Original Research Article

Evaluation of ameliorative effects of *Phyllantus amarus* and *Senna alata* in acetaminophen-induced toxicity in rats

Abstract

Back ground: Liver and kidney are basic organs in the body because they perform vital functions in the system, the preventing of injury to them is necessary as well as the management of injury on them is vital. *P. amarus* and *S.alata* were plants that had been used to protect the liver and traditionalist used them in management of jaundice but there was no study to evaluate their ameliorative effects on liver and kidney damage and to comparatively identify the one that has more efficacy in managing the organs.

Study Design: Experimental Design

Place and Duration of Study: Department of Pharmacology and Toxicology, National institute of Pharmaceutical Research and Development, Idu, Abuja, between February to April 2023.

Methods: The hydroethanolic (70%) extract of both plants were obtained by maceration. Experimental animals were divided in to 9 groups, group I were given distilled water, group II were given Sylimarin and group III were given acetaminophen only, group IV-VI were given ACP (850mg/kg) before PAM 100mg/kg, 300mg/kg,and 900mg/kg respectively while group VII-IX were given ACP before SA at same three doses for 14 days. Acetaminophen (850mg/kg) was administered a day after fasting before the extract(s); Gross weighing of the organs was done, sample for biochemical analysis and organs for histopathological evaluation were collected.

Results: *P. amarus* at all the doses demonstrated ameliorative effects, reducing the weight of the liver at 300mg/kg and 900mg/kg (P≤.01), decreased significantly (P≥.0001) the elevated level of all the parameters (ALP,AST,ALT,BUN,T.BIL) with no visible lesion in the histopicture of the organs. *S alata* significantly decreased (P≥.0001) the biochemical parameters at 300mg/kg

Conclusion: The result of this study showed that both plants have ameliorative effects but *P. amarus* has more ameliorative potentials.

Key words: ameliorative, toxic, histopathology, biochemical, comparative

1. INTRODUCTION

The liver is a vertebrate organ that purifies different metabolites, generates proteins, and creates biochemicals required for digestion [1]. It is engaged in carbohydrate digestion; it generates and accumulates glycogen via glycogenesis; and it is in charge of protein metabolism, including production and breakdown [2]. The liver additionally plays an important role in lipid metabolism, as it produces cholesterol, lipogenesis, and triglycerides [3]. It is essential for digestion because it creates and excretes bile (a yellowish liquid) that is needed for emulsifying fats and aiding in the absorption of vitamin K from the food. The liver is in charge of insulin and other hormone breakdown [4]. In a process known as drug metabolism, it is crucial for eliminating or altering harmful compounds (such as methylation) and the majority of pharmaceuticals, which occasionally causes toxication when the metabolite is more harmful than its precursor and caused drug-induced hepatotoxicity [5].

Acetaminophen is used as analgesic/antipyretic, it is associated with drug-induced liver injury (DILI) at an overdose [6,7]. Approximately half of all occurrences of acute liver failure today in the United States and Great Britain are caused by acetaminophen toxicity which can lead to a fatal hepatic centrilobular necrosis. Cytochrome P450 enzymes biologically activate it, resulting in the reactive metabolite N-acetyl-p-benzoquinone imine (NAPQI), that liver damage is caused by low levels of glutathione (GSH), which can be prevented by replenishing the substance [8,9].

The herb *Phyllanthus amarus*, which is also known as stone breaker, is a member of the Euphorbiaceae family, and it is widespread in central and southern India [10]. For more than 2000 years, ayurveda medicine has utilized this plant to treat secondary hepatitis and other illnesses [9]. It has been applied topically as a poultice for skin ulcers, lesions, swelling, and itching [11]. It has also been used for the treatment jaundice, gonorrhea, frequent menstruation, and diabetes.

Senna alata, referred to as Gelenggang, is a significant Fabaceae flowering tree that is used for both ornamental and therapeutic purposes. It is an annual and, sometimes, biennial herb that grows in tropical regions with warm, humid climates, such as Southeast Asia and Africa [12]. According to studies by Dahie et al. [13] and Uwazie et al. [14], the pharmacological actions of *Senna alata* include antibacterial, cytotoxic, anti-inflammatory, anti-malaria, antifungal, hepatoprotective effects, antiseptic, and antiviral properties.

There is a need to evaluate the efficacy of both plants in the management/amelioration of liver and kidney damage caused by accidental acetaminophen ingestion at overdose or acetaminophen toxicity in order to determine which is preferable. The majority of research has focused on the protective effects of the plants on either the liver or the kidney.

2. MATERIAL AND METHOD

2.1 Plant material

About 3000g of the whole plant of *P. amarus* was collected from the medicinal plant department of NIPRD. The leaves of *P. amarus* was protected from heat and direct sunlight for about 4 weeks. The dried leaves and stems were homogenized to fine powder using Laboratory Hammer mill (Zhen Chang Equipment SFS P66), 200g of the powder was macerated in hydroethanolic solution (70%) at room temperature, for 72h with occasional shaking. After maceration, the material was filtered and the solvent was eliminated in a rotatory evaporator to obtained dried extract of *P. amarus*.

