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EFFECT OF CO-ADMINISTRATION OF METFORMIN AND AQUEOUS LEAF EXTRACT OF *BRYOPHYLLUM PINNATUM* ON KIDNEY FUNCTION INDICES IN DIABETIC RATS

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ABSTRACT
The aim of this study was to evaluate the effect of co-administration of metformin and *Bryophyllum pinnatum* (BP) on kidney function parameters in diabetic rats. Forty male Wistar rats were randomly divided into 5 groups with 8 rats each namely; A (Control), B (Diabetic untreated), C (120 mg/kg b. wt Metformin only), D (400 mg/kg b. wt. BP only), and E (400 mg/kg b. wt. BP and 120 mg/kg b. wt. Metformin). Results showed urea, BUN and serum ALP significantly increased ($p < 0.05$) while Na^+, K^+, Ca^{2+}, PO_4^{3-}, Cl^- and kidney ALP decreased significantly ($p < 0.05$) in group B but were not altered in groups C, D and E when compared with A. However, Ca^{2+} level was significantly reduced ($p < 0.05$) in group E compared with groups A, C and D. Kidney sections for groups A, B, C, D and E showed renal tissue with preserved architecture. There are no vascular lesions seen and no features of acute or chronic damage. In conclusion, co-administration of metformin and *B. pinnatum* in diabetic rats reversed diabetes-induced alterations in selected kidney function parameters in experimental rats.

Keywords: Hypoglycemic agents, Metformin, *Kalanchoe*, Streptozotocin, Diabetes mellitus.

INTRODUCTION

Diabetic nephropathy, a debilitating complication of diabetes mellitus, remains a leading cause of chronic kidney disease globally (Lim, 2014). In 2014, 8.5% of adults aged 18 years and older had diabetes. In 2019, diabetes was the direct cause of 1.5 million deaths and 48% of all deaths due to diabetes occurred before the age of 70 years. Another 460 000 kidney disease deaths were caused by diabetes, and raised blood glucose causes around 20% of cardiovascular deaths (GBDCN, 2020). With the escalating prevalence of diabetes, there is an increasing need for innovative therapeutic strategies to address not only hyperglycemia but also the associated complications affecting vital organs such as the kidneys.

Metformin, a first-line oral hypoglycemic agent, exerts its effects through various mechanisms, including the inhibition of hepatic gluconeogenesis and improvement of insulin sensitivity (Foretz, 2023). It is primarily excreted unchanged by the kidney, and renal impairment may cause the accumulation of metformin leading to an elevated metformin concentration, and this has been proposed to lead to lactic acidosis (Lipska *et al.,* 2011). *Bryophyllum pinnatum*, commonly known as "Patharchatta" or "Life Plant," has been recognized for its anti-diabetic properties, with studies highlighting its potential benefits in ameliorating hyperglycemia and associated complications (Ezuruike and Prieto, 2014). The leaves of *Bryophyllum pinnatum* are widely used in traditional and ethnomedicinal practices for treatment of urinary insufficiency and stone disorders. The fresh leaf juice or along with the powder of 2-3 black peppers (*Piper nigrum* Linn.) is used as folklore medicine for treatment of kidney and gall bladder stones (Lans, 2006; Yadav *et al*., 2016). However, the co-administration of metformin and *Bryophyllum pinnatum* raises questions about potential drug-herb interactions and their impact on renal function in the context of diabetes. Limited research has explored the simultaneous administration of metformin and *Bryophyllum pinnatum,* especially concerning their combined effects on kidney functions in diabetic conditions.

The aim of this study therefore was to evaluate the effect of co-administering metformin, a widely prescribed anti-diabetic medication, and *Bryophyllum pinnatum*, a traditional medicinal plant, on kidney functions in diabetic rats. The findings will contribute valuable insights into the safety and efficacy of integrating herbal supplements with conventional anti-diabetic medications, guiding future considerations for diabetic care that embraces a holistic approach.

MATERIALS AND METHODS

Plant collection and authentication

Fresh leaves of *Bryophyllum pinnatum* were gotten from a garden (8˚31'37.12116''N 4˚35'19.21848''E) within Ilorin, Kwara State, Nigeria on 2nd of April, 2023 at 7 am. It was authenticated at the Department of Plant Biology, University of Ilorin, Ilorin, Nigeria and a voucher specimen was deposited under a voucher number; UILH/001/909/2023, for reference purpose.

