Trop J Nat Prod Res, September 2023; 7(9):4084-4091 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Changes in Hepatic Function Indices in Ulcerated Rats Treated with *Persea americana* Seed and *Bryophyllum pinnatum* Leaf Ethyl Acetate Fraction

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ARTICLE INFO

Article history:
Received 10 February 2023
Revised 03 May 2023
Accepted 05 June 2023
Published online 01 October 2023

ABSTRACT

The present study investigated the changes in hepatic function indices in ulcer*ated* rats pre-treated with *P. americana* seed (PAM) and *B. pinnatum* leaf (BPI) ethyl acetate fraction. The study consisted of fifty (50) male wistar rats divided into 10 groups of five animals each as follows: The groups 1-5 were designated normal control (NC), ulcer control (UC), Omeprazole 20mgkg^{-1} (OMEP), PAM and BPI respectively. Binary mixtures of PAM and BPI were pre-treated to groups 6-10 in the ratios PAM + BPI (1:1), PAM + BPI (1:2), PAM + BPI (1:3), PAM + BPI (2:1) and PAM + BPI (3:1) respectively. Rats in groups 4 – 10 were pre-treated for 21 days with 400 mgKg^{-1} body weight/day of single or binary mixtures of the fraction by intubation. Gastric ulcer was induced in rats of group 2-10 on 22nd day by administration of indomethacin (30mg/kg body weight) in a single dose. Results show that administration of PAM, PAM+BPI (1:1), and PAM+BPI (3:1) resulted in the elevation of AST and ALT activity; while ALT, AST activity, and globulin concentration were significantly ($p<0.05$) reduced in OMEP, BPI, PAM+BPI (1:2) PAM+BPI (2:1), PAM+BPI (1:3) and UC, OMEP, PAM, PAM+BPI (1:2), PAM+BPI (2:1) and PAM+BPI (3:1) treated groups respectively. Also, serum albumin concentration and albumin/globulin ratio were increased in all groups exposed to indomethacin except for BPI and PAM+BPI (1:3) groups. The present study demonstrated that pre-treatment of the animals with binary combinations of PAM+BPI (1:1), PAM+BPI (3:1), and indomethacin may result in significant derangement of liver function and histopathological structures.

Keywords: *P. americana, B. pinnatum, indomethacin, gastric ulcer, hepatotoxicity*

Introduction

Peptic ulcer disease (PUD) is a disorder of the digestive tract, affecting either the stomach, duodenum or both; characterized by erosion of the gastric mucosa, with mucosal breaks >3 mm *in* size, extending into the submucosa.[1]. Peptic ulcers are usually acid-induced, resulting from negative balance in protective mechanisms and causative factors in the *gastric mucosal*. Peptic ulcer disease (duodenal and gastric) is highly ranked among prevalent gastroenterological diseases. The disease impacts approximately 8.09 million people annually; the estimated prevalence ranges from 5−10% globally.[2, 3] Regional and national burden have been studied and reported [2], including prevalence in Africa. Information on geographical patterns are scanty due to inefficient reporting system, poor health facilities and trained experts.[4]

The risk of gastric disorders has been found to increase with age and associated with economic development and lifestyle in developed countries.[5] Positive correlation between age-standardized prevalence, occurrence, mortality, disability-adjusted life years and socio-economic profiles has been established.[2]

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Citation: Asiwe ES, Ugenyi AU, Enenebeaku UE, Opara PO, Alisi CS, Ekezie J. Changes in Hepatic Function Indices in Ulcerated Rats Treated with *Persea americana* Seed and *Bryophyllum pinnatum* Leaf Ethyl Acetate Fraction. Trop J Nat Prod Res. 2023; 7(9):4084-4091 http://www.doi.org/10.26538/tjnpr/v7i9.36

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

Also, prevalence has been reported to decrease with age among young males while a reverse trend was seen among women.[4] Although, males had a larger age-standardized PUD prevalence rate than females in all years from 1990 to 2019; statistics obtained from Nigeria and Ghana showed that a larger proportion of females accounted for approximately 54–57% incidence.[5]

The occurrence of gastric ulcer (GU) disease is hinged primarily on non-steroidal anti-inflammatory drugs (NSAIDs) and *Helicobacter pylori* infection.[7] Other underlying risk factors have been implicated in the etiologies of PUD, including stress, lifestyle habits such as smoking and alcohol consumption, and genetic characteristics. Gastric ulcers are mainly characterized by hemorrhage and abdominal pain; preventing symptoms include nausea, vomiting, and weight loss.[8] Numerous studies revealed other factors such as tumor necrosis factor-α (TNF-α), interlukin-1β (IL-1β) and matrix metalloproteinases (MMP) are involved in the pathogenesis of gastric ulcer.[9,10] Despite the successes of orthodox medicine in the management of GU disease, traditional medicine is still significantly applied globally in GU management due to cost-effectiveness, cultural acceptability and lesser side effects.[11] The liver is an important organ of xenobiotics metabolism and homeostasis; and is markedly affected in metabolic disease conditions, toxic, microbial, circulatory, and neoplastic injury.[12] Some traditional medications pose the risk of liver cells lesion with lethal clinical implications. *Helicobacter pylori* infection, a major gastric ulcer risk factor has been associated with liver dysfunction and damage.[13,14]