2.2 Pilot Toxicity Study

Acute toxicity study was done using 2000mg/kg body weight the extract. The animals were observed for changes in behavior and mortality for 24 hours and a week.

2.3 Experimental Animals and their care

A total of 45 rats of both sexes weighing 90-220g were purchased and kept in Experimental Animal Unit of the Department of Pharmacology and Toxicology, NIPRD where the study was also carried out. The animals were handled in accordance with international principles guiding the Use and Handling of experimental animals after an ethical approval was obtained with number NIPRD/05:03:05-41. The rats were be maintained on standard rat feed (Vital Feeds from Grand Cereals Limited, Jos Nigeria) The rats were maintained at an ambient temperature between 28-30^{0}c, humidity of 55±5%, and standard (natural) photoperiod of approximately 12 hours of lighting (06:30 hours – 18:30 hours) alternating with approximately 12 hours of darkness (18:30 hours – 06:30 hours)

2.4 Acetaminophen induced toxicity in animals

The animals were grouped in to 9 of 5 animals each;

Group 1 received acetaminophen for the first two days, Group 2 which served as normal control received distilled water orally for 14 days, Group 3 received acetaminophen (850 mg/kg) on day 1 and 2 before Sylimarin (100mg/kg) till day 14, group 4 animals received acetaminophen (850 mg/kg)on day 1 and 2 before PA (100mg/kg) till day 14, group 5 received acetaminophen (850 mg/kg) on day 1 and 2 before PA (300mg/kg) till day 14, group 6 received acetaminophen (850mg/kg) on day 1 and 2 before PA (900mg/kg) till day 14; the same dose for *S.alata* for group 7-9

2.5 Biochemical Analysis

After each experiment animals were anaesthesized with diethyl ether and blood samples were collected from retro-orbital vein into plain bottles for the determination of biochemical parameters such as Alkaline phosphatase (ALP), Aspartate transaminase (AST), Alanine transaminase (ALT), Blood urea (BUN), Creatinine (CRT), Total bilirubin (T. Bil), Direct bilirubin (D.Bil), uric acid (UA) and Total protein (T.PRO). Well-labeled plain bottles were used to collect 10 mL of blood sample, which was allowed to clot for 4 hours before centrifuging using Uniscope Laboratory Centrifuge (Model SM 112, Surgifriend Medicals, England) at 2000 revolution per minute for 20minutes to separate the sera from clotted blood cells. Each serum was carefully separated in the plain bottles that were well label accordingly at room

temperature of 23-26^{o}C. The activities of AST, ALP and ALT were estimated as described by Reitman and Frankel (1957). The activities of T. Bil were determined by colorimetric method using a kit supplied by Randox test kit (UK). The blood urea was determined using urease-Berthelot (enzymatic) colorimetric method and serum total protein was evaluated based on the Gornall *et al* (1949) method; Uric acid and creatinine were also investigated using standard procedures.

2.6 Gross and histopathology

The organs were identified (kidney and liver), sectioned and rinsed in normal saline. The tissue was be fixed in 10% formal-saline, dehydrated with 100% ethanol solution and embedded in paraffin and sectioned at 5µm, stained using H&E method, cleared in xylene and mounted in a mountant as was described by Akanbi & Taiwo in 2014, using the magnification of 400x.

2.7 Data analysis

The results were expressed as mean± standard error of mean (SEM) of 5 animals. One way analysis of variance (ANOVA) was used to compare the means between groups. It was followed by Dunnet test using GraphPad Prism software. P<0.05 was considered statistically significant.

3.0 Results

3.1 Biochemical Analysis of both extracts

3.1.1 Ameliorative effects of *P. amarus and S.alata* (100mg/kg)

The 100mg/kg of *P. amarus* significantly decreased (P≥ .0001) ALP, D.BIL, Urea, and CRT; with reduction (P=.0564) in T.Bil, (P≥ .50) in ALT, AST and uric acid but no significant difference in T.Pro when compared with the acetaminophen group. While *S. alata* at 100mg/kg decreased significantly (P≥ .0001) ALP but no significant difference in T.Pro, ALT and Uric acid when compared with the toxic group as shown in Fig 1