Experimental Animals

Forty male Wistar rats (125 ± 5 g) of norvegicus strain were obtained from the animal breeding section of Biochemistry Department, University of Ilorin, Ilorin, Nigeria. The rats were housed in well ventilated plastic cages and allowed to acclimatize to animal house conditions (temperature 28 – 31°C; 12 hours of natural light and 12 hours darkness and humidity 50 – 51%) for 14 days before commencement of experiment. The rats were fed with rat chow (Top Feeds Ltd., Ogorode Industrial Estate, Sapele, Delta State, Nigeria) and clean tap water *ad libitum*

Ethical approval

Handling of animals was done in accordance with guidelines provided by the University of Ilorin ethical committee for the use and care of animals for research and protocol identification code UERC/LSC 078 was issued.

Drugs, Assay kits and reagents

Metformin (Eden U.K. Pharmaceutical Ltd.) was purchased from a local pharmacy store in Ilorin, Nigeria. Assay kits for phosphate and calcium ions were determined using spectrum kits (Egyptian Company of Biotechnology, Egypt). Assay kits for alkaline phosphatase activity, urea, potassium ion and chloride ion were products of Fortress diagnostics limited, Unit 2C Antrim technology, park, Antrim, BT41 IQS, United Kingdom. Sodium ion assay kit was a product of Atlas medical, William James House, Cowley rd, Cambridge, CB4 0WX. Streptozotocin and D-Fructose were products of Sigma-Aldrich, St. Louis, Mo, USA and Molychem, Mumbai, India respectively. Other reagents were of analytical grade and were purchased from reputable chemical vendors in Ilorin, Nigeria.

Plant preparation and extraction

Bryophyllum pinnatum leaves were rinsed with distilled water, air-dried, and ground into fine powder using an electric blender (Super Master® Model SMB-2977, Japan). The powdered plant material was percolated in 6 L of distilled water and kept refrigerated for 72 hours with intermittent stirring. The mixture was filtered using a strainer and complemented with Whatman No. 1 filter paper. The resulting filtrate was lyophilized (SJIA-18N Branch Manifold Model, Ningbo SjiaLab Equipment Co., Ltd, Ningbo City, China) and the resulting concentrate reconstituted in distilled water to arrive at the dose used in this study.

Induction and confirmation of type 2 diabetes

All experimental animals except those in group A (control group) were exposed to 10% w/v fructose solution for 14 days. Afterwards, rats were fasted for 8 hours before freshly prepared streptozotocin (STZ) (45 mg/kg b.wt. in 0.1M sodium citrate buffer [pH 4.5]) was administered to them as a single intra-peritoneal injection to induce diabetes mellitus (Wilson and Islam, 2012). Forty-eight (48) hours after STZ administration, Fasting Blood Glucose (FBG) level of the rats was determined using Accu-check active glucometer and compatible strips. Rats showing FBG level above 200 mg/dL were considered diabetic and recruited for the experiment.

Experimental design

Forty male Wistar rats obtained were randomly divided into 5 groups consisting of 8 rats each treated as follows:

Group A (Control) – Non-diabetic rats that received distilled water

Group B (Negative control) - Diabetic rats that received distilled water

Group C - Diabetic rats that received standard drug metformin (120 mg/kg b.wt.)

Group D - Diabetic rats administered BP (400 mg/kg b.wt.)

Group E - Diabetic rats administered BP (400 mg/kg b.wt.) + standard drug metformin (120 mg/kg b.wt.)

Rats were treated for 28 days and sacrificed 24 hours after. Their kidneys and blood were collected and prepared for kidney function parameters and histopathology analyses.

Animal sacrifice and tissue collection

Rats were euthanized by exsanguination under dichloromethane anesthesia. The fur and skin in the neck region of each rat was quickly cleared to expose the jugular vein. The slightly displaced jugular veins were then incised using a sterilized sharp blade. Blood samples were collected into plain sample bottles and was allowed to clot at room temperature and the serum was collected using Pasteur pipette. Kidneys were carefully detached, cleaned with tissue paper, and weighed. The kidneys were then preserved in 0.25 M sucrose solution (for biochemical analysis) or 10% w/v formalin (for histopathological analysis).

Determination of kidney function parameters

ALP activity, urea, sodium, potassium, chloride, calcium and phosphate ions were determined by following the manufacturing instructions in the various assay kits, while BUN was calculated by dividing the value of urea (mg/dL) by 2.14 (Fawcett and Scott, 1960). Kidney b.wt. ratio was determined by dividing weight of kidney (g) by b.wt. of animal (g).