Persea americana Mill. is a perennial, evergreen tree growing up to about 15–20 m in height. It is native to the Caribbean, the fruits are consumed for nutritive values and different parts are applied in traditional medicine mixtures. The seed of *P. americana* is applied by

traditional medicine healers in Nigeria as a potent antiulcer and anti-inflammatory remedy. Biological activity and many attributed medicinal properties have been investigated [15, 16,17].

B. pinnatum belongs to the crassulaceae family, known for their xeromorphic character, efficient *vegetative propagation through any part*, and unique form of photosynthetic ability known as crassulacean acid metabolism. They have been extensively researched for their therapeutic potential; and applied traditionally in the management of gastritis, cough and many diseases conditions.[18, 19] The chemical composition, hepatoprotective activity, anti-ulcer activity, *in vitro* and *in vivo* anti-inflammatory and free radical scavenging activities of the leaf, root has also been reported.[20, 21, 22, 23] Changes in serum liver function enzymes markers characterize hepatic injury. The present study investigates the effect of binary mixtures of *B. pinnnatum* and *P. americana* ethyl acetate fraction to hepatic function indices in indomethacin induced gastric ulcer.

Methods

Chemicals/Reagents

Indomethacin (Sigma-Aldrich Mo USA), Omeprazole extended-release capsules (Sanofi-aventis, Switzerland), Ethanol and ethyl acetate (JHD, China), aspartate aminotransferase (AST) test kit, alanine aminotransferase (ALT) test kit, alkaline phosphatase (ALP) test kit, total protein test kit, albumin test kit and bilirubin test kits were purchased from Randox Laboratories, United Kingdom. All of the other reagents and chemicals employed were of high analytical quality.

Plant materials

Apparently healthy, matured fruits of *P. americana* were obtained from a local farm in Ugiri-Ike autonomous community, Ikeduru L.G.A., Imo State. while fresh *B. pinnatum* leaves were collected from stands planted around the Department of Biochemistry, Federal University of Technology Owerri, Nigeria in April, 2019. Prof. F. N. Mbagwu, a plant taxonomist authenticated the plant materials at Imo State University, Owerri, Department of Plant Science and Biotechnology Nigeria. Plant specimens were placed in the institution's herbarium and given the voucher numbers IMSUH 0225 and IMSUH 0226 respectively.

Experimental animals

Apparently healthy Male wistar rats (*Rattus norvegicus*) weighing 80-120g (averaging 8 weeks old) were purchased from the Department of Veterinary Medicine, University of Nigeria, Nsukka's animal house. The animals were housed under standard laboratory conditions (temperature, (25±2^{o}C) and relative humidity, (55 ± 5%)) with stainless steel cages. the animals were fed conventional rat pellets (Vital finisher, Nigeria) with free access to water. The entire study was conducted in strict accordance with the department ethics committee rules for the care and use of laboratory animals, with approval number BIOSC-BCH-EC-019.

Preparation of ethyl acetate fraction:

The extract preparation was carried out as described by Asiwe *et al.*,[15] and Asiwe *et al.*,[16] Fresh, healthy *B. pinnatum* leaves were acquired and cleaned with water to eliminate surface-adherent dust particles; fresh seeds of *P. americana* were gathered by delicately cutting apart the fruits and sliced into cubes to create large surface area for drying. For 14 days, the samples were left to air dry at ambient temperature (25 ± 2^{o}C). The dried plant matter was pulverized to coarse powder in a commercial mill. A Soxhlet extractor was used to extract about 500 g of each plant powder with 2 litres of 80% ethanol. The ethanol extract was partitioned in an ethyl acetate/water combination, and the soluble ethyl acetate component was recovered through distillation under decreased pressure at 49oC using a rotary evaporator (Buchi rotavapour, Switzerland). The extract was further dried in a vacuum desiccator to a semi-solid slurry state and then stored at 4.0 ^{0}C until it was needed for the study.

Experimental design

The ulcero-protective study of ethyl acetate fraction was carried out in ten (10) groups of five (5) animals each; grouped according to body weight (100-120mgKg^{-1} b. wt.) as described in our earlier studies.[16] The rats were fed standard rat pellets with access to drinking water *ad libitum* for 21 days. Groups 3-10 were pre-treated with respective protective treatment shown in Table 1 for 21 days. After the last treatment, the rats were starved, and groups 2-10 were induced for gastric ulcer by administration of 30mg/kg body weight indomethacin and allowed for 4 hrs before sacrificing. The groups and administration regimen was organized as shown in Table 1.

