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Renal Function Indices of Ulcerated Rats Pre-Treated with Binary Combinations of *Persea americana* Seed and *Bryophyllum pinnatum* Leaf Ethyl Acetate Fraction

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ABSTRACT

The use of certain medications and herbal remedies poses a significant risk to renal function. The study aimed to investigate the effect of binary combinations of *Persea americana* seed (PAEF) and *Bryophyllum pinnatum* leaf (BPEF) ethyl acetate fractions on renal function indices of ulcerated rats. Wistar rats were divided into ten groups of five rats each. The groups were designated: normal control (NC) (Group 1), ulcer control (UC) (Group 2), Omeprazole (OMEP) (Group 3), PAEF (Group 4), BPEF (Group 5), PAEF + BPEF (1:1) (Group 6), PAEF + BPEF (1:2) (Group 7), PAEF + BPEF (1:3) (Group 8), PAEF + BPEF (2:1) (Group 9), and PAEF + BPEF (3:1) (Group 10). Groups 4-10 were pre-treated with 400 $mgkg^{-1}$ body weight/day of ethyl acetate fractions of the plants, and their binary combinations orally for 21 days. On the 22nd day, after 12 h fasting, gastric ulcer was induced in the rats in groups 2-10 by a single oral dose of indomethacin (30 mg/kg body weight). Results showed that the administration of PAEF + BPEF (1:2), PAEF + BPEF (1:3), PAEF + BPEF (2:1), PAEF + BPEF (3:1), and omeprazole caused significant ($p<0.05$) elevation of serum urea when compared to NC and UC. The binary combinations PAEF + BPEF (1:1), PAEF + BPEF (1:3), PAEF + BPEF (2:1), PAEF + BPEF (3:1) caused normalization of serum calcium, magnesium, and zinc concentrations comparable to normal control. Therefore, the binary combinations of PAEF and BPEF may distort kidney function and electrolyte balance.

Keywords: *Persea americana, Bryophyllum pinnatum,* Indomethacin, Gastric ulcer, Nephrotoxicity.

Introduction

Gastric and duodenal ulcers are classified as peptic ulcer disease (PUD) and are recurrent gastroenterological disorders affecting approximately 8.09 million people annually, with an estimated 5−10% prevalence worldwide.[1,2] Peptic ulcers are usually acid-induced, resulting from an imbalance between gastric mucosal protective and causative factors. Abuse of non-steroidal anti-inflammatory drugs (NSAIDs), *Helicobacter pylori* infection, Oxidative stress, lifestyles such as alcoholism, smoking and genetic characteristics are identified risk factors.[3] The risk of the disease in developed countries is reportedly age, economic development, and lifestyle dependent.[1] There is an increasing link between gastric ulcer predisposing factors and nephrotic injury. The Cytotoxin-associated gene A (CagA) promotes the induction of underglycosylation of IgA1 of *H. pylori* and secretion of serum immunoglobulin A1 (IgA1), key factors implicated in nephropathy.[4] Acute *H. pylori* infection have been touted as a possible risk factor in nephropathy, hypertension, and diabetes patients.[5,6]

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The reduction in gastric mucosal cytoprotection by indomethacin follows mechanisms that involve reduced prostaglandin synthesis and production of free radicals. The event of a low luminal pH favours free radical oxidation of intestinal matrix focal tissue necrosis. High doses or long-term exposure to NSAIDs are implicated in heightened nephrotoxicity risk, especially acute kidney injury in the elderly and chronic kidney disease (CKD) in long-term exposure.[7] NSAIDs may induce renal dysfunction in high-risk subjects with lowered renal blood perfusion, leading to electrolyte imbalance and metabolic acidosis resulting from decreased activity of COX-1 and COX-2.[8,9]

Persea americana Mill., is a common fruit transcending the Nigerian landscape, it is known by various local names such as ube-beke, orewépia, and ganyen piya by the Igbo, Yoruba, and Hausa tribes of Nigeria, respectively.[10] *P. americana* tree is evergreen, Indigenous to the Caribbean, and commonly known as the avocado. The plant's seed, leaves, and stem bark have been widely studied for their antioxidant, anti-inflammatory, antihypertensive, gastroprotective, hypoglycaemic, and hypolipidemic effects.[11-13]

Bryphyllum pinnatum, a Crassulaceae family member, is largely distributed in the rainforest belt of tropical countries. The plant is native to Madagascar, known by names such as "life plant", "love plant", "miracle leaves", and "air plant", all describing their unique characteristics.[14] The chemical composition, hepatoprotective activity, pain-relieving effects, gastroprotective activity, and radical inhibitory activities have been reported.[15-19] The use of certain orthodox medications and herbal remedies in disease management poses a significant risk to renal function and electrolyte balance.[20] Our previous study examined the ulcer-protective potential and toxicity of the binary combination of the plant fractions.[19,21] There is a need to investigate the effect of these traditional anti-ulcer remedies in renal homeostasis and turnover, given the attendant feedback of some medication being disguised as poisoned chalice. This work aims to assess the effect of binary mixtures of *B. pinnnatum* and *P. americana* ethyl acetate fraction

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on kidney function indices and electrolyte balance in indomethacin-induced gastric ulcers in rats.