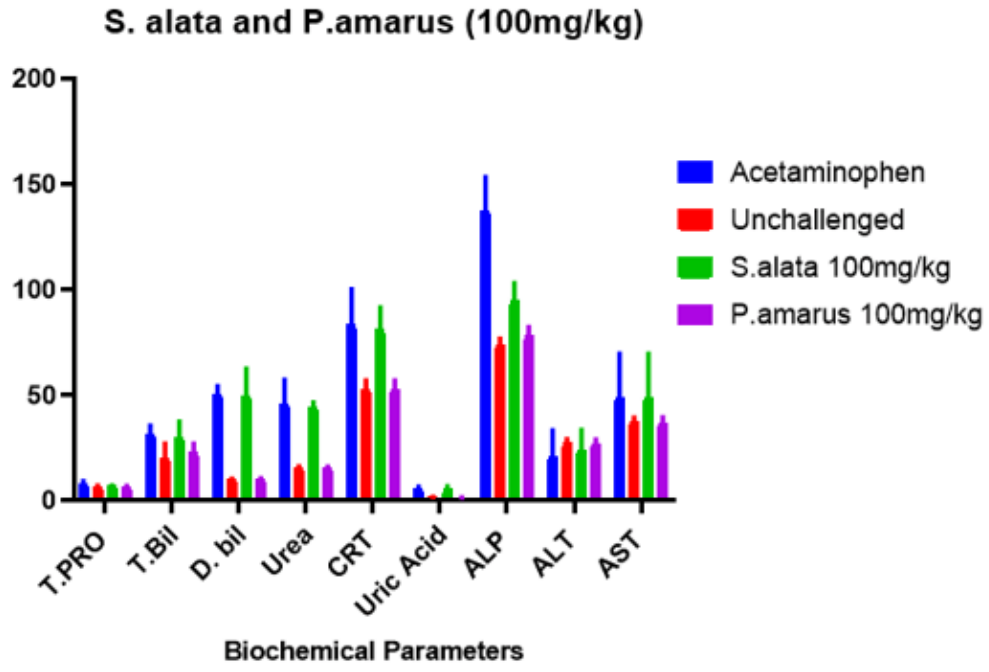


Fig.1. Effects of the extracts at 100mg/kg on the biochemical parameters

3.1.2 Ameliorative effect of *P. amarus and S.alata* 300mg/kg

In the 300mg/kg group, *P. amarus* significantly reduced (P≥ .0001) AST, ALT, ALP, BUN, CRT, T. Bil, and D.Bil; but there is no significant difference in T.Pro, and Uric acid when

compared with acetaminophen. *S. alata* also reduced (P≥ .001) significantly all the parameters except T.Pro, T.Bil, Urea and Uric acid when compared with the toxic group as demonstrated in Fig 2.

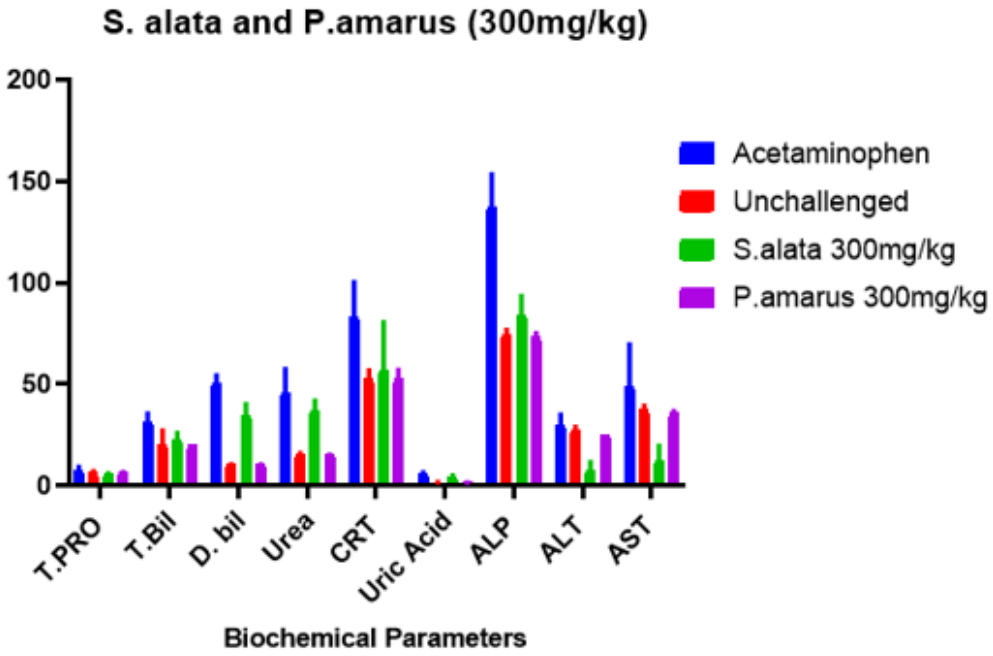


Fig. 2. Effect of the extracts at 300mg/kg on the biochemical parameters

3.1.3 Ameliorative effect of *P. amarus and S.alata* (900mg/kg)

The extract of *P.amarus* and *S. alata* at 900mg/kg showed significant reduction (P≥0.0001) in ALP , CRT, Urea, and D. Bil with no significant difference (P≥0.9000) in AST, ALT, T.Bil,Uric acid and T.Pro as shown in Fig 3.