Kidney histopathological processing and examination

Thin slices (5 mm) of the fixed organ were cut, put in tissue cassettes and labelled appropriately; they were processed using paraffin wax tissues processing method with the aid of LEICA PT 1020 Automatic Tissue Processor as follows: The tissues were dehydrated in ascending grades of alcohol (70% v/v alcohol — absolute alcohol), cleared in two changes of xylene with infiltration and impregnation in two changes of thermostatically regulated paraffin wax. After tissue processing, the tissues were embedded with molten paraffin wax, cooled and ribbon sections of the tissue were cut with a LEICA RM 2135RT rotary microtome at five microns. The sections were floated and attached to the slide with the aid of the floating bath and floating fluid (20% alcohol). The attached sections on the slides were then labelled with the corresponding number on the tissue cassettes. The slides were then dried at 65˚C in a hot air oven to allow for staining (Drury and Wallington, 1980; Avwioro, 2014).

Tissue sections were de-waxed in xylene for one hour, hydrated in descending grades of alcohol (absolute alcohol — 70% v/v alcohol) and rinsed in distilled water. They were then stained with Mayer's Haematoxylin for 15 minutes, rinsed in water, differentiated in 1% acid-alcohol briefly and rinsed again in distilled water. Thereafter, the sections were blued in running tap water for 10 minutes, stained in Eosin for 2 minutes, dehydrated in ascending grades of alcohol (70% v/v alcohol — absolute alcohol), cleared in xylene then mounted with dibutyl phthalate xylene (D.P.X) (Drury and Wallington, 1980).

The stained tissue sections were examined using x10 objective lens of the microscope (Binocular microscope WKT-LE4, Jiangsu, China) for focusing and later viewed with the x40 objective lens of the microscope for a higher magnification. Photomicrographs were taken at a magnification of x100 and high power of x400.

Statistical analysis

Data were expressed as mean of 6 determinations ± SEM. The data were subjected to statistical analysis using the IBM® statistical package for social sciences (SPSS) software version 20. All significant differences were determined by one way analysis of variance (ANOVA). Post hoc multiple comparisons were done using Duncan's multiple range test. The level of significance was set at $p < 0.05$ (confidence level = 95 %).

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RESULTS

Kidney and serum alkaline phosphatase (ALP) activity in diabetic rats co-administered aqueous leaf extract of *Bryophyllum pinnatum* and metformin

Table 1 shows the result of kidney and serum ALP activity in diabetic rats co-administered aqueous leaf extract of *Bryophyllum pinnatum* and metformin. There was a significant reduction ($p < 0.05$) in kidney ALP activity of rats in groups B compared to the control (A). Groups D and E however showed significant increase ($p < 0.05$) in ALP activity compared to B but was not significantly different ($p > 0.05$) to A. There was no significant difference ($p > 0.05$) in serum ALP activity for the experimental groups compared to A except B which increased significantly ($p < 0.05$) compared to control (A).

Table 1: Kidney and serum alkaline phosphatase activity in diabetic rats co-administered aqueous leaf extract of *Bryophyllum pinnatum* and metformin

Group	Alkaline phosphatase activity (U/L)	
	Serum	Kidney
A	55.96 ± 1.27^{a}	63.03 ± 0.60^{a}
B	62.45 ± 0.98^{b}	54.08 ± 1.42^{b}
C	58.86 ± 0.94^{a}	60.61 ± 1.53^{a}
D	57.54 ± 1.69^{a}	61.83 ± 1.51^{a}
E	57.50 ± 1.33^{a}	64.40 ± 1.15^{a}

Values are mean of six determinants ± SEM: Comparison is down the columns. Values with superscript are significantly different ($p < 0.05$). A = Positive Control (Non diabetic rats); B = Negative Control (Untreated diabetic rats); C = Reference drug treated rats (Metformin 120 mg/kg b.wt.); D = *Bryophyllum pinnatum* aqueous extract treated rats (BP 400 mg/kg b.wt.); E = *Bryophyllum pinnatum* aqueous extract co-administered with Metformin (BP; 400 mg/kg b.wt. And Metformin; 120 mg/kg b.wt.)