Materials and Methods

Chemicals/Reagents

Indomethacin and Omeprazole capsules were acquired from Sigma-Aldrich Sigma-Aldrich Mo USA and Sanofi-aventis, Switzerland, respectively. Ethanol and Ethyl acetate (JHD, China), Zinc test Kit (Randox Lab, UK), urea, creatinine, calcium, and magnesium reagents were obtained from Biosystems, Spain, while sodium, potassium, chloride, and bicarbonate reagents were from TECO Diagnostic, USA. All other chemicals and reagents used were of analytical grade.

Collection of plant materials

Matured fruits of *P. americana* and fresh, healthy leaves of *B. pinnatum* were collected from a local farm at Ugiri-Ike autonomous community, Ikeduru L.G.A. Imo State, Nigeria, in April 2019. The plant materials were identified and authenticated by Prof. F. N. Mbagwu, a professor of plant taxonomy at Imo State University, Owerri, Nigeria, Department of Plant Science and Biotechnology. The plant specimens were allotted voucher numbers IMSUH 0225 and IMSUH 0226, respectively, and catalogued in the institution's herbarium. The plants were use according to the guidelines of the Plant Variety Protection Act of the Federal Republic of Nigeria, 2021.

Experimental animals

Male Wistar rats weighing between 80 -100 g were procured from animal friend farms in Owerri, Nigeria. Prior to the study, the rats were acclimatized for seven (7) days at room temperature (25 ± 2ºC), and relative humidity of 55 ± 5% in stainless steel cages, fed standard rat pellets (Vital Finisher, Nigeria), and portable water *ad libitum*.

Ethical approval

The protocol was approved by the animal rights and ethical committee of the Department of Biochemistry, Federal University of Technology, Owerri, Nigeria, with (approval number: BIOSC-BCH-EC-019. Animal handling strictly followed the principles of laboratory animal care (NIH Publication, 1985 to 1993; revised, 1985). The study was conducted in strict compliance with the department ethics committee rules for using experimental animals

Preparation of ethyl acetate fraction

The preparation of the plant materials followed the method described by Asiwe *et al.* (2021c),[18] and Asiwe *et al.* (2022).[19] The leaves of *B. pinnatum* were sorted to remove unwanted materials and thoroughly washed free of dust particles with clean water. The seeds of *P. americana* were obtained by cutting open the fruit pod. The seeds were sliced into cubes of 20 mm in diameter to ease drying. After air-drying at 25 ± 2ºC for 14 days, the dry plant matter was crushed into coarse powdered form using a commercial mill. About 500 g of the crushed plant matter was extracted with 1 L of 80% ethanol using a soxhlet extractor. The extract obtained was partitioned in ethyl acetate/water mixture, and the ethyl acetate soluble component was recovered and dried to solid forms in a vacuum desiccator and stored at 4ºC until needed for the experiment.

Experimental design

Male Wistar rats were grouped into ten (10) groups of five (5) rats each, they were treated as outlined in Table 1. Group 1: normal control, rats in this group did not receive any treatment. Group 2: ulcer control, indomethacin-induced ulcer. Group 3: pre-treated with omeprazole 20 mg/kg for 21 days. Groups 4 – 10: pre-treated with respective plant fractions and binary mixtures for 21 days. On the last day of treatment, animals were fasted overnight, and on the 22nd day, the animals in groups 2-10 were treated with 30 mg kg^{-1} body weight indomethacin by intubation in a single dose. After 4 hours of ulcer induction, the animals were humanely sacrificed under dichloromethane anaesthesia. Blood samples were collected via cardiac puncture and allowed to clot at room temperature (25± 2ºC). The serum was separated after retraction of cells and centrifugation at 4000 rpm for 10 minutes. A serum was collected and used to determine kidney function parameters. The kidneys were surgically removed, and used for histopathological analysis.

Table 1: Animal grouping and treatment

Group number	Group name	Treatment
1	Normal control (NC)	Standard rat diet and drinking water *ad libitum* for 21 days
2	Ulcer control (UC)	Indomethacin 30 $mgkg^{-1}$ b. wt. on the 22nd day after overnight fasting
3	Omeprazole (OMEP)	Omeprazole 20 $mgkg^{-1}$ b. wt. for 21 days + indomethacin 30 $mgkg^{-1}$ b. wt. on the 22nd day after overnight fasting.
4	PAEF	*P. americana* ethyl acetate fraction (PAEF) 400 $mgkg^{-1}$ b.wt. for 21 days + indomethacin 30 $mgkg^{-1}$ b. wt. on the 22nd day after overnight fasting
5	BPEF	*B. pinnatum* ethyl acetate fraction BPEF 400 $mgkg^{-1}$ b.wt for 21 days + indomethacin 30 $mgkg^{-1}$ b. wt. on the 22nd day after overnight fasting.
6	PAEF + BPEF (1:1)	*P. americana* + *B. pinnatum* ethyl acetate fraction (1:1) 400 $mgkg^{-1}$ b. wt. for 21 days + indomethacin 30 $mgkg^{-1}$ b. wt. on the 22nd day after overnight fasting.