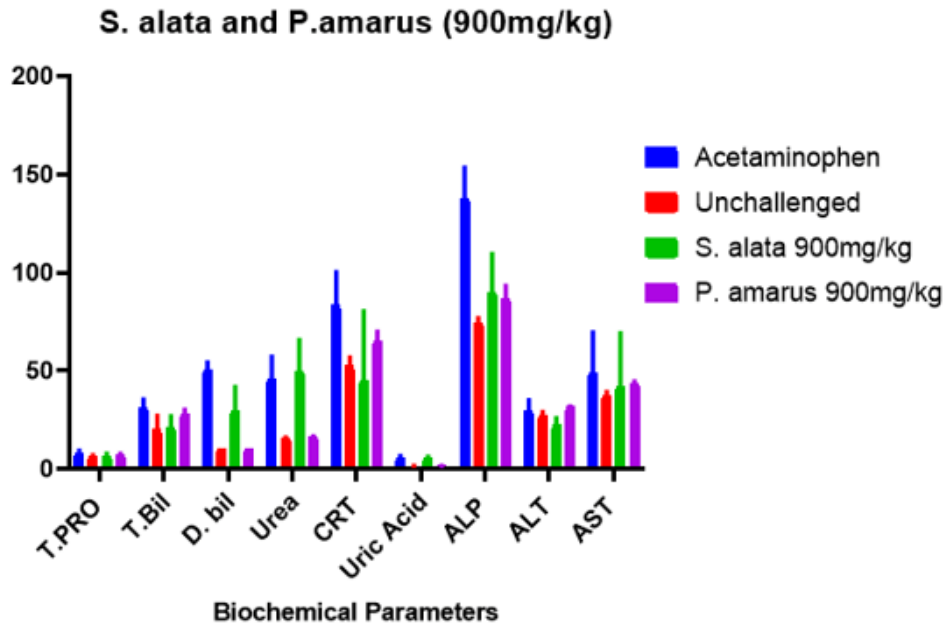


Fig.3. Effect of the extracts at 900mg/kg on the biochemical parameters

3.2 Gross weighing of the organs

The *P. amarus* at 300mg/kg and 900mg/kg showed significant reduction (P≤0.01) in the weight of liver while others showed no significant reduction when compared with acetaminophen group. There was no significant reduction in the weight of the kidney all the groups when compared with the toxic group as demonstrated in Fig 4.

