



Hepatoprotective Activity of *Ficus Pseudopalma* Blanco against Acetaminophen-Induced Liver Toxicity in Sprague-Dawley Rats

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Research Article

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ABSTRACT

The hepatoprotective activity of *F. pseudopalma* was evaluated against acetaminophen-induced liver toxicity and was compared with N-acetyl cysteine (NAC) and Lupeol. Acetaminophen (500mg/kg BW) was induced daily to the rats for seven days and the plant extract (200mg/kg and 400mg/kg), NAC (100mg/kg) and lupeol (10mg/kg) were administered four hours after acetaminophen induction. The liver index, biomarkers of liver damage (ALT, AST, ALP and albumin) and antioxidant enzyme levels were (GSH and catalase) all determined. Histopathological analysis of the liver samples was performed to further assess the protective ability of *F. pseudopalma*. Following the 7-day oral administration of *F. pseudopalma* at 200mg/kg and 400 mg/kg BW ameliorated the toxic effects brought by the overdose of acetaminophen. The liver enzymes in the sera were normalized, the liver index improved, glutathione and catalase levels restored and lipid peroxides remarkably reduced in the liver homogenate although albumin levels remained unaltered. Histopathological examination indicated an improved condition of the hepatocytes though not completely healed or repaired as result of the 7-day continued acetaminophen-induced injury in the liver. These data suggest that administration of antioxidants such as *F. pseudopalma*, lupeol and NAC may be important in the repair of damage brought by acetaminophen-induced hepatotoxicity.

Keywords: *Ficus pseudopalma*, N-acetylsytein, Lupeol, hepatoprotective, acetaminophen, antioxidant

Introduction

Hepatotoxicity and drug-induced injury account for a big number of death, hospital admission and acute liver failure (ALF) worldwide. Acetaminophen is among the commonly available non-prescription analgesics in the Philippines. This over-the-counter (OTC) drug serves as an active ingredient in most popular pain medications and muscle relaxants that are readily consumed in therapeutic doses over time or even too much in committing suicide without realizing that the drug may cause subclinical damage to the liver. Acetaminophen produces a potentially fatal, hepatic centrilobular necrosis when taken in overdose [1]. Acetaminophen overdose, publicized to trigger the most number of ALF cases in the United States, leads to mitochondrial dysfunction and nuclear DNA fragmentation, resulting in necrotic cell death [2]. Acetaminophen is metabolically activated by a group of enzymes located in the endoplasmic reticulum by cytochrome P450 in the liver to a reactive metabolite identified to be N-acetyl-p-benzoquinone imine (NAPQI) [3,4] instigating glutathione (GSH) depletion that led to the development of the currently used antidote N-acetylcysteine (NAC) in the market for acetaminophen overdose.

Previous study showed that *F. pseudopalma* Blanco of the Moraceae family, an endemic medicinal plant widely cultivated in the country, is a potent antioxidant and scavenger of various free radicals such as DPPH, nitric oxide and lipid peroxides [5,6] in which lupeol, a pentacyclic triterpene, was found to be one of the major bioactive compounds [7]. Toxicity study also revealed that *F. pseudopalma* has no toxic effects towards Sprague-Dawley rats at 2000mg/kg dose [8]. In addition to that, the plant was also studied for its anti-urolithiatic activity and was shown to effectively lessen the effect of ethylene glycol-induced kidney stone formation in rats [9]. Lastly, *F. pseudopalma* was also demonstrated to have a good cytotoxic activity towards hepatocellular carcinoma (HpeG2) and prostate (PRST2) cancer cell lines [10,11]. However, *F. pseudopalma* has not been investigated in liver-damaged animal model. This study aimed to demonstrate whether the administration of this



plant can ameliorate the effects of acetaminophen-induced hepatotoxicity in rats by studying the levels of biomarkers of liver damage compared to lupeol and NAC.

Materials and Method

Experimental Animals and Induction of Liver Damage using Acetaminophen

The crude ethanolic leaf extract of *F. pseudopalma* was evaluated for its hepatoprotective activity using female Sprague-Dawley rats induced with acetaminophen.

The animal experiment was approved by the University of Santo Tomas Institute of Animal Care and Use Committee (UST-IACUC) and certified by the Bureau of Animal Industry (BAI) and the experimental animals were purchased from the Food and Drug Administration.

