds and grind into fine powder using a mechanical blender (Osterizer ® Commercial Blender). The dried plant was powdered and extracted with 99% methanol for 72 hours in room temperature. Using a magnetic stirrer, the suspension was stirred from time to time for the powder to be fully dissolve in the methanol. The extract was filtered through cheesecloth followed by filtration through Whatman No. 1 filter paper and then evaporated using the rotary evaporator.

Liver histopathological examinations was carried out by the use of a light microscope. Liver is fixed in 10% buffered formaldehyde, and then processed by programmed tissue processing machine and after that was embedded in paraffin wax. Following embedding a 5 μm thick section is prepare and stain with hematoxylin-eosin (HE) for photomicroscope assessment.

RESULTS AND DISCUSSION

Table 1 shows the results of the screening test for flavonoids, the presence of yellow color indicates the occurrence of flavonoids. In Willstatter method there is an appearance of red color which indicates the presence of flavonoids.

Table 1. Screening Test for Flavonoids

Tests	Theoretical Results	Actual Results	Interpretation
Test for flavonoids	Yellow Color	Presence of Yellow color	Presence of Flavonoids (+)
Willstatter test	Orange to Red, Crimson and Magenta or Greenish to Blue	Presence of Red Color	Presence of Flavonoids (+)

(Aguinaldo et al,2006)

The results in the hepatosomatic index (HSI) were significantly higher with the experimental rats belonging to the positive control group when compared with those in the negative control group. This explained that there is an enlargement of liver or otherwise known as hepatomegaly which is caused by toxin poisoning. HSI values of the *Ipomea aquatica* extract treated groups was significantly comparable with the HSI values of the negative control and silymarin treated group. One of the rats in the group treated with 1g/kg of the *I. aquatic* extract showed generalized cloudy swelling of the liver. The rest of the rats in

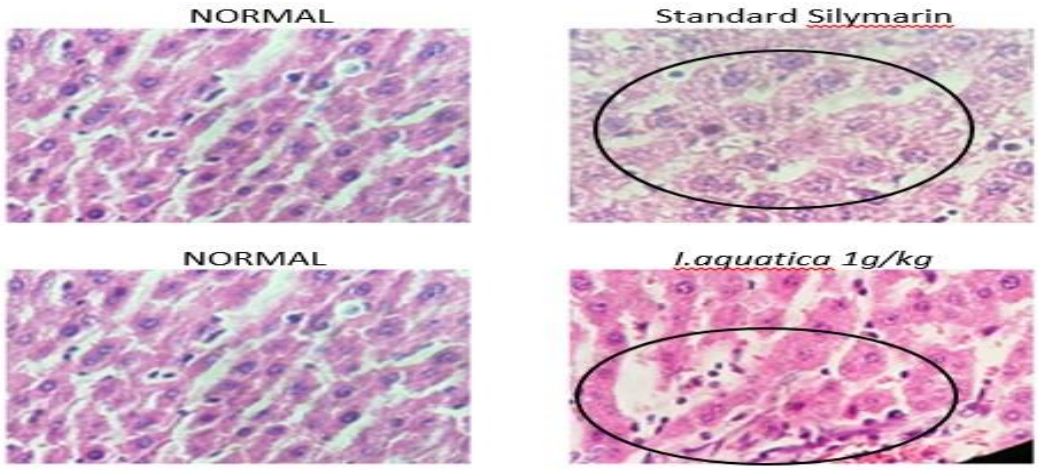
the same treatment group exhibited no apparent lesion, while all the rats in the second treatment group of 2g/kg of the plant extract also manifested no cytoplasmic granulation.

This kind of minor lesion was prevented in most of the liver given *kangkong* extract. Many of the slides showed No Apparent Lesion. No Apparent Lesion (NAL) means there were no lesions noted in any form like vascular changes (congestion, edema, hemorrhages), degenerative lesion (atrophy, aplasia), proliferative lesions (fibrosis, leucocytosis, hemosiderosis), inflammatory lesions (hepatitis, pancreatitis) or even neoplastic lesions (carcinoma, lymphoma). The only lesion observed was the presence of generalized cloudy swelling or cytoplasmic granulation in the standard Silymarin slide and *I. aquatic* extract.

Table 2. Comparison of the Hepatosomatic Index of Rats among Different Treatment Groups

Treatment	Weight of Rat in Grams	Weight of Liver in Grams	Hepatosomatic Index
Negative control			
Animal No.1	201.5	6.29	3.12
Animal No.2	200	6.74	3.37
Positive control			
Animal No.1	211	8.13	3.85
Animal No.2	197	6.74	3.42
Silymarin			
Animal No.1	202	6.17	3.05
(/)Animal No.2	216.5	6.96	3.21
Animal No.3	200	5.97	2.99
Ipomea aquatica extract (1g/kg)			
(/)Animal No.1	190	6.07	3.19
Animal No.2	195	6.14	3.15
Animal No.3	185	5.48	2.96
Ipomea aquatica extract (2g/kg)			
Animal No.1	191	5.85	3.06
Animal No.2	210	7.04	3.35
Animal No.3	200	6.57	3.29

The results on the histopathological slides showed No Apparent Lesion (NAL) means that there were no lesions noted in any form like vascular changes (congestion, edema, hemorrhages), degenerative lesion (atrophy, aplasia), proliferative lesions (fibrosis, leucocytosis, hemosiderosis), inflammatory lesions (hepatitis, pancreatitis) or even neoplastic lesions (carcinoma, lymphoma). The only lesion observed was the presence of generalized cloudy swelling or cytoplasmic granulation in the standard Silymarin slide and *I. aquatica* extract as shown above. Cloudy swelling is a very minor lesion which is the first reaction of the liver cell (hepatocyte) to minor irritation or stimulus. According to the veterinary, “there are certain factors that could have affected the function of the liver such as age, diet and abnormal genes that are inherited thus producing the minor lesions. This kind of minor lesion was prevented in most of the liver given kangkong extract, since the hepatocyte images shows results comparable to normal



CONCLUSION AND RECOMMENDATIONS

The study suggested that *I. aquatica* has hepatoprotective property comparable with the food supplement silymarin, thus frequent consumption can counteract the cellular oxidative damage that is caused by acetaminophen intoxication. Findings showed that the *I. aquatica* extract provides a promising hepatoprotective property that can also be considered for other oxidative stress related liver diseases.

Therefore, to further improve the study the following actions may be take into considerations such as the length of time for induction of liver damage using any hepatotoxic material be prolonged together with any extra that will have any hepatoprotective action. This kind of hepatic toxicity takes time to develop since it is chronic induction that can take more than 10 months for proper observation and interpretation. Using Blood test as another basis of liver function analysis and testing the hepatoprotective property of the plant extract against other medications that could induced hepatic damage.

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