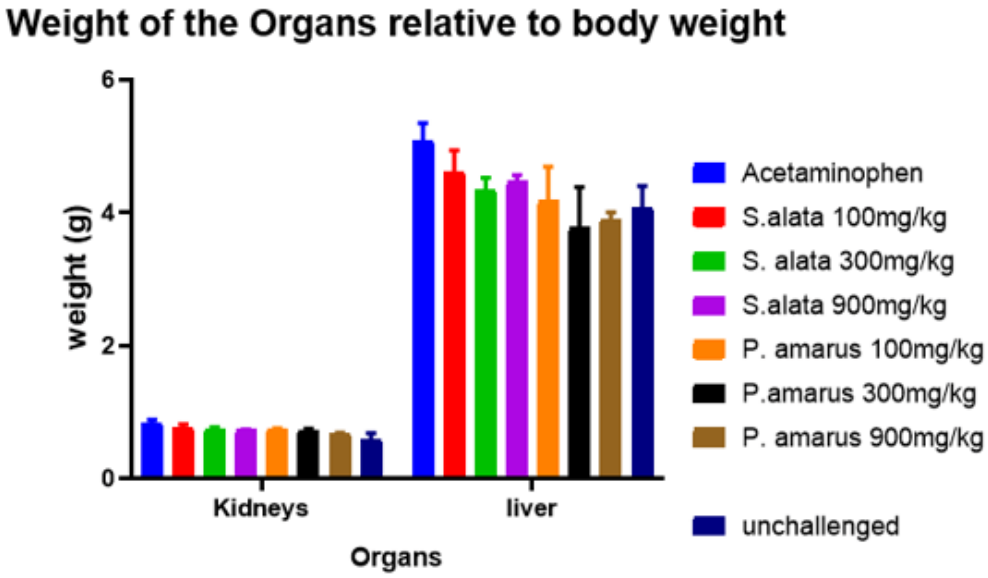
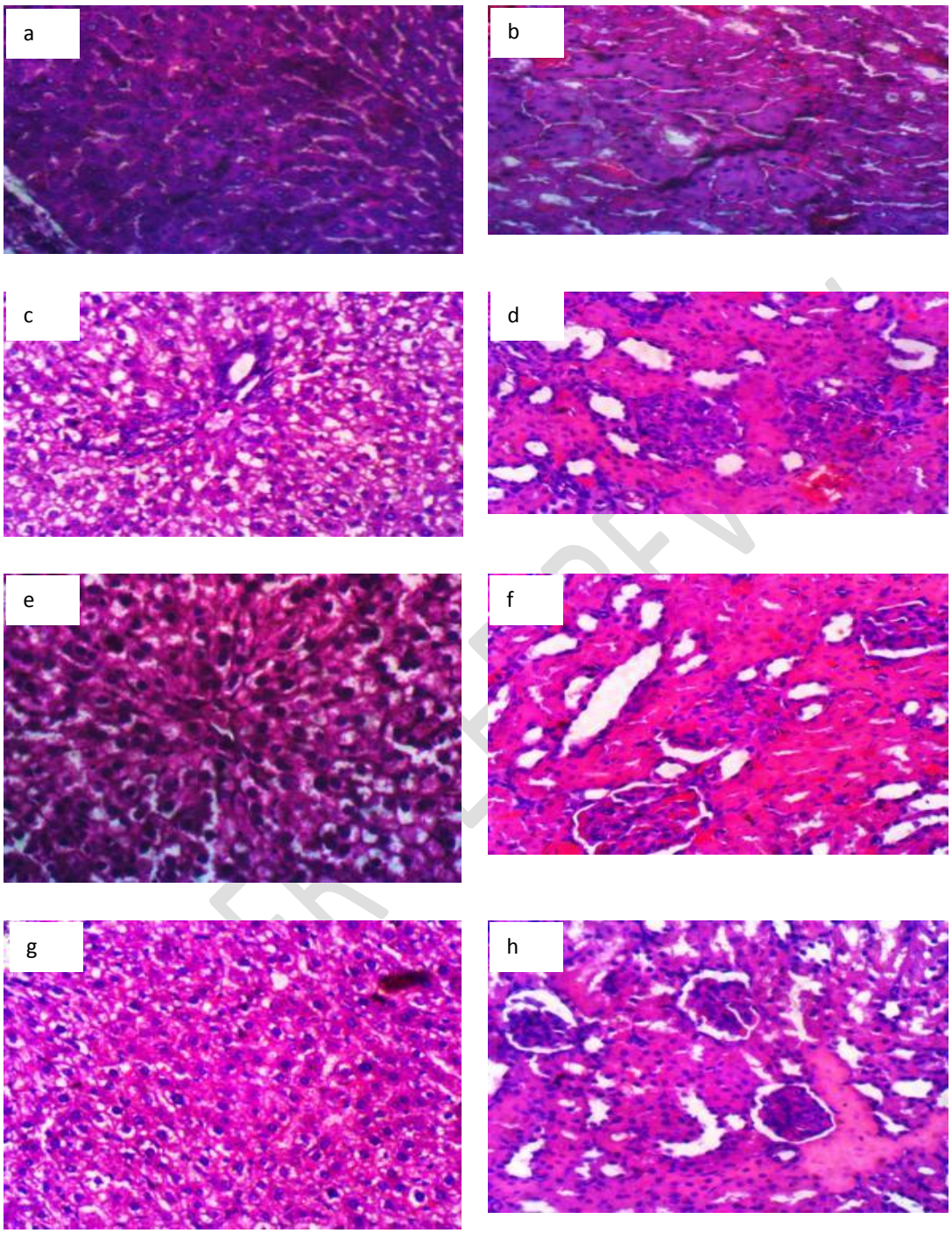


Fig. 4. Weight of the organ relative to body weight at 100mg/kg, 300mg/kg and 90mg/kg of both extracts.

