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Renal Protective Properties of Aqueous Extract of *Bryophyllum pinnatum* (Lam.) Oken Leaf against Petrol Vapour – Induced Toxicity on Male Albino Rats

Friday O. Uhegbu[1*], Caleb J. Nwaogwugwu[1], Chibuzo H. Onwuegbuchulam[1], Chinwe Edith Oriaku[1], Okechukwu C. Atasie[1] and Kelechi U. Akatobi[1]

[1]Department of Biochemistry, Faculty of Biological and Physical Sciences, Abia State University, P.M.B. 2000, Uturu, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author FOU designed and supervised the study and wrote the first draft of the manuscript. Author CJN and CHO performed the experiments and managed the literature searches. Author CEN and OCA prepared plant extract and animal house maintenance, Author KUA performed the statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

The aim of this study was to investigate the renal protective properties of aqueous leaf extract of *Bryophyllum pinnatum* from Nigeria against petrol vapour – induced toxicity on the kidney of male albino rats. Fifty apparently healthy male albino rats aged 8 weeks and weighing between 165 g – 185 g were randomly divided into five groups of ten animals each. Group 1 served as control and were not treated with the plant extract or exposed to gasoline vapour. Group 2 served as negative control and were exposed to the gasoline vapour but not treated with the plant extract. Groups 3, 4

**Corresponding author: E-mail: fouhegbu@yahoo.com, fouhegbu@abiastateuniversity.edu.ng;*

and 5 were treated with the plant extract at 20 mg/kg body weight, 40 mg/kg body weight, 60 mg/kg body weight respectively and exposed to the gasoline vapour for 5 hrs daily for 21 days. Results show that mean body weight of treated animals increased significantly ($p \leq 0.05$) compared to negative control. Haematological parameters; PCV, Hb, Total bilirubin, WBC and RBC increased, while renal function biomarkers Na^{+}, Ca^{2+}, Mg^{2+} and Cl^{-} also increased in the treated animals compared to the negative control respectively. The effect of the plant extract is dose dependent. The results show that the plants extract has renal protective potential on the experimental animals.

Keywords: Toxicity; kidney; body fluids; metabolic waste; homeostatic; medicinal plants.

1. INTRODUCTION

The internal environment of the cells of the body is made up of the extracellular fluid. The vital activities of cells are therefore carried out within the extracellular fluid. The extracellular fluid is maintained relatively constant in composition, since changes in extracellular fluid are reflected by change in the environment within the cells and thus also in cell function [1].

Two organs, the lungs and the kidneys are responsible for the regulation of the internal environment. The lungs control the concentrations of oxygen and carbon-dioxide while the kidneys maintain optimal chemical composition of the body fluids. The kidney thus is involved in removal of metabolic waste and performance of homeostatic functions [2].

Metabolic acidosis is a characteristic complication of renal disease. It is caused by a decrease in the glomerular filtration rate, which leads to retention of fixed acid catabolites such as phosphates and sulfates which is accompanied by a rise in non-protein nitrogen in the blood. There may also be a failure of the renal tubular mechanism for excretion of hydrogen ions and for the manufacture of ammonia, which would also lead to a considerable loss of cation into the urine. Under these circumstances reduction in sodium content of the plasma may become so severe that dehydration secondary to the electrolyte depletion will result. The consequent fall in extracellular fluid volume is a factor in reducing blood flow to the kidney, which further impairs its function [3,4].

Petrol (gasoline) is a complex mixture of hydrocarbons (typically of chain length C4-12). It is produced by mixing fractions obtained from the distillation of crude oil with brand-specific additives to improve performance. Under normal conditions, it is a volatile liquid with a characteristic odour. Like other xenobiotics, the chemical pollutants from petrol vapour may be metabolically transformed into several other metabolites as it gets into the body [5]. These metabolites may become very reactive and interact with the excreting and metabolizing tissues of the body to elicit toxic effects, especially the liver, lungs and kidneys [6]. Cellular injuries to tissues are caused by interaction of tissues with these metabolites. Some components of petrol such as volatile nitrates, benzene, other hydrocarbons and lead have been reported to produce toxic effects on lymph nodes, bone marrow and spleen [5]. Impairment of renal function as a result of serum electrolyte derangement due to exposure to crude oil, diesel and petrol vapour in albino rats has been reported [7,8].

During the course of usage of these products, individuals are usually exposed to pollutants from petroleum products in their environments [9]. A recent study had found respiratory problems in 21 out of 75 petrol pump workers studied; as 20.69% of workers with five years experience and 22.73% of employees who had put in up to 10 years of work at petrol pumps had problems. The study also found changes in brain function, respiratory and menstrual problems in petrol pump workers [10]. Problems of memory, intellectual capacity, and psychomotor ability in petrol pump workers have been reported [9]. Studies also show petrol pump workers are prone to lung diseases with cough, sputum production, breathlessness and chest pain and kidney problems [11,12].

Bryophyllum pinnatum is a genus of African and Madagascan plants in the