Table 1: Grouping and experimental design for indomethacin-induced gastric ulcer study

Group	Number of animals	Treatment	Group name
1	5	Standard rat diet and drinking water *ad libitum* for 21 days	Normal control (NC)
2	5	30mgKg^{-1} body weight (b. wt.) indomethacin	Ulcer control (UC)
3	5	20mgKg^{-1} b. wt. omeprazole for 21 days + 30mgKg^{-1} b. wt. indomethacin.	Omeprazole (OMEP)
4	5	400mgKg^{-1} b. wt. of *P. americana* ethyl acetate fraction (PAM) for 21 days + 30mgKg^{-1} b. wt. indomethacin.	*P. Americana* (PAM)
5	5	400mgKg^{-1} b.wt of *B. pinnatum* ethyl acetate fraction (BPI) for 21 days + 30mgKg^{-1} b. wt. indomethacin.	*B. pinnatum* (BPI)
6	5	400mgKg^{-1} b. wt. of *P. americana* + *B. pinnatum* ethyl acetate fraction (50% : 50% (1:1) for 21 days + 30mgKg^{-1} b. wt. indomethacin.	PAM + BPI (1:1)
7	5	400mgKg^{-1} b. wt. of *P. americana* + *B. pinnatum* ethyl acetate fraction (33% : 67% (1:2) for 21 days + 30mgKg^{-1} b. wt. indomethacin.	PAM + BPI (1:2)
8	5	400mgKg^{-1} b. wt. of *P. americana* + *B. pinnatum* ethyl acetate fraction (25% : 75% (1:3) for 21 days + 30mgKg^{-1} b. wt. indomethacin.	PAM + BPI (1:3)
9	5	400mgKg^{-1} b. wt. of *P. americana* + *B. pinnatum* ethyl acetate fraction (67% : 33% (2:1) for 21 days + 30mgKg^{-1} b. wt. indomethacin.	PAM + BPI (2:1)
10	5	400mgKg^{-1} b. wt. of *P. americana* + *B. pinnatum* ethyl acetate fraction (75% : 25% (3:1) for 21 days + 30mgKg^{-1} b. wt. indomethacin.	PAM + BPI (3:1)

Sacrificing, blood collection and isolation of stomach tissues

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

The animals were humanely sacrificed 4 hrs post-induction under light anaesthesia using dichloromethane. Cardiac puncture technique was used for collection of blood sample. The whole blood was allowed to coagulate at ambient temperature (25± 2°C) for 30 min and serum obtained by centrifuging samples at 3500 rpm at 30°C for 10 min. Serum was aspirated out and used for biochemical studies; liver samples were excised and preserved in 10% formal saline for histopathological studies.

Assessment of liver function parameters

Liver function enzymes (aspartate aminotransferase (AST) and alanine aminotransferase (ALT)) were determined according to the methods of Reitman and Frankel, [24] serum alkaline phosphatase (ALP) activity and bilirubin were assayed by the methods described by Englehardt,[25] and Jendrassik and Grof, [26] respectively. The serum total protein and albumin concentration were assessed as described by Gornall *et al.*,[27] and Doumas *et al.*,[28] respectively. Serum globulin and albumin/globulin ratio were derived as follows:

$$Albumin\ (mg/L) = serum\ total\ protein\ (mg/L) - serum\ albumin\ (mg/L)$$

$$\text{Albumin/globulin ratio} = \frac{Albumin(mg/L)}{Globulin\ (mg/L)}$$

Histopathological studies

Liver tissues of all the groups were assessed for any evidence of histopathological changes as described by Abebe and Gebru.[29] The tissues were fixed with 10% formal saline and dehydrated continuously with increasing concentrations of ethanol (30%, 50%, 70%, 90%, and 100% alcohol for 1hr, 2hrs, and 3hrs). The tissue samples were cleared by immersion in xylene for 3 hours; after which it was immersed in paraffin wax. Tissues were sectioned to about 3–6 μm thickness; Eosin and Hematoxylin (E & H) were applied stains and histopathological changes examined with light microscope fitted with digital camera (Nikon, ECLIPSE, TS100, Japan).

Data/statistical analysis

The collected data were processed in Microsoft Excel and expressed as the mean ± standard deviation of five (n=5) measurements. The results were analyzed using the Statistical Package for Social Sciences (SPSS) version 20 (One-way-ANOVA). Statistical comparison of results was considered at $p<0.05$ using the turkey and Duncan homogeneity of variance test.