Histopathological Analysis



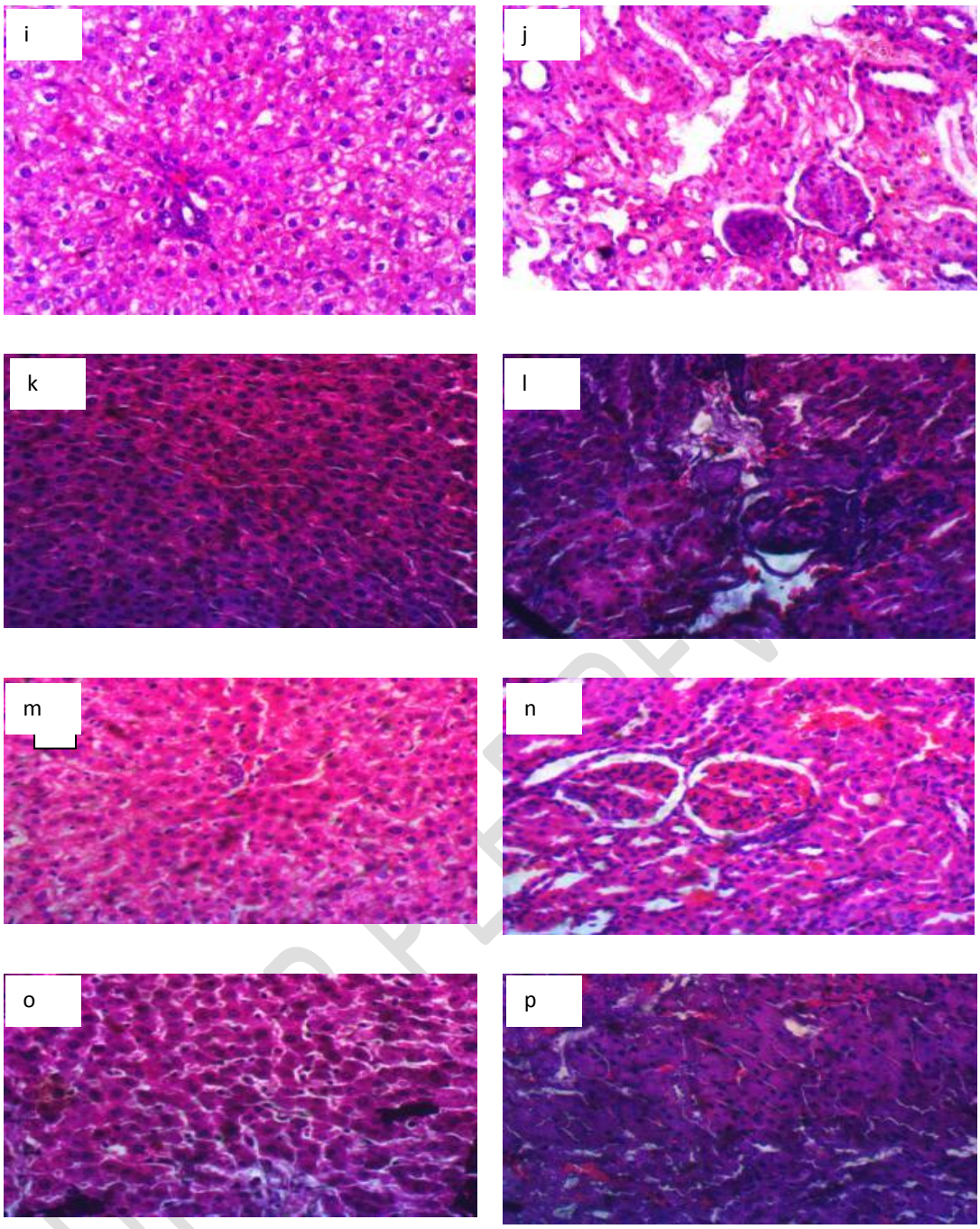


Plate 1. Histopathological Analysis

a. UNC Liver- There is no observable lesion. HE x400
b. UNC Kidney- There is no observable lesion. HE x400
c. ACP Liver- There is periportal hepatocellular degeneration and necrosis (arrows). HE x400
d. ACP Kidney-There is attenuation of tubular epithelium and luminal ectasia (arrows). HE x400
e. SA100MG/KG-Liver-There is centrilobular hepatocellular degeneration and necrosis. HE x400
f. SA100mg/kg Kidney- There is attenuation of tubular epithelium and luminal ectasia (arrows). HE x400
g. SA 300mg/kg Liver- There is no observable lesion. HE x400

h. SA 300mg/kg Kidney- There is no observable lesion. HE x400
i. SA 900mg/kg Liver- There is no observable lesion. HE x400
j. SA 900mg/kg Kidney- There is atrophy of tubular epithelium. HE x400
k. PAM 100mg/kg Liver- There is no observable lesion. HE 400
l. PAM 100mg/kg Kidney- There is no observable lesion. HE 400
m. PAM 300mg/kg Liver- There is no observable lesion. HE x400
n. PAM 300mg/kg Kidney- There is no observable lesion. HE x400
o. PAM 900mg/kg Liver- There is moderate Kupffer cell hyperplasia. HE x400
p. PAM 900mg/kg Kidney- There is no observable lesion. HE x400

Discussion

The liver is a beneficial organ that typically guards against xenobiotic chemical damage to individuals [17]. Because the liver frequently serves as the site of metabolism and is where some chemicals concentrate and get bioactivated, it is vulnerable to damage from chemical compounds [18]. The liver is involved in many other crucial physiological functions, including nutrient homeostasis, glucose regulation, cholesterol synthesis and uptake, and synthesis of clotting factors [1]. Though its capacity for repair and regeneration makes the liver a quite efficient organ, liver damage may progress to its failure and death if the ability to regenerate is insufficient or if injury to the liver is very severe [18].