The rats were numbered and randomly grouped into six (6), consisting of six (6) rats each:

Group 1-Normal Control (2% Tween 80)

Group 2-Acetaminophen (500mg/kg BW)

Group 3- Acetaminophen + *F. pseudopalma* (200mg/kg BW)

Group 4- Acetaminophen + *F. pseudopalma* (400mg/kg BW)

Group 5- Acetaminophen + Lupeol (10mg/kg BW)

Group 6- Acetaminophen + N-acetylcysteine (100mg/kg BW)

All treatments were done orally for seven (7) days following a previously described protocol with some modification [12]. Acetaminophen was administered four (4) hours before administration of the *F. pseudoaplma* extract, lupeol and NAC. Single overdose of acetaminophen or repeated and excessive ingestion of therapeutic doses may lead to its accumulation in the body that further lead to hepatotoxicity. Acetaminophen toxicity can be explained by its metabolism inside the body, which involves its oxidation to form N-acetyl-p-benzoquinoneimine (NAPQI) [13]. NAPQI is a highly reactive intermediate alkylating metabolite that is when produced in higher amounts (due to acetaminophen overdose) bonds covalently with various cell proteins forming inactive conjugates [14]. These inactive conjugates contribute to the irreversible hepatocyte injury and necrosis. Also during this certain period, levels of reduced glutathione (GSH) and other antioxidant enzymes such as catalase is lower compared to that of lipid peroxides, which are produced via the augmentation of oxidative stress [15].

To understand the mechanism of action of *F. pseudopalma* this study was undertaken. In this experiment, the initial weight of each rats were noted and blood was collected in order to determine the initial ALT, AST, albumin and alkaline phosphatase levels in the serum. The rats were then treated daily with the specified doses of the chemicals and extract using an oral gavage and were allowed to feed with ordinary rat chow and drinking water. Blood serum was again collected on 8th day and the final weight of each rats was also noted.

The rats were killed via cervical dislocation and the livers were collected. Two livers from each group were placed in separate container with 10% buffered formalin and were sent to Hi-Precision Diagnostics for histopathological analysis. The other four livers from each group were placed in a plastic container

with potassium phosphate buffer (pH 7.4) and were used for the antioxidant assays (GSH, lipid peroxidation). All colorimetric assays were done using Corona micro plate reader SH-1000 (Hitachi, Japan).

Assessment of the Biochemical Parameters and Antioxidant Enzyme Levels

Colorimetric analysis of the biochemical parameters were performed using commercially available test kits. These included the measurement of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and albumin. In addition to that, levels of antioxidant enzymes such as the reduced glutathione (GSH), lipid peroxides and catalase were also estimated.

Statistical Analysis

Results were expressed as mean± SD and statistical analysis was performed using ANOVA, to determine the significant differences between the groups with $p < 0.05$ implied significant.

Results & Discussion

It has long been established that NAPQI-mediated mitochondrial injury of hepatocytes, an important reactive intermediate and a big source of initiating reactive oxygen species (ROS) and reactive nitrogen species, play important role in acetaminophen-induced hepatotoxicity.

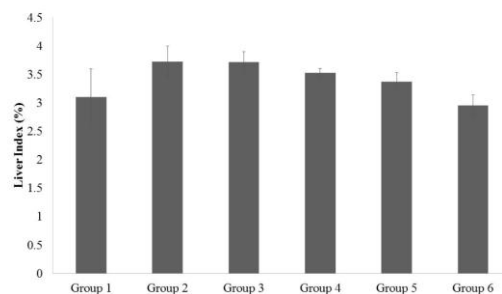


Figure 1. Liver index (%) of the different treatment groups. Values (n=6) represent mean±SEM, $p < 0.05$).

Liver Index

The weight of each rats were noted prior to cervical dislocation. The livers were obtained and weighed to determine the liver index. The liver index is an indicator of liver toxicity and was evaluated by obtaining the ratio of the liver weight to the body weight of each rats [17]. Figure 1 presents the mean liver index for each group, where Group 2 (Acetaminophen [500mg/kg] control) had the highest liver index followed by Group 3 (200mg/kg *F. pseudopalma*). The liver index for Group 6 (100mg/kg NAC) was closely similar to that of Group 1 (Normal control).