Serum Urea, Blood Urea Nitrogen and Electrolytes Concentrations in Diabetic Rats Co-administered Aqueous Leaf Extract of *Bryophyllum pinnatum* and Metformin

Table 2 shows the concentrations of serum urea, BUN, and selected electrolytes in diabetic rats co-administered aqueous leaf extract of *Bryophyllum pinnatum* and metformin. There was a significant increase ($p < 0.05$) in the serum urea and BUN concentrations in the diabetic untreated group (B) compared to control (A), while groups C, D and E were not significantly different ($p > 0.05$) to A. Na^{+}, K^{+}, Ca^{2+}, PO^{3-}_{4} and Cl^{-} concentrations were decreased significantly ($p < 0.05$) in diabetic untreated group (B) but no significant difference ($p > 0.05$) was recorded in them compared to group A. However Ca^{2+} level was significantly reduced ($p < 0.05$) in group co-administered with metformin and BP compared to group A, C and D.

Table 2: Serum urea, blood urea nitrogen and electrolytes concentrations in diabetic rats co-administered aqueous leaf extract of *Bryophyllum pinnatum* and metformin

Groups	Urea (mg/dl)	BUN (mg/dl)	Na^{+} (mmol/l)	K^{+} (mmol/l)	Cl^{-} (mmol/l)	PO^{3-}_{4} (mmol/l)	Ca^{2+} (mmol/l)
A	7.63 ± 1.30^{ac}	3.56 ± 0.84^{a}	146.14 ± 2.46^{a}	5.51 ± 0.14^{a}	76.31 ± 1.06^{a}	2.04 ± 0.10^{a}	2.39 ± 0.01^{a}
B	14.25 ± 0.90^{b}	6.65 ± 0.42^{b}	140.82 ± 1.40^{b}	4.47 ± 0.21^{b}	70.13 ± 0.78^{b}	1.10 ± 0.06^{b}	1.40 ± 0.01^{b}
C	7.53 ± 0.40^{ac}	3.51 ± 0.18^{a}	152.91 ± 3.94^{a}	5.78 ± 0.14^{a}	80.32 ± 0.76^{c}	1.89 ± 0.18^{a}	2.38 ± 0.03^{a}
D	5.99 ± 0.61^{a}	2.80 ± 0.28^{a}	151.18 ± 2.62^{a}	6.30 ± 0.12^{b}	81.32 ± 1.10^{c}	2.25 ± 0.07^{b}	2.40 ± 0.01^{a}
E	8.11 ± 0.26^{c}	3.79 ± 0.81^{a}	152.00 ± 3.98^{a}	5.73 ± 0.14^{a}	80.06 ± 1.24^{c}	2.04 ± 0.04^{a}	2.33 ± 0.01^{c}

Values are mean of six determinants ± SEM: Comparison is down the columns. Values with superscript are significantly different ($p < 0.05$). A - Positive Control (Non diabetic rats); B - Negative Control (Untreated diabetic rats); C - Reference drug treated rats (Metformin 120 mg/kg b.wt.); D - *Bryophyllum pinnatum* aqueous extract treated rats (BP 400 mg/kg b.wt.); E - *Bryophyllum pinnatum* aqueous extract co-administered with Metformin (BP; 400 mg/kg b.wt. and Metformin; 120 mg/kg b.wt.); BUN – blood urea nitrogen; Na^{+} – Sodium ion; K^{+} – Potassium ion; Cl^{-} - chloride; P – Phosphorus; Ca^{2+} - calcium ion

Kidney-body weight ratio of diabetic rats co-administered aqueous leaf extract of *Bryophyllum pinnatum* and metformin

Table 3 shows the result of kidney-b.wt. ratio of diabetic rats co-administered aqueous leaf extract of *Bryophyllum pinnatum* and metformin. The kidney-b.wt. ratio of diabetic rats showed no significant difference ($p > 0.05$) when compared to the control group.

Table 3: Kidney-b.wt. ratio in diabetic rats co-administered aqueous leaf extract of *Bryophyllum pinnatum* and metformin

Group	Kidney-b.wt. ratio (x10^{-3})
A	6.99 ± 0.54^{a}
B	6.16 ± 0.69^{a}
C	6.86 ± 0.52^{a}
D	7.10 ± 0.16^{a}
E	7.16 ± 1.10^{a}

Values are mean of six determinants ± SEM: Comparison is down the columns. Values with superscript are significantly different ($p < 0.05$). A - Positive Control (Non diabetic rats); B - Negative Control (Untreated diabetic rats); C - Reference drug treated rats (Metformin 120 mg/kg b.wt.); D - *Bryophyllum pinnatum* aqueous extract treated rats (BP 400 mg/kg b.wt.); E - *Bryophyllum pinnatum* aqueous extract co-administered with Metformin (BP; 400 mg/kg b.wt. and Metformin; 120 mg/kg b.wt.)