Acetaminophen is regarded as safe at therapeutic levels, but at greater quantities, it can cause a deadly centrilobular liver necrosis [7]. The mechanism is a result of a complicated chain of events involving its metabolism by CYP to a reactive metabolite that depletes glutathione and binds covalently to proteins; the loss of glutathione causes an increase in the formation of reactive oxygen and nitrogen species in hepatocytes and subsequent necrotic changes [19]; Increasing oxidative stress, abnormalities in calcium homeostasis, and the onset of signal transduction reactions that result in the switch of mitochondrial permeability [20]; This increase in oxidative stress, loss of mitochondrial membrane potential, and reduction in the ability of the mitochondria to produce ATP all contribute to the change in mitochondrial permeability [21]; It now results in necrosis. This mechanism is linked to inflammatory mediators, such as certain cytokines and chemokines that can alter the toxicity [22].

It has been determined that the presence of cytochrome P-450 mixed function oxidase isoenzymes contributes to the pathogenesis of renal damage in acetaminophen poisoning [22], despite the fact that additional processes, such as the function of the enzymes prostaglandin synthase and N-deacetylase, have been discovered [24]. Kidney damage can result from the system's loss of gluthatione caused by liver damage led on by acetaminophen poisoning [23]

Phyllantus amarus and *Senna alata* were able to reduce the level of elevated biochemical parameters due to acetaminophen toxicity and restore the anatomical structure of the liver and kidney especially at 300mg/kg. *P. amarus* was able to effectively ameliorates both organs even at lower doses 100mg/kg and the kidney at 900mg/kg. It was able to reduce the inflamed liver to reverse to normal. The *S. alata* at 100mg/kg and 900mg/kg could not effectively decrease the level of the biomarkers as that of *P. amarus.*

In earlier research, phytochemical examination of an ethanol extract of *S. alata* indicated the existence of significant secondary metabolites that are mostly responsible for its therapeutic potentials, these include tannins, steroids, alkaloids, anthraquinones, terpenes, carbohydrates, and saponins [25] while a different investigation found additional mild amounts of cardiac glycosides, phylobatanin, and flavonoids [26]; From the leaf and root bark, the methanol extract was found to contain alkaloid, saponin, flavonoid, tannin, and phenol, respectively [27]. These phytochemicals in the plant extracts were able to inhibit the depletion of gluthatione in the liver thereby preventing the release of reactive species that led to oxidative stress and increase in mitochondrial permeability; they therefore ameliorated the

damage induced by acetaminophen and alter the release of inflammatory factors thus managing inflammation of the organs.
P. amarus contains the active phytochemicals flavonoids, alkaloids, terpenoids, lignans, polyphenols, tannins, coumarins and saponins [28] as well as cyanogenic glycosides and oxalates [29]. These secondary metabolites have antioxidants that are efficient at removing free radicals from the body, lowering oxidative stress on organs, and mitigating the negative effects of substances like acetaminophen on the liver and kidney even at lower dose. Additionally, they have antimicrobial functions [28] that help in the healing process and preventing secondary bacterial infections. This may therefore be responsible for the ameliorative activities of the plant ethanol extract in this study.

Conclusion

This study showed that both plants have ameliorative potentials in acetaminophen induced toxicity on the liver and kidney at 300mg/kg. *P. amarus* has ameliorative potentials on the kidney at all the doses thus it is preferred and can therefore be considered in drug development for the management of liver and kidney injury.

ETHICAL APPROVAL

"All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee"

Conflict of interest declaration

Declaration of the source of funding for the project

Declaration of the role played by each author

Acknowledgements – authors should thank any individual or group that helped the authors

References

1. Lala V, Goyal A, Minter DA. Liver function tests. StatPearls [internet]. StatPearls Publishing. 2021 [write the reference in full and provide the link]
2. Mitra V, Metcalf J. Metabolic functions of the liver. Anaesthesia & Intensive Care Medicine. 2012; 13, 54-55.
3. Uhlén M, Fagerberg, L, Hallström B M., Lindskog C, Oksvold PM . "Tissue-based map of the human proteome". Science.2015; 347 (6220):1260419.
4. Abdel-Misih SRZ, Bloomston M. "Liver Anatomy". Surgical Clinics of North America. 2010; 90 (4): 643–653. doi:10.1016
5. Renz JF, Kinkhabwala M . "Surgical Anatomy of the Liver". In Busuttil, Ronald W.; Klintmalm, Göran B. Transplantation of the Liver. Elsevier. 2014; pp. 23–39. ISBN 978-1-4557-5383-3.
6. Ghobadi SD, Dastan M, Soleimani A Nili-Ahmadabadi. Hepatoprotective potential and antioxidant activity of Allium tripedale in acetaminophen-induced oxidative damage; Research in Pharmaceutical Sciences, 2019; 14 (6), p. 488
7. Agrawal S, Khazaeni B, 2022. Acetaminophen toxicity. Statpearls [internet]. StatPearls Publishing, 2022. [write the reference fully]
8. Abdelmegeed MA., Jang SA. Banerjee JP, Hardwick B.-J. Song. Robust protein nitration contributes to acetaminophen-induced mitochondrial dysfunction and acute liver injury; Free Radical Biology and Medicine, 2013; 60: 211-222