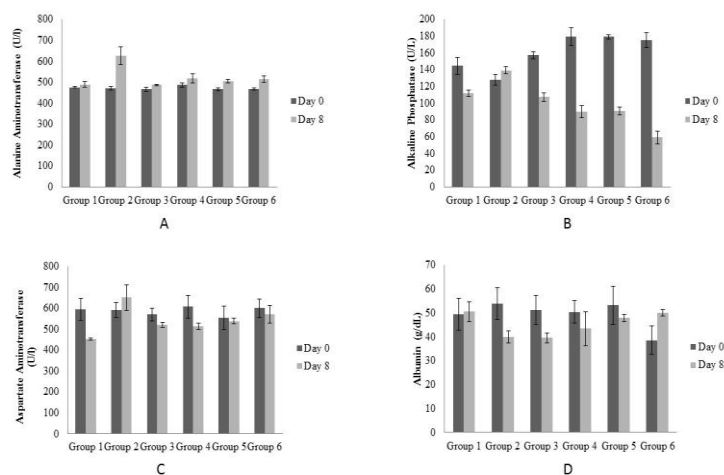


Figure 2. Measurement of liver enzyme levels in rats expressed as mean±SEM after induction of acetaminophen and treatment of *F. pseudopalma*, lupeol and NAC. n=6; p<0.05

The significant increase in the liver weight in paracetamol toxicity may be related to the accumulation of red blood cells in the sinusoids that resulted from the decreased intra-hepatic and portal vein pressure [17].

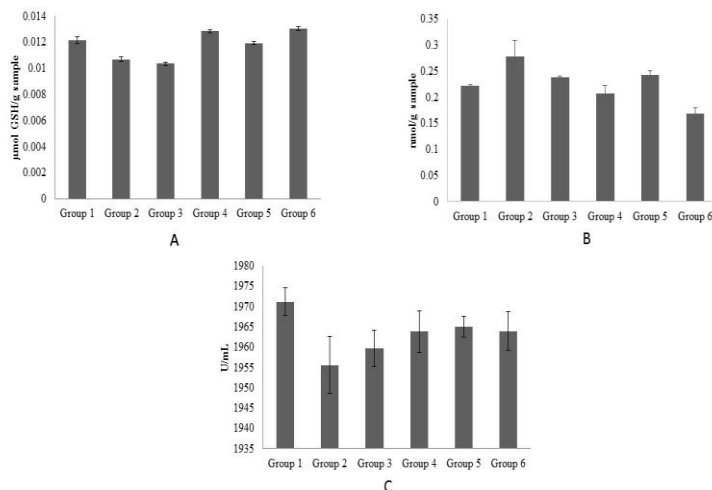


Figure 3. Measurement of antioxidant enzyme levels in rats expressed as mean±SEM after induction of acetaminophen and treatment of *F. pseudopalma*, lupeol and NAC. n=6; p<0.05

Biochemical Parameters and Antioxidant Enzyme Levels

Alanine aminotransferase (ALT) is the enzyme involved in the metabolism of cellular nitrogen and amino acid as well as in the liver gluconeogenesis [18]. The major function of the enzyme is the conversion of alanine to glucose which is then transported out of the liver to the other organs. Normally, ALT levels are low and elevation of serum ALT indicates liver damage since this protein is specifically and abundantly present in the liver [19]. As shown in Figure 2A, there is a significant increase in ALT levels in acetaminophen treated

group (Group 2) while the rest of the groups had a lower value of ALT that still fall within the normal range.

Aspartate aminotransferase is responsible for the catalyzing the transfer of amino and keto groups between α -amino and α -keto acids. This enzyme is widely distributed in the body tissues and any damage or injury on the organs may result to the increase levels of AST in the serum [20]. As shown in Figure 2B, AST levels of the acetaminophen treated group (Group 2) had the highest AST levels in Day 8 as compared to that of the other groups. The damage that was inflicted by acetaminophen on the other groups of rats were somehow decreased by the crude ethanolic leaf extract of *F. pseudopalma* using both concentrations (200 and 400mg/kg). These observed lowering of AST are comparable to that of the NAC- and lupeol-treated rats.