7	PAEF + BPEF (1:2)	*P. americana* + *B. pinnatum* ethyl acetate fraction (33%:67%) or (1:2) 400 mgkg^{-1} b. wt. for 21 days + indomethacin 30 mgkg^{-1} b. wt. on the 22nd day after overnight fasting.
8	PAEF + BPEF (1:3)	*P. americana* + *B. pinnatum* ethyl acetate fraction (25%:75%) or (1:3) 400 mgkg^{-1} b. wt. for 21 days + indomethacin 30 mgkg^{-1} b. wt. on the 22nd day after overnight fasting.
9	PAEF + BPEF (2:1)	*P. americana* + *B. pinnatum* ethyl acetate fraction (67%:33%) or (2:1) 400 mgkg^{-1} b. wt. for 21 days + indomethacin 30 mgkg^{-1} b. wt. on the 22nd day after overnight fasting.
10	PAEF + BPEF (3:1)	*P. americana* + *B. pinnatum* ethyl acetate fraction (75%:25%) or (3:1) 400 mgkg^{-1} b. wt. for 21 days + indomethacin 30 mgkg^{-1} b. wt. on the 22nd day after overnight fasting.

Assessment of kidney function parameters

The concentration of kidney function indices, including urea, creatinine, and electrolytes (sodium, potassium, chloride, and bicarbonate) were determined using respective test kits. Urea concentration was assayed spectrophotometrically as described by Searcy *et al.* (1967);[22] creatinine concentration was assessed as described by Bartels and Boehmer (1971),[23] and Fabiny and Ertingshausen (1971).[24]

Potassium and Chloride concentration determination followed the method described by Terri and Sesin (1958)[25] and Skeggs and Hochstrasser (1964),[26] respectively. The serum sodium and bicarbonate concentrations were assessed based on the method of Maruna (1957),[27] and Trinder (1951),[28] and Kaplan and Glucose (1974),[29] respectively. The serum calcium and magnesium concentrations were measured spectrophotometrically as described by Gindler and King (1972),[30] and Chauman and Ray (1969),[31,] respectively. Serum zinc concentration was determined following the method described by Abe *et al.* (1989),[32] and Makino (1991).[33]

Histopathological analysis

Histopathological analysis of kidney tissues was assessed as described by Abebe and Gebru (2015).[34] The kidney tissues were fixed in 10% formal saline and continuously dehydrated with 30%, 50%, 70%, 90%, and absolute ethanol at intervals of 1 h, 2 h, and 3 h. The tissue samples were immersed in xylene for 3 hours and embedded in paraffin wax before cutting into 3–6 µm thick sections. The sections were stained with eosin and hematoxylin (E & H) and examined under a light microscope fitted with a digital camera (Nikon, ECLIPSE, TS100, Japan).

Statistical analysis

Statistical analysis of data was done using the Statistical Package for Social Sciences (SPSS) version 20. The results were expressed as mean ± standard deviation of five (5) determinations. Differences between means were analyzed using one-way analysis of variance (ANOVA), followed by Turkey and Duncan homogeneity of variance test. Statistical significance was determined at $p<0.05$.

Results and Discussion

Kidney function markers are determinants of the presence of renal disease, indicators of the kidney's response to medication, detoxification ability, and overall health of the kidney. The results of the effect of *P. americana* seed and *B. pinnatum* leaf ethyl acetate fraction administration on kidney function parameters in indomethacin-induced ulcerated rats are summarized in Table 2. The results showed that urea concentration was not significantly altered by indomethacin administration, but, omeprazole and ethyl acetate fraction combinations of PAEF + BPEF (1:2), PAEF + BPEF (1:3), PAEF+BPEF (2:1), and PAEF + BPEF (3:1) caused a significant elevation in serum urea when compared to those of NC and UC groups. Uremia is characteristic of kidney injury or compromised kidney function. On the other hand, serum creatinine concentration did not significantly vary across the treatment groups compared to the normal control, except for the omeprazole group, which expressed a marked four-fold increase in creatinine concentration.