Results and Discussion

Liver tissue damage is usually indicated by increased activity of serum marker enzymes such as ALT, AST and ALP which correspond to a decrease in the tissue enzyme activity.

The result of the effect of *P. americana* seed and *B. pinnatum* leaf ethyl acetate fraction administration on liver function enzymes of the indomethacin-induced ulcerated rats are summarized in Figure 1.

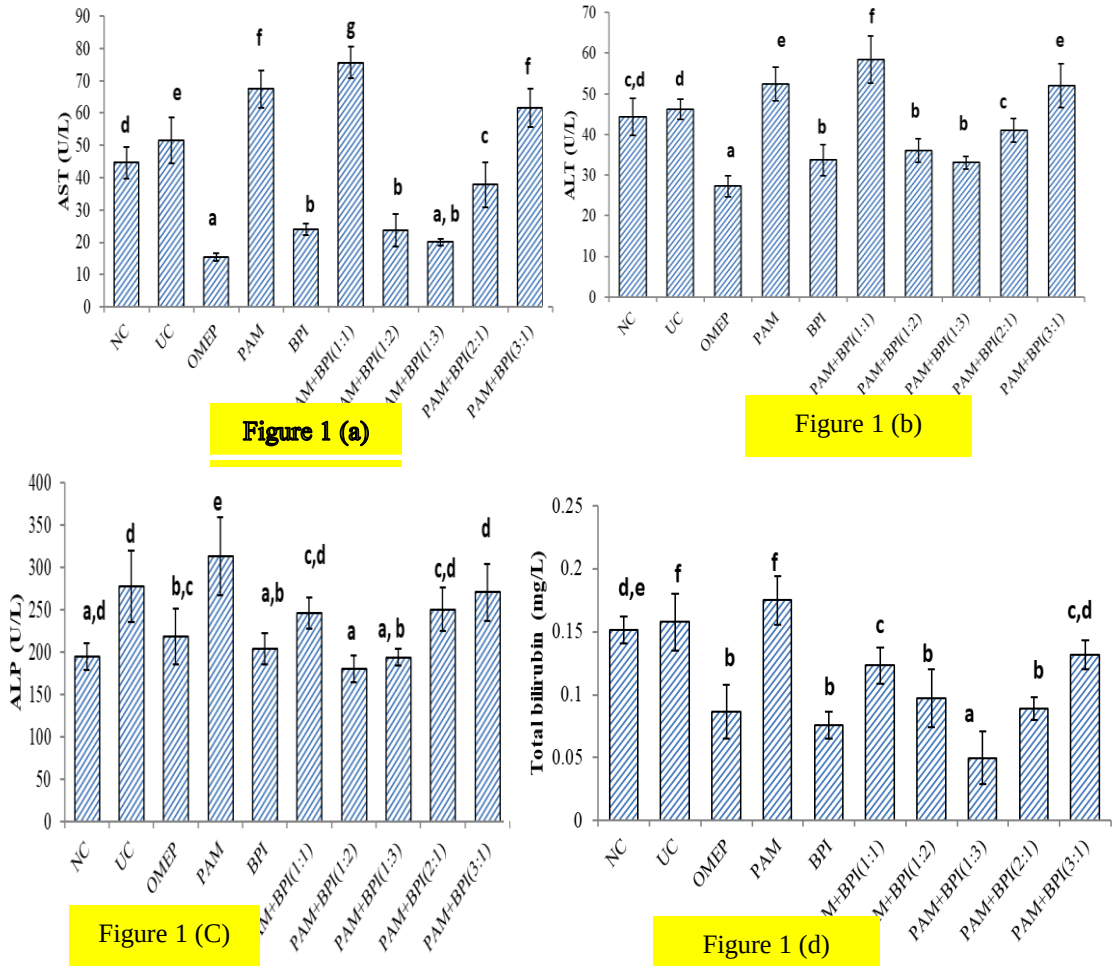

Figure 1: Effect of P. americana seed and B. pinnatum leaf ethyl acetate fraction administration on serum AST , ALT, ALP activity and total bilirubin concentration of male albino rats exposed to indomethacin-induced gastric ulcer. Bars are mean ± SD of 5 determinations. Bars bearing different superscript letters across groups are significantly different ($p<0.05$).