Histopathology of kidneys of diabetic rats co-administered aqueous leaf extract of *Bryophyllum pinnatum* and metformin

Figure 1 shows photomicrograph of hematoxylin-eosin-stained (X100) section of the kidneys of representative experimental rats co-administered aqueous leaf extract of *Bryophyllum pinnatum* and metformin. Sections for groups A, B, C, D and E shows renal tissue with preserved architecture composed of normal glomeruli, tubules and unremarkable interstitium. There are no vascular lesions seen and no features of acute or chronic damage.

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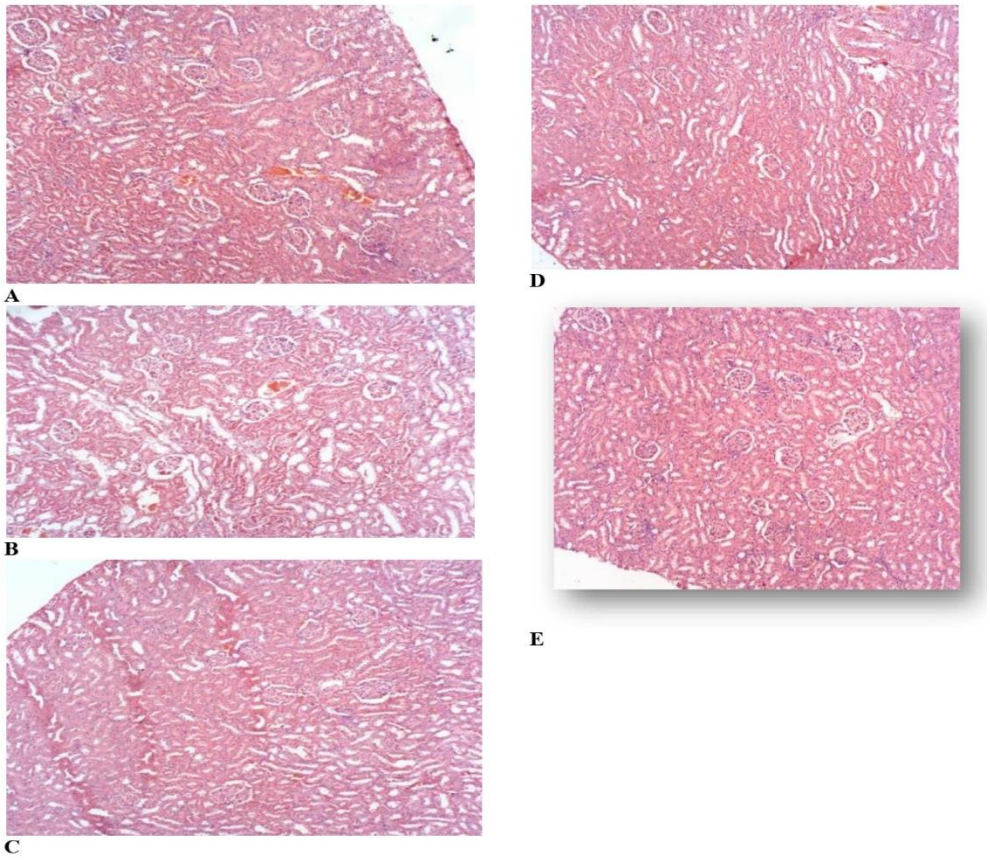

Figure 1: Photomicrographs showing representative hematoxylin-eosin-stained (100x) sections of the kidneys of diabetic and control rats co-administered aqueous leaf extract of *Bryophyllum pinnatum* and metformin

A - Positive Control (Non diabetic rats); B - Negative Control (Untreated diabetic rats); C - Reference drug treated rats (Metformin 120 mg/kg b.wt.); D - *Bryophyllum pinnatum* aqueous extract treated rats (BP 400 mg/kg b.wt.); E - *Bryophyllum pinnatum* aqueous extract co-administered with Metformin (BP; 400 mg/kg b.wt. and Metformin; 120 mg/kg b.wt.);