Results of the electrolyte profile (Table 2) showed that serum potassium concentrations were significantly reduced in groups exposed to indomethacin. However, in treatment groups like PAEF, and PAEF+BPEF (2:1), potassium concentration was restored to normal values by the administration of the ethyl acetate fraction. The decreased potassium concentration may be attributed to increased renal loss of potassium ions. However, sodium concentration was not significantly altered by indomethacin administration. The results showed that indomethacin administration caused no significant alteration in the anions concentrations. Serum chloride and bicarbonate concentrations were not significantly altered, except in the group PAEF+BPEF (3:1) where chloride concentration was elevated above that of the normal control. Administration of omeprazole resulted in an increased serum urea, creatinine, and a slight but non-significant increase in sodium concentration, coupled with a corresponding decrease in serum potassium ion concentration. Derangements in serum urea, creatinine, and electrolyte balance are associated with renal function impairment.[35,36] These results suggest a disruptive effect of omeprazole on kidney function. The result of serum calcium concentration (Figure 1A) showed that calcium was significantly ($p<0.05$) elevated in male Wistar rats with indomethacin-induced gastric ulcer. The administration of ethyl acetate fraction binary mixture in the ratios PAEF + BPEF (1:3), PAEF + BPEF (2:1), PAEF + BPEF (3:1), and OMEP resulted in a significant ($p<0.05$) normalization of calcium concentration comparable to normal control. In addition**,** the results presented in Figure 1B showed that indomethacin administration significantly ($p<0.05$) resulted in a depletion of serum magnesium concentration compared to normal control. However, the combinations of *P. americana* seed and *B. pinnatum* leaf ethyl acetate fraction in the ratios PAEF, PAEF + BPEF (1:1), PAEF + BPEF (1:2), and PAEF + BPEF (3:1) resulted in significant restoration of magnesium concentration.

Magnesium is an important element associated with blood pressure control; it regulates smooth muscle contraction via activation of protein kinase C.[37,38] This is mediated through calcium ion influx. The observed indomethacin-induced reduced magnesium ion concentration may trigger increased blood pressure through calcium ion influx into vascular endothelial smooth muscle cells, narrowing blood vessels and, in turn, activating cardiomyocytes.[39] Prolonged use of PPIs may further exacerbate the danger of CKD onset, promoting secretion of atherogenic and inflammatory markers associated with endothelial dysfunction of the renal tissue.[40] There is a strong association of PPI use with mild, moderate, and severe hypomagnesemia,[41] suggesting that PPI-associated CKD may be mediated through the onset of hypomagnesemia.[40,42,43] This may account for the renal loss of magnesium due to poor resorption and absorption caused by PPI-altered gastrointestinal tract pH, decreasing TRPM6 and TRPM7 transporters absorption.[42]

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Table 2: Effect of *P. americana* seed and *B. pinnatum* leaf ethyl acetate fraction binary mixtures on kidney function indices of indomethacin-induced ulcerated rats

	NC	UC	OMEP	PAEF	BPEF	PAEF + BPEF (1:1)	PAEF + BPEF (1:2)	PAEF + BPEF (1:3)	PAEF + BPEF (2:1)	PAEF + BPEF (3:1)
Urea (mg/dL)	29.79 ± 1.34[a]	32.14 ± 1.53a	88.52 ± 6.52*	36.34 ± 1.84[a,b]	32.92 ± 4.74[a]	39.88 ± 5.19[b,c]	60.53 ± 4.19**	50.12 ± 6.02d	45.47 ± 1.87[c,d]	65.10 ± 9.26**
Creatinine (mg/dL)	1.50 ± 0.30*	1.29 ± 0.16**	7.42 ± 0.73b	1.08 ± 0.17**	0.79 ± 0.28[a]	1.42 ± 0.29b*	1.29 ± 0.25**	1.08 ± 0.32**	1.13 ± 0.16a*	1.17 ± 0.14**
Potassium (mmol/L)	9.19 ± 0.97[f]	7.57 ± 0.14[c,d]	5.91 ± 1.36[a]	8.36 ± 0.80[d,e]	6.19 ± 0.40[a,b]	7.05 ± 0.14[a,b,c]	6.06 ± 0.83[a,b]	5.89 ± 0.51[a]	8.35 ± 0.42[d,e]	6.31 ± 0.63[a,b]
Sodium (mmol/L)	126.06 ± 5.64[a,b]	133.43 ± 11.89[b,c]	123.41 ± 4.17[a,b]	133.00 ± 6.30[b,c]	126.28 ± 6.06[a,b]	122.98 ± 17.95[b,c]	133.81 ± 21.35[b,c]	114.80 ± 2.64[a]	144.26 ± 6.98c	124.82 ± 6.91[a,b]
Chloride (mmol/L)	61.26 ± 5.38[a,b]	56.22 ± 4.98[a]	63.95 ± 5.42[a,b]	64.45 ± 5.80[a,b]	61.68 ± 1.89[a,b]	57.06 ± 7.06a,b	61.60 ± 4.17[a,b]	63.78 ± 5.88[a,b]	63.53 ± 6.79[a,b]	76.22 ± 2.01[c]
Bicarbonate (mmol/L)	43.70 ± 0.53[a,b]	44.35 ± 1.04[a,b]	44.13 ± 0.25[a,b]	43.70 ± 0.36[a,b]	44.70 ± 0.24[b]	43.61 ± 1.72[a]	44.06 ± 0.41[a,b]	43.93 ± 0.35[a,b]	44.08 ± 0.45[a,b]	44.14 ± 0.32[a,b]