9. Kalaskar MG, Surana SJ. Ethnomedicinal plants used against liver diseases among the tribes of India. Journal of Biological Sciences 2014;14(3):154-168.
10. Mali FN, Negoita IA, Mali DESN, Robu G. The evaluation of hepatoprotective effect of silymarin, Phyllanthus niruri extract and choline combination.The Medical Surgical Journal 2018;122(2):267-275
11. Onocha P, Ali M. 2021. Antileishmaniasis, phytotoxicity and cytotoxicity of Nigerian Euphorbiaceous Plants 2: Phyllanthus amarus and Phyllanthus muellerianus Extracts. African Scientist 2021.; 11: [write the reference properly and fully]
12. Adelowo F, Oladeji O. An overview of the phytochemical analysis of bioactive compounds in Senna alata. Advances in Biochemistry. *2017;* 5: 102-109.
13. Dajic SZ, Pljevljakusic D. Challenges and Decision Making in Cultivation of Medicinal and Aromatic Plants. Medicinal and Aromatic Plants of the World, 2015; [include volume / edition]: 145-164.
14. Uwazie JN, Yakubu MT, Ashafa AOT, Ajiboye TO. Identification and characterization of anti-diabetic principle in Senna alata (Linn.) flower using alloxan-induced diabetic male Wistar rats. Journal of ethnopharmacology, 2020; 261, 112997.
15. Soldatow VY, LeCluyse EL, Griffith LG, Rusyn I. In vitro models for liver toxicity testing. Toxicology research,2013; 2, 23-39.
16. Bischoff K, Mukai M, Ramaiah SK. Liver toxicity. Veterinary toxicology. Elsevier, 2018; 239-257.
17. Blieden M, Paramore LC, Shah, D, Ben-Joseph, R. A perspective on the epidemiology of acetaminophen exposure and toxicity in the United States. Expert review of clinical pharmacology, 2014; 7, 341-348.
18. Zhang J, Song S, Pang Q, Zhang R, Zhou L, Liu S, Meng F, Wu Q, Liu C. Serotonin deficiency exacerbates acetaminophen-induced liver toxicity in mice. Scientific reports, 2015; 5, 1-12.
19. Ulger O, Kubat GB, Cicek Z, Celik E, Atalay O, Suvay S, Ozler M. The effects of mitochondrial transplantation in acetaminophen-induced liver toxicity in rats. Life Sciences, 2021; 279, 119669.
20. Uchida NS, Silva-Filho SE, Cardia GFE, Creme E, Silva-Comar FM, Silva EL, Bersani-Amado, CA et al., Hepatoprotective effect of citral on acetaminophen-induced liver toxicity in mice. Evidence-Based Complementary and Alternative Medicine 2017. [write the reference fully]
21. Seok PR, Kim JH, Kwon HR, Heo JS, Choi JR, Shin J.-H. Protective effects of Gastrodia elata Blume on acetaminophen-induced liver and kidney toxicity in rats. Food science and biotechnology, 2018; 27, 1445-1454.
22. Mazer M, Perrone J. Acetaminophen-induced nephrotoxicity: pathophysiology, clinical manifestations, and management. Journal of Medical Toxicology, 2008; 4, 2-6.
23. Rahim NA, Ferdosh S, Sarker MZI. Extraction Methodologies, Phytochemical Constituents, and Biological Activities of *Senna alata* Linn: A Review. The Natural Products Journal, 2023; 13, 2-18.
24. Oladeji SO, Adelowo FE, Odelade KA. Mass spectroscopic and phytochemical screening of phenolic compounds in the leaf extract of Senna alata (L.) Roxb.(Fabales: Fabaceae). Brazilian Journal of Biological Sciences, 2016; 3, 209-219.
25. Abubakar I, Mann A, Mathew J. Phytochemical composition, antioxidant and anti-nutritional properties of root-bark and leaf methanol extracts of Senna alata L. grown in Nigeria. African Journal of Pure and Applied Chemistry, 2015; 9, 91-97.
26. Verma S, Sharma H, Garg M. Phyllanthus amarus: A review. Journal of pharmacognosy and Phytochemistry, 2014; 3, 18-22.
27. Oyebamiji AK, Soetan EA, Akintelu SA, Ayeleso AO, Mukwevho E, 2022. Alpha-glucosidase activity of phytochemicals from *Phyllanthus amarus* leaves via in-silico approaches. Pharmacological Research-Modern Chinese Medicine 2022; 2, 100054.
28. Adegoke A, Iberi P, Akinpelu D, Aiyegoro O, Mboto C. Studies on phytochemical screening and antimicrobial potentials of *Phyllanthus amarus* against multiple

antibiotic resistant bacteria. International Journal of Applied Research in Natural Products, 2010; 3, 6-12.