Alkaline phosphatase (ALP) is a non-specific metalloenzyme responsible for the hydrolysis of various types of phosphate esters at alkaline pH in the presence of zinc and magnesium. The ALP activity was determined based on a colorimetric method using p-nitrophenylphosphate as substrate. The ALP activity is directly proportional to the rate of increased absorbance of the reaction mixture. As observed in Figure 2C, Group 2 (acetaminophen, 500mg/kg) had the highest ALP levels as compared to that of the rest of the groups. The alkaline phosphatase levels in groups 3, 4, 5 and 6 were significantly decreased during the 8th day.

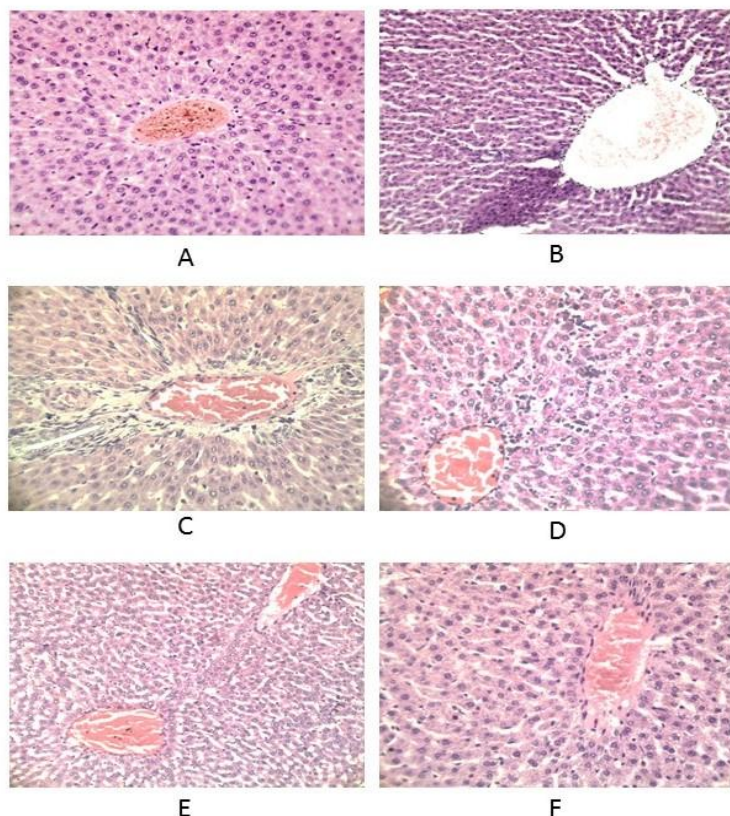
Lastly, albumin is the most abundant plasma protein (60%) in the body. It carries out many important physiological functions including maintenance of plasma osmotic pressure and the transport of nutritional substances [21]. Colorimetric assay was performed to determine the levels of serum albumin, which involves the use of bromocresol green that changes its color to green when it reacts with albumin in the presence of citrate buffer [22]. As demonstrated in Figure 2D, Groups 5 (10mg/kg Lupeol) has a comparable albumin level as to that of Group 6 (100mg/kg NAC), whereas Group 2 (Acetaminophen, 500mg/kg) and Group 3 (200mg/kg *F. pseudopalma*) had the lowest. As presented in Figure 3A, reduced glutathione levels of Group 4 (400mg/kg *F. pseudopalma*) and Group 6 (100mg/kg NAC) had an almost similar values with group 2 (500mg/kg Acetaminophen) and Group 3 (200mg/kg *F. pseudopalma*) as the lowest. GSH is a direct scavenger of NAPQI and is responsible for detoxifying ROS such as H₂O₂ and reactive nitrogen species (RNS) such as nitric oxide radicals (NO•). However in case of acetaminophen overdose, there is a higher level of NAPQI that cannot be suppressed by GSH and thus, lead to the production of ROS and RNS due to oxidative stress.