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

The restoration effect of the ethyl acetate fractions on magnesium concentration may be attributable to their inhibitory effect on pathways that induce hypocalcemia or calcium ion influx. Analysis of serum zinc concentration indicated a significant decrease in serum zinc concentration by indomethacin induction compared to normal control (Figure 1C). However, the administration of omeprazole (OMEP) and the ethyl acetate fractions of PAEF, BPEF, PAEF+BPEF (1:1), PAEF+BPEF (1:2), PAEF+BPEF (1:3), and PAEF+BPEF (3:1) resulted in significant restoration of zinc concentration in comparison to the ulcer control group. The ethyl acetate fraction binary mixtures and the single fractions effectively ensured a balance in serum zinc concentration. Serum zinc levels have been documented to be reduced in gastritis caused by *H. pylori*, peptic ulcer, and gastric cancer.[44,45] Also, Meng-Chieh *et al.* [46] reported a non-significant decrease in trace elements such as zinc and copper levels in *H. pylori*-infected patients. Zinc is an essential micronutrient for survival, optimal cellular function, and membrane barrier integrity.[45] Zinc deficiency may impair gastric output depending on the severity and increase the lethality of infectious diseases and cellular damage.[47] Restoration of zinc level by the binary mixtures of *P. americana* and *B. pinnatum* in the present study strongly suggests protection of membrane barrier function integrity against inflammatory factors. The increases in zinc level by the binary mixtures ethyl acetate fraction were important in promoting gastric wound healing.[48,49]

Figure 2 shows the histological sections of rats' kidney tissues: Plate 1 (Normal control group) show a section typical of normal architecture of the kidney. The tuft of the glomerulus was seen within the cortex with a slightly loose basement membrane. The interstitium and collecting tubules appear normal, with no histopathological lesions. In the ulcer control group, the architecture of the kidney was normal except for mild necrosis (Plate 2). However, in the omeprazole group (group 3), the micrograph shows a section of the kidney with reduced cell mass, large urinary space necrosis (N), and loose glomerular membrane (LGM) (Plate 3). These features are indicative of an inflammatory response. In plate 4, the micrograph shows a more organized cellular architecture than group 2. There were no apparent histopathological lesions in the kidney section of rats pre-treated with *P. americana* (PAEF) 400mg/kg b. wt. and later received indomethacin 30 mg/kg b. wt. However, in the groups induced with indomethacin 30 mg/kg b. wt., rats pre-treated with *B. pinnatum* (BPEF) 400 mg/kg b. wt. (figure 3, Plate 5) showed an organized architecture of the kidney when compared to the ulcer control group (group 2). There were no apparent histopathological lesions seen. Also, in plate six (6), a better organized kidney structure than that of group 2 was seen in indomethacin-induced ulcerated rats pre-treated with (PAEF + BPEF) (1:1) 400 mg/kg b. wt.; no apparent histopathological lesions were seen. Plate 7 shows the histopathology of induced rats pre-treated with (PAEF + BPEF) (1:2) 400 mg/kg b. wt., the micrograph shows an organized kidney structure except for the appearance of a large urinary space **(**US) and loose glomerular membrane (LGM) (Plate 7).

In the induced group, pre-treated with (PAEF + BPEF) (1:3) 400 mg/kg b. wt. (Plate 8), the photomicrograph showed a normal kidney structure when compared to group 2. Furthermore, in the group pre-treated with (PAEF + BPEF, 2:1) 400 mg/kg b.wt. and induced with indomethacin 30 mg/kg b. wt., the micrograph showed normal urinary space (US) with a distorted glomerulus (Plate 9).