The pretreatment of rats with PAM, PAM+BPI (1:1) and PAM+BPI (3:1) resulted in elevation of AST and ALT activity when compared to NC and UC. However, the ALP activity result shows that activity was statistically significant ($p < 0.05$) and higher in the groups UC, PAM, PAM+BPI (2:1) and PAM+BPI (3:1); while other groups did not vary from normal control. In the results presented in Figure 1 (d), total bilirubin concentration was not statistically significantly ($p>0.05$) altered by extract fraction administration prior to indomethacin induction, except among the group that received PAM which showed a slight elevation of total bilirubin concentration. Total bilirubin in the other groups OMEP, BPI, PAM+BPI (1:1), PAM+BPI (1:2), PAM+BPI (1:3), PAM+BPI (2:1) and PAM+BPI (3:1) were markedly reduced. This result suggests that indomethacin administration did not significantly alter liver function; however, administration of the ethyl acetate fractions and their binary combinations resulted in varying degrees of liver lesion. Prominent changes was observed in groups receiving PAM, PAM+BPI (1:1) and PAM+BPI (3:1), which resulted in a significant elevation of ALT and AST activities as compared to NC and UC. It is possible that the elevation of this enzyme activity in the groups that received ethyl acetate fractions of *P. americana* seed and *B. pinnatum* leaf may be attributed to the toxicity of the ethyl acetate fractions on hepatocytes. This result corroborates with the serum ALP activity of the group indicating a possible hepatotoxic effect of the administration of PAM, and the binary mixture of *P. americana* and *B. pinnatum* ethyl acetate fraction in the ratios PAM+BPI (1:1) and PAM+BPI (3:1) respectively. Elevation of ALP activity usually occurs as an indicator of bile duct obstruction or intrahepatic cholestasis. Other groups BPI, PAM+BPI (1:2), PAM+BPI (1:3) and PAM+BPI (2:1) did not significantly alter liver function enzyme activity in the respective treated animals. The ALT, AST and ALP activities of these groups were comparable to those of NC and OMEP. ALT is a liver compartmentalized enzyme, increase in the serum ALT and AST activity following pretreatment of rats with PAM and binary combinations of ethyl acetate fraction in the ratios PAM+BPI (1:1) and PAM+BPI (3:1) may be attributed to hepatocellular destruction resulting in the release of the enzyme into the extracellular space.[30] Previous studies on leaf and seed extracts of *B. pinnatum* and *P. americana* seed have been reported to ameliorate hepatotoxicity in chemically-induced hepatotoxic models.[31,32, 33] The variation in findings with our results may be attributed to differences in extraction solvents. The ethyl acetate fraction may contain phytochemical groups that can induce hepatotoxicity.

The result of other indicators of liver function reinforces trends seen in serum liver enzyme activities, indicating that indomethacin administration did not significantly ($p>0.05$) alter direct and indirect bilirubin concentration as seen in UC when compared to normal control (Table 2). Although direct bilirubin concentration was observed to be significantly ($p<0.05$) altered by the ethyl acetate fractions administration to rats in the groups PAM, PAM+BPI (1:1) and PAM+BPI (1:2); the group that received PAM showed a slight elevation of total bilirubin concentration which is consistent with earlier reported increase in serum transaminases. The total and indirect bilirubin concentrations were significantly ($p<0.05$) lowered in OMEP and groups that received the ethyl acetate fraction binary combinations of PAM, BPI, PAM+BPI (1:1), PAM+BPI (1:2), PAM+BPI (1:3), PAM+BPI (2:1) and PAM+BPI (3:1). Reduction in serum total and indirect bilirubin concentrations is a pointer to non-toxic effect of these ethyl acetate fractions to liver tissue and may be an indication of a possible regenerative effect on hepatocytes or increasing potential of bilirubin transport to the liver for conjugation.[34] Also, these ethyl acetate fractions may possibly inhibit haem oxygenase, involved in bilirubin biosynthesis, reducing serum total bilirubin in treated animals.

Furthermore, an assessment of the effect on liver detoxifying ability presented in table 2.0, showed that serum total protein concentration was not significantly ($p>0.05$) altered by indomethacin administration. However, a significant ($p<0.05$) decrease in globulin concentration was seen in the groups UC, OMEP, PAM, PAM+BPI (1:2), PAM+BPI (2:1) and PAM+BPI (3:1) compared to normal control. On the contrary, the group PAM+BPI (1:3) showed a significant ($p<0.05$) increase in serum globulin concentration. Similarly, serum albumin concentration was increased in groups exposed to indomethacin with few exceptions in the groups BPI and PAM+BPI (1:3). However, significant decrease in globulin concentration was seen in the groups UC, OMEP, PAM, PAM+BPI (1:2), PAM+BPI (2:1) and PAM+BPI (3:1) groups compared to normal control with exception in the group PAM+BPI (1:3) as presented in table 2.0. Overall, the serum albumin/globulin ratio was generally significantly increased in groups exposed to indomethacin with few exceptions in the groups BPI and PAM+BPI (1:3). Albumin is an important factor of xenobiotic detoxification mechanism in the liver. Elevation of the serum albumin concentration and the marked increased in albumin-globulin ratio show that although liver enzyme markers were released into circulation, the liver biosynthetic ability was not altered by extract and indomethacin administration.[35]

Photomicrographs of liver tissues of groups that received indomethacin presented in Fig. 2 and Fig. 3.0 (Plate 2-10) showed sections with enlarged/dilated central and portal veins, formation of reticulocytes and mild necrosis of the hepatocytes. These changes were also presented in the groups pretreated with 400mg/Kg b. wt. of ethyl acetate fractions of PAM, BPI, PAM+ BPI (1:1), and PAM+ BPI) (3:1). Liver sections of these groups showed varying degree of mild degenerative changes, necrosis of hepatocytes and enlarged central portal vein as presented in the photomicrographs.