DISCUSSION

Many orthodox drugs have their origin from herbs. Herbs contain a large number of compounds, rather than a single pharmacologically active substance unlike orthodox drugs which contain chemically pure substances; hence components of both herbal and orthodox medicines may act on one another to moderate, oppose, or enhance an effect (De Smet, 1997; Houghton, 2009). Concurrent use of herbal and orthodox medicines according to Neustadt (2006) causes interactions between these two forms of medicines which can lead to undesirable pharmacokinetic and pharmacodynamic effects (Neustadt , 2013). In this study, the decrease in kidney ALP activity and concomitant increase in the serum ALP activity suggest possible damage in plasma membrane of the kidney. Although kidney is not the only possible source of ALP, it is found in the cytosol of liver cells and the canalicular membrane of hepatocytes (Green and Sambrook, 2020). It is also found in decreasing concentrations in various organs such as the placenta, ileal mucosa, and bone. Upon administration of BP singly and BP together with metformin, an increase in the activity of ALP was recorded which compared favourably to the positive control. This suggests recovery from the damage earlier recorded in group B. The leaf of *Bryophyllum pinnatum* is reportedly used in traditional and ethnomedicinal practices for treatment of urinary insufficiency and stone disorders (Prachi *et al*., 2009). The medicinal and pharmacological properties of *Bryophyllum pinnatum* were ascribed to the presence of alkanes, alkanols, triterpenes and sterols (Nozawa *et al.,* 1984), triterpenoids and phenanthrenes (Jardillier, 1981), flavonoids (Iino, 1995), bufadienolides (Bowen and Remaley , 2014), alkaloids, glycosides and lipids (Knaak *et al*., 1997).

In this study, the significant increase in the level of serum urea concentration in diabetic rats suggests renal dysfunction. Elevated level of urea in diabetic rats might be due to increased catabolism of protein or deamination of amino acid for gluconeogenesis (Yamaguchi and Weitzmann, 2009; Omoniwa and Yakubu, 2017). Administration of BP and coadministration of BP and metformin resulted in significant decrease in urea concentration, suggesting improvement in the renal function. However, between group D and E, group E had a significant increase in urea level compared to D. Based on this finding, co-administration of metformin and BP might

interfere with total renal function recovery. The improvement in renal function with the extract in this work may be attributed to its antidiabetic property. This finding is in agreement with an earlier study where the aqueous extract of *Bryophyllum pinnatum* exhibited inhibitory effect on serum creatinine and urea (Shukla *et al.,* 2014).

Diabetes mellitus is characterized by abnormally high blood glucose levels which may lead to microvascular changes via activation of non-enzymatic glycosylation of collagen and tissue proteins in kidney. Elevated glycemic levels affects the nephrons which are the filtering units of kidney leading to renal dysfunction (Mauricio *et al*., 2023). If the kidneys are not functioning properly, BUN formed will not be cleared off from the kidney and will increase abnormally in the serum (Seki *et al*., 2019). In our study, serum BUN was elevated for diabetic untreated group. Studies conducted by Singh *et al.* (2014) and Bamanikar *et al.* (2016) recorded a similar finding. Administration of treatments however resulted in significant decrease in BUN levels for groups D and E and this decrease compared favourably to control (A), suggesting recovery from the renal dysfunction. There was no difference in the levels of BUN for rats administered metformin and BP singly and those co-administered both metformin and BP as seen for urea.
The electrolytes evaluated in this study showed there was an imbalance in the electrolyte concentrations in the diabetic untreated group (B). In diabetes mellitus, hyperglycemia causes glucose-induced osmotic diuresis with resultant loss of body fluids and electrolytes (Ojiako and Chikezie, 2015). This imbalance was however restored to normal upon treatment with metformin (C), BP (D) and BP plus metformin. There was no difference in the electrolyte levels between the individual treatment groups (C, D and E). This finding is similar to that of Uhegbu *et al.* (2017) who had earlier reported the renal protective potential of BP in their study. However, co-administration of metformin and BP was not restored to control in this study (Table 2).

Findings from this study showed that the biochemical changes recorded for the untreated diabetic rats and the treated groups did not result in concomitant change in the histopathology of the kidneys section examined, as well as the kidney to b.wt. ratio of the rats. This observation is similar to reports from previous study (Yadav *et al*., 2016; Sharma and Choudhary, 2023).

Conclusion

Co-administration of 120 mg/kg b.wt. metformin and 400 mg/kg b.wt. *B. pinnatum* for 28 days in diabetic rats restored altered kidney function indices arising from diabetes mellitus in experimental rats in this study and did not cause any noticeable changes to the kidney architecture. Therefore, the concurrent use of these two agents might not cause deleterious effect on the kidney.

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