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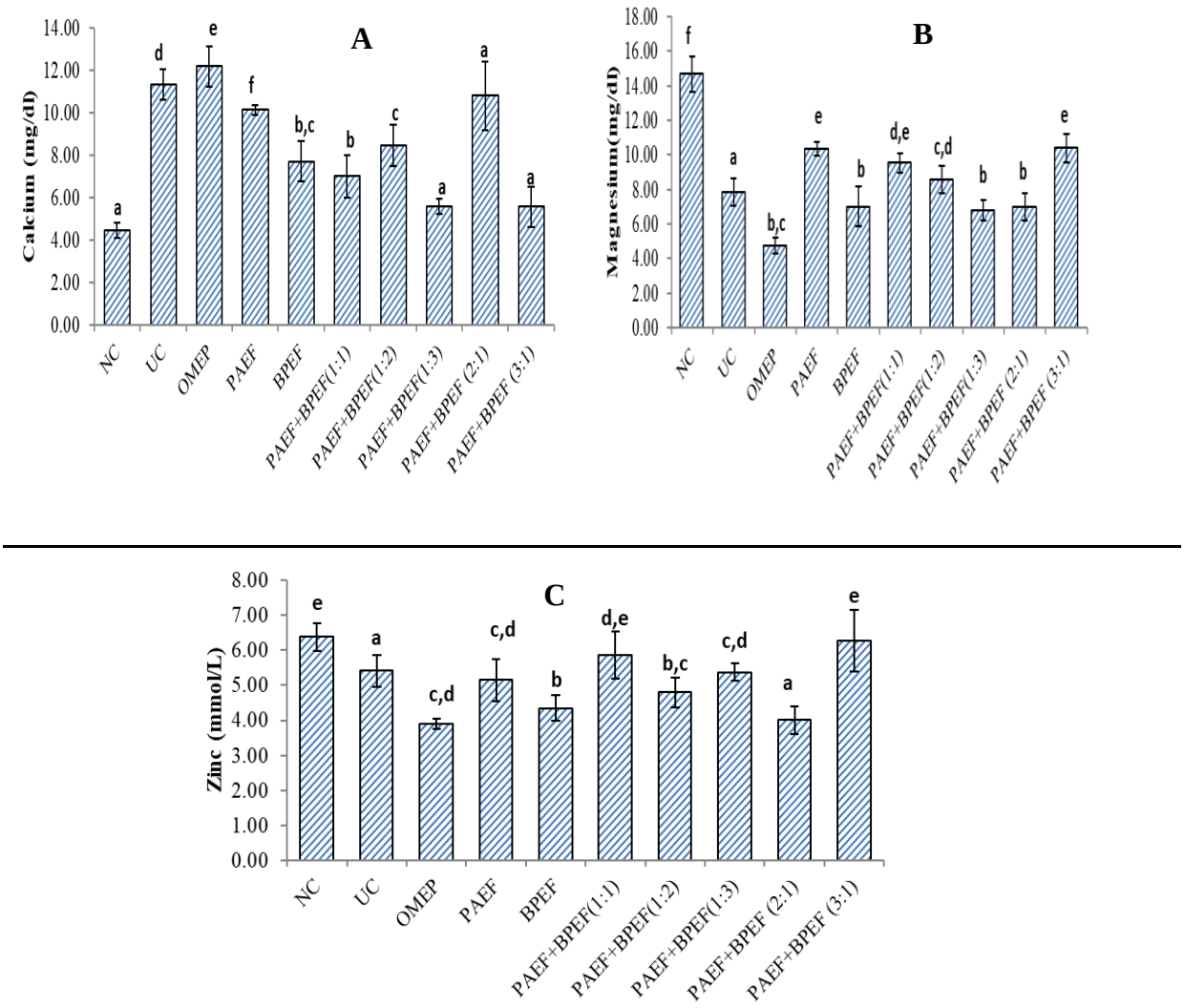

Figure 1: Effect of *P. americana* seed and *B. pinnatum* leaf ethyl acetate fraction binary mixture on serum calcium **(A)**, magnesium **(B)**, and zinc **(C)** concentrations in indomethacin-induced ulcerated rats. Values are mean ± SD (n = 5). Bars bearing different superscript letters across groups are significantly different ($p<0.05$).

In the induced group pre-treated with (PAEF + BPEF, 3:1) 400 mg/kg b. wt., the micrograph (Plate 10) showed a normal architecture of the kidney, but the proximal renal tubule was not well contrasted with the bowman's capsule. Histopathological examination of the kidney tissues revealed kidneys with reduced cell mass, necrosis, and loosed glomerular membrane in the standard (omeprazole) group. However, other groups pre-treated with ethyl acetate fraction of the binary mixtures showed slight changes in kidney architecture but did not differ significantly from the normal control. Compared to the ulcer control group, these groups demonstrated organized architecture with mild necrosis. The observations did not culminate in significant changes in the kidney function indices. This study, however, did not completely align with the findings of Pacifici [50] who reported that indomethacin treatment elevated creatinine concentration in infants with body weight <1 kg. Lee *et al.*[51] reported that the severity of indomethacin's deleterious effects increases with infant immaturity. A high risk of developing nephrotoxicity has been reported in elderly patients receiving NSAIDs.[7] This may involve reduced glomerular filtration rate, hyperkalemia, chronic kidney disease, acute interstitial nephritis, sodium retention, edema, and renal papillary necrosis in aged patients.[9,52] Histopathological sections of the kidneys in the present study are suggestive of a possible nephrotoxic effect. The disparity between the present study and previous study may be attributable to differences in duration of administration. Other groups, such as PAEF + BPEF (2:1) and PAEF + BPEF (3:1), showed normal kidney architecture but mild glomerulus distortion. From the above observations, it is clear that the administration of indomethacin results in a possible toxic effect on the kidney. The alteration in kidney function by omeprazole administration seen in this study is consistent with that reported in the literatures.[53-56]

Omeprazole use has been implicated in triggering pre-acute kidney injury.[56] The long-term use of proton pump inhibitors (PPIs) may increase the risk of chronic kidney disease.[53-56] These may be possible through some proposed mechanisms involving the bioaccumulation of PPIs and their bye-products in renal tissue, culminating in renal interstitial fibrosis. This disruption directly marked the initiation of chronic kidney disease (CKD), and decreased nitric oxide synthesis was attributed to lysosomal proton pump inhibition. The resultant effect is the generation of cytotoxic superoxide radicals, which causes hypomagnesemia and renal tissue endothelial dysfunction.[56,57]