Table 2: Effect of *P. americana* seed and *B. pinnatum* leaf ethyl acetate fraction administration on serum total protein, globulin, albumin, albumin/globulin ratio, direct bilirubin and indirect bilirubin concentration of male albino rats exposed to indomethacin-induced gastric ulcer

Groups	Direct bilirubin (mg/L)	Indirect bilirubin (mg/L)	Total Protein (mg/L)	Globulin (mg/L)	Albumin (mg/L)	Albumin/Globulin ratio
NC	0.04 ± 0.01^{c}	$0.11 \pm 0.01^{f,g}$	84.04 ± 3.40^{a}	54.51 ± 3.06^{a}	29.53 ± 2.67a	0.54 ± 0.07^{b}
UC	0.04 ± 0.00^{c}	0.12 ± 0.00^{g}	80.40 ± 2.82^{a}	44.27 ± 3.13^{b}	36.13 ± 5.12^{b}	0.83 ± 0.17^{e}
OMEP	0.01 ± 0.00^{a}	$0.07 \pm 0.01^{d,e}$	78.86 ± 3.13^{a}	45.44 ± 3.49^{b}	33.42 ± 3.34^{a}	0.74 ± 0.12^{e}
PAM	0.07 ± 0.00^{d}	$0.10 \pm 0.01^{f,g}$	82.41 ± 4.19^{a}	46.57 ± 4.90^{b}	35.84 ± 4.10^{d}	0.78 ± 0.15^{e}
BPI	0.02 ± 0.00^{b}	$0.05 \pm 0.01^{b,c}$	76.47 ± 7.04^{a}	50.88 ± 6.16^{a}	25.59 ± 5.92^{a}	0.51 ± 0.08^{b}
PAM+BPI(1:1)	0.09 ± 0.00^{e}	0.04 ± 0.01^{b}	86.24 ± 1.69^{a}	55.05 ± 5.56^{a}	31.20 ± 4.36^{c}	$0.58 \pm 0.14^{b,c}$
PAM+BPI(1:2)	0.06 ± 0.00^{d}	0.03 ± 0.01^{b}	83.27 ± 2.13^{a}	61.14 ± 6.54^{d}	22.13 ± 6.48^{a}	$0.73 \pm 0.14^{c,d}$
PAM+BPI(1:3)	0.04 ± 0.01^{c}	0.01 ± 0.00^{a}	86.05 ± 1.84^{a}	51.21 ± 3.63^{d}	34.84 ± 3.54^{e}	0.35 ± 0.07^{a}
PAM+BPI(2:1)	0.02 ± 0.00^{b}	$0.07 \pm 0.0^{c,d}$	81.93 ± 1.69^{a}	47.86 ± 7.65^{b}	34.07 ± 9.17^{e}	$0.71 \pm 0.04^{c,d}$
PAM+BPI(3:1)	0.04 ± 0.01^{c}	$0.10 \pm 0.0^{e,f}$	84.42 ± 2.70^{a}	46.91 ± 6.11^{c}	37.51 ± 6.07^{e}	0.80 ± 0.06^{e}

Results are mean ± SD of 5 determinations. Bars bearing different superscript letters across groups are significantly different ($p<0.05$).

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Notably, the group pre-treated with PAM+ BPI (1:1) 400mg/Kg b.wt and 30mg/Kg b.wt indomethacin, showed mild changes in liver architecture with mild necrosis of hepatocytes (Fig. 3: Plate 6). Also, in the group that was pre-treated with PAM+ BPI (1:2) 400mg/Kg b. wt and later received 30mg/Kg b.wt indomethacin (Fig. 3: Plate 7), the plate showed a normal liver architecture similar to those of Plate 1. While pre-treatment with 400mg/Kg b. wt. PAM+ BPI (2:1) and (PAM+ BPI) (1:3) before indomethacin induction resulted in regenerative changes in liver tissues (Fig. 3: Plate 8 and 9). The micrograph showed better regenerative changes in plate 9 when compared to plates 7 and 8. However, pre-treatment with PAM+ BPI (3:1) 400mg/Kg b. wt. and prior to indomethacin induction showed a preservation of normal architecture of the liver except for mild necrosis (Fig. 3.0: Plate 10). It is possible that the degenerative changes seen in the liver tissue of some of the groups maybe not be attributed to indomethacin toxicity, but associated with the effect of the ethyl acetate fractions on hepatocytes. However, other groups such as PAM+BPI (1:2), PAM+BPI (1:3), PAM+BPI (2:1) and PAM+BPI (3:1) clearly indicated regenerative tendency with normal liver architecture (Fig. 3.0). This may be attributed to a possible protection against liver degeneration; and are consistent with our findings in the alterations of liver function enzymes markers. Xenobiotics interfere with detoxification mechanisms of hepatocytes; impairing protein, carbohydrate and lipid metabolism. Consequently, cells or cytoplasmic organelles are damaged, resulting to distortions in metabolic enzymes activity, vacuolar degeneration, and necrosis of hepatocytes.[36,37,38] These metabolic distortions in the liver or excessive hemolysis impair bilirubin processing resulting to hyperbilirubinaemia.