Table 1. Surgical Pathology Report of Rat Livers

Group	Gross Description	Microscopic Description
1-Normal	The liver sample measured 3.0x3.0x1.0cm, tan brown, smooth, soft, multilobulated tissue	Polyhedral cells with well-defined cytoplasm with prominent nucleus and nucleolus. Intact central vein filled with red blood cells and surrounded by healthy hepatocytes
2-Acetaminophen induced control (500mg/kg)	The liver sample measured 3.5x3.2x1.5cm, brown, soft, multilobulated tissue	There is a widespread lysis of the cytoplasm of hepatocytes and loss of hepatocytic individualization; some apoptotic and pyknotic nuclei seen. There is a prominent sinusoidal congestion and dilation. There are minimal aggregates of inflammatory cells predominantly lymphocytes and some neutrophils in the portal tracts and around blood vessels.
3-Acetaminophen + Low dose crude FP (200mg/kg)	The liver sample measured 3.7x3.1x1.0cm, brown, smooth, soft, multilobulated tissue	There is lysis of the cytoplasm of hepatocytes and partial loss of hepatic individualization. Necrosis is not observed. There are aggregates of inflammatory cells predominantly lymphocytes, some neutrophils and macropahges in the portal tracts and around blood vessels.
4-Acetaminophen + High dose FP (400mg/kg)	The liver sample measured 4.0x2.8x1.5cm, brown, smooth, soft, multilobulated tissue	There is lysis of the cytoplasm of hepatocytes and partial loss of hepatic individualization. Some sinusoidal congestion is present but not heavily distributed throughout the tissue.
5-Acetaminophen + Lupeol (10mg/kg)	The liver sample measured 4.5x3.5x2.0cm, lobulated, dark brown and soft on-cut	There is a moderate coagulative necrosis prominently in hepatocytes surrounding central vein. There is minimal bile duct hyperplasia and moderate infiltration of fibrous connective tissue around bile duct. Few lymphoid aggregates in portal tract.
6-Acetaminophen + NAC control (100mg/kg)	The liver sample measured 4.0x3.0x2.0cm, lobulated, dark brown and soft on-cut	There is a moderate coagulative necrosis prominently in hepatocytes surrounding central vein. The bile ducts are within normal limits. There is minimal infiltration of fibrous connective tissue around bile duct. Moderate number of lymphoid infiltrates in portal tract.

Figure 4. Histopathological examination of rat livers. Representative micrographs of liver from Group 1 (A), Group 2(B), Group 3 (C), Group 4 (D), Group 5 (E) and Group 6 (F)



This would imply that there is a need of a good scavenger of these reactive species in order to maintain the homoestasis inside the body. This event is shown in Figure 3A, wherein acetaminophen treated group (500mg/kg) Group 3 had low levels of GSH as compared to that of Group 4-7, which are treated with *F. pseudopalma* (200mg/kg and 400mg/kg), Lupeol (10mg/kg) and NAC (100mg/kg), respectively.

As demonstrated in Figure 3B, there is high levels of lipid peroxides that were produced in acetaminophen-induced groups (Group 2) and lower levels in Groups 3 to 6. This would also show the antioxidant capacity of *F. pseudopalma* that is comparable to that of NAC. As previously determined, the crude ethanolic leaf extract of *F. pseudopalma* is a good scavenger of lipid peroxides *in vitro* [5].

As shown in Figure 3C, catalase level of rats in Group 2 (500mg/kg acetaminophen) was lower compared to the other groups. Catalase levels of the Groups 4, 5 and 6 have almost similar values but is lower compared to that of the normal control group.

Histopathological Analysis

Histopathological analysis was performed and interpreted in the Hi-Precision Diagnostics



laboratory. The results of the analysis are summarized in Table 1. The analysis was reviewed by a registered medical technologist and certified by a pathologist.

Microscopic examination of liver samples often reveals histopathological changes after acetaminophen overdose [17].

Conclusion

The antioxidant biomarkers as well as the liver enzymes were somehow normalized by the treatment of *F. pseudopalma* extract. There seemed to have no remarkable change or reversion of damage induced by acetaminophen in the histopathological examination of the rat livers in the *F. pseudopalma*, lupeol and NAC groups. This is perhaps due to the short duration of the study that was only limited to eight (8) days following seven days of repeated acetaminophen assault.

Anyhow, the results on the plant's effect on the liver enzymes and antioxidant biomarkers support a probable hepatoprotective activity of *F. pseudopalma* and would therefore indicate that it may be a useful antidote for acetaminophen overdose like N-acetylcysteine (NAC).

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AUTHORS' CONTRIBUTIONS

Authors contributed equally to all aspects of the study.

PEER REVIEW

Not commissioned; externally peer reviewed.

CONFLICTS OF INTEREST

The authors declare that they have no competing interests.