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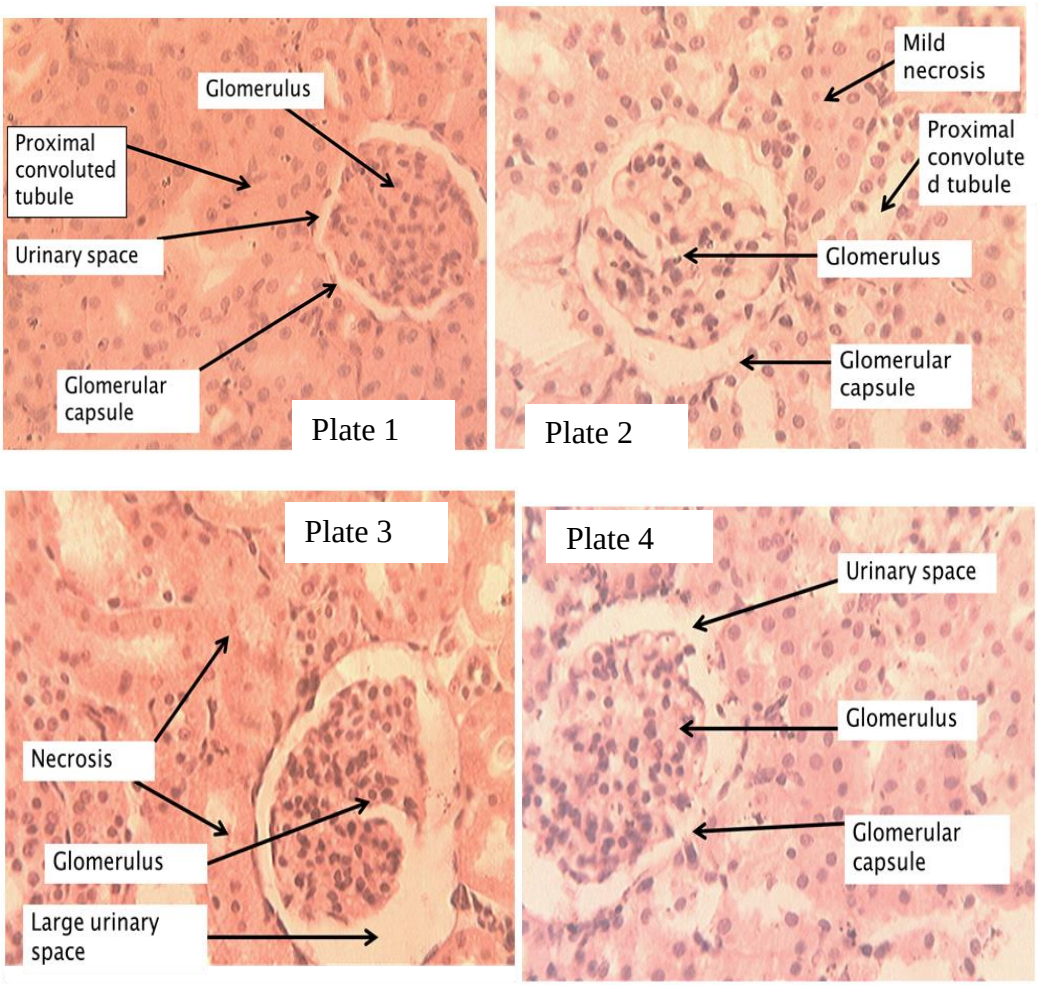
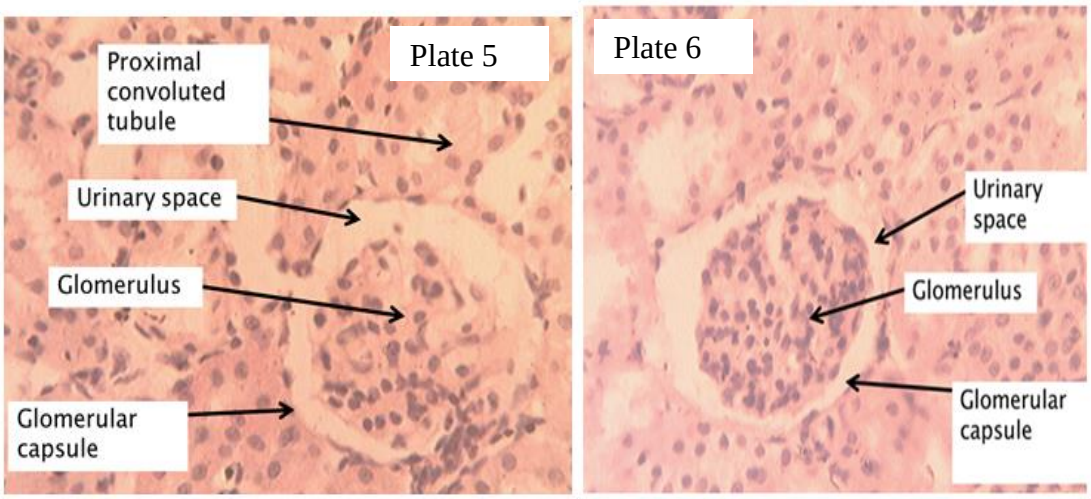