Conclusion

The present study demonstrated that pre-treatment of the rats with single and binary mixtures of ethyl acetate fraction of *P. americana* seed and *B. pinnatum* leaf before gastric ulcer induction using indomethacin resulted in significant derangement of liver function indices and histopathological changes. The changes were dependent on the mixture ratios of the binary fractions; results suggest that binary combinations of the two plant fractions in the ratios PAM, PAM+BPI (1:1) and PAM+BPI (3:1) may present mild liver changes. The binary ratios of PAM + BPI (1:2), PAM + BPI (1:3), PAM + BPI (2:1) and PAM + BPI (3:1) increased regeneration of hepatocytes and decreased liver cells distortions and are considered generally safe to the liver.

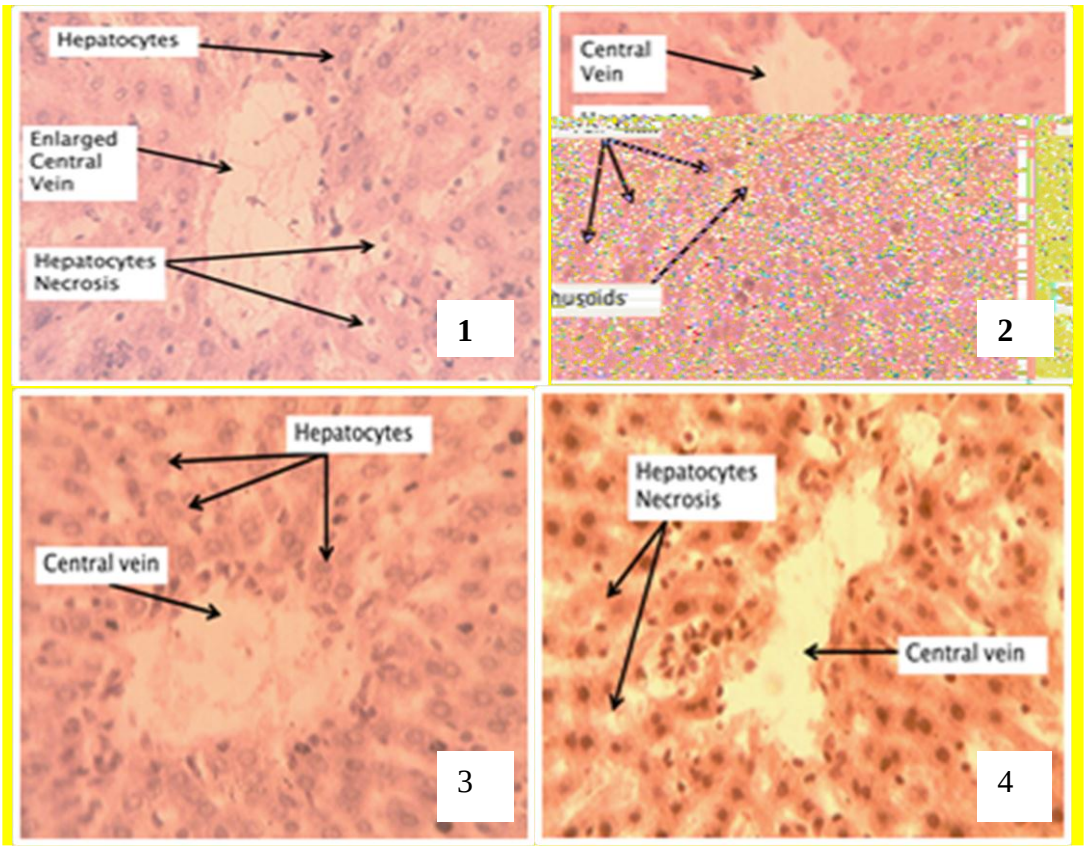

Figure 2: Photomicrograph of liver tissue sections of groups 1-4 presented in plates 1-4.

Legend: Plate 1: Group 1 (Control), Plate 2: group 2 (Ulcer control)

Plate 3: group 3 (Standard group), Plate 4: group 4 ((PAM) 400mg/Kg b.wt.)

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

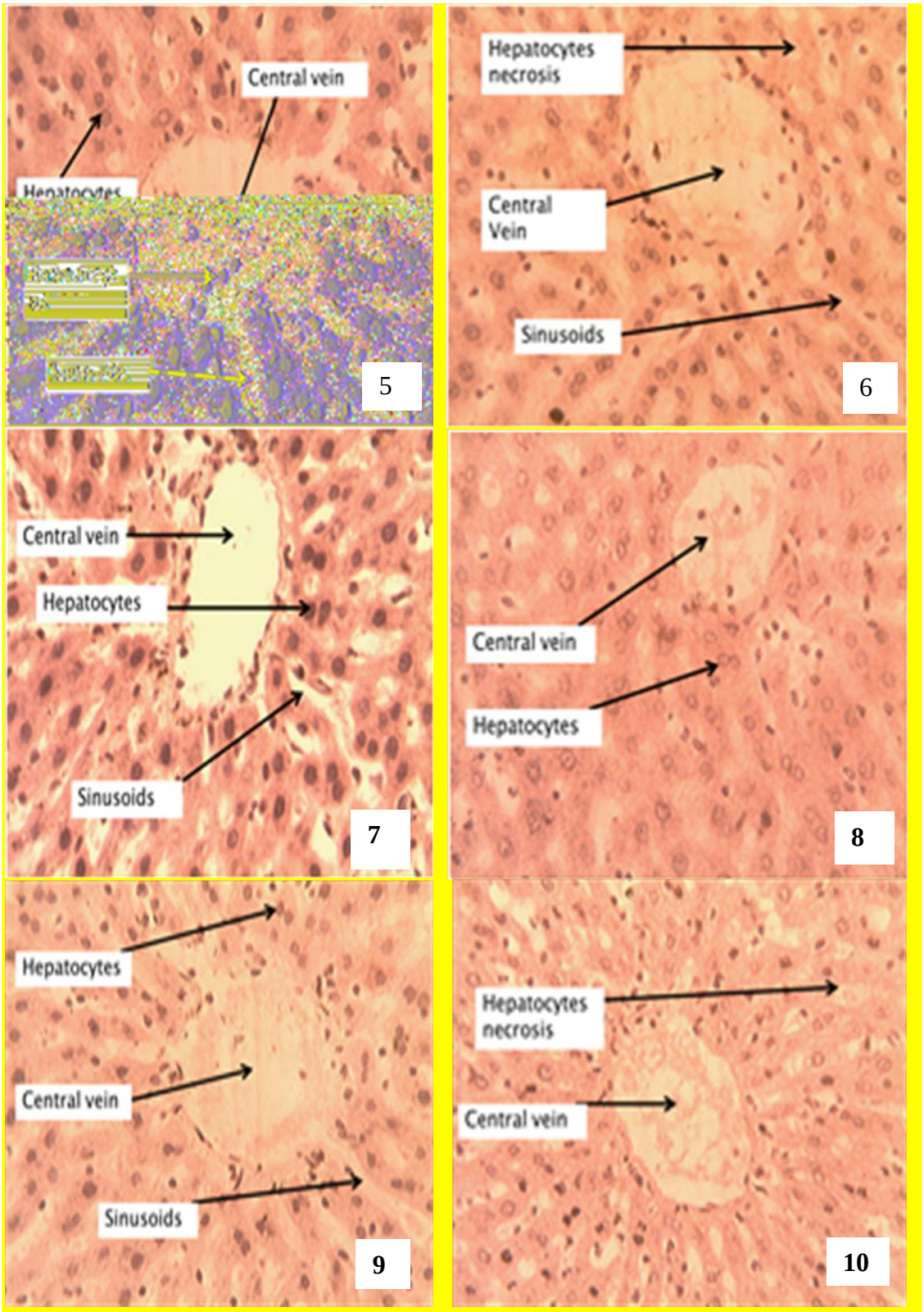

Figure 3: Photomicrograph of liver tissue sections of groups 5-10 presented in plates 5-10.
Legend: Plate 5: group 5 ((BPI) 400mg/Kg bwt),Plate 6: group 6 (PAM + BPI) (1:1) 400mg/Kg, Plate 7: group 7 (PAM + BPI) (1:2) 400mg/Kg, Plate 8 group 8 (PAM + BPI) (1:3) 400mg/Kg, Plate 9: group 9 (PAM + BPI) (2:1) 400mg/Kg, Plate 10: group 10 (PAM + BPI) (3:1).

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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