Figure 2: Photomicrograph of rat kidneys following treatment with *P. americana* seed and *B. pinnatum* leaf ethyl acetate fraction binary mixture
Key: Plate 1: Group 1 (Control), Plate 2: Group 2 (Ulcer control), Plate 3: Group 3 (Standard Omeprazole) 20 mg/kg b.wt, Plate 4: Group 4 ((PAEF) 400 mg/kg bwt)



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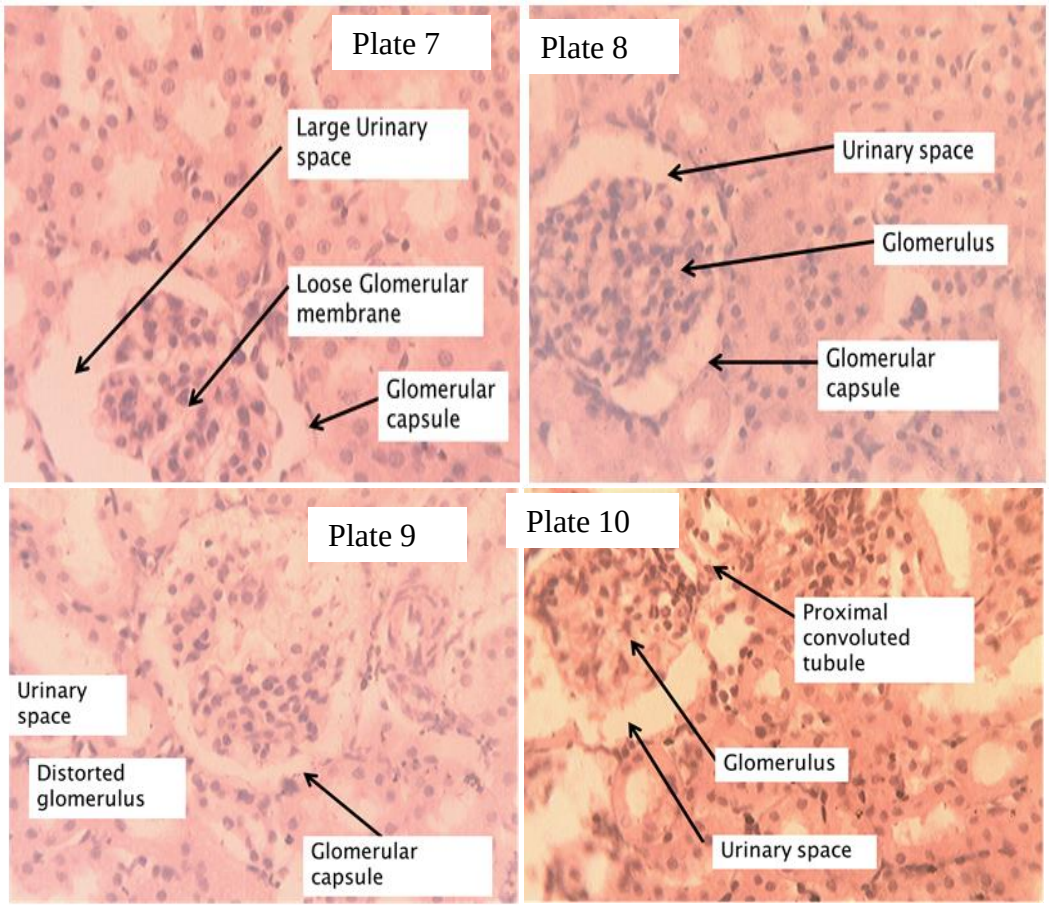

Figure 3: Photomicrograph of rat kidneys following treatment with *P. americana* seed and *B. pinnatum* leaf ethyl acetate fraction binary mixture
Key: Plate 5: Group 5 (BPEF), Plate 6: Group 6 (PAEF + BPEF) (1:1), Plate 7: Group 7 (PAEF + BPEF) (1:2), Plate 8: Group 8 (PAEF + BPEF) (1:3), Plate 9: Group 9 (PAEF + BPEF) (2:1)
Plate 10: Group 10 (PAEF + BPEF) (3:1)

Conclusion

The present study has shown that pre-treatment of rats with binary combinations of *P. americana* seed and *B. pinnatum* leaf ethyl acetate fraction significantly elevated serum urea concentration. The ethyl acetate fraction of the binary combination in the ratios PAEF+BPEF (1:1), PA+BP (1:3), PAEF+BPEF (2:1), and PAEF+BPEF (3:1) distorted kidney function and electrolyte balance. However, the fractions significantly normalized serum calcium, magnesium, and zinc concentrations. These observations suggest that the binary combinations of PAEF and BPEF may distort kidney function and electrolyte balance.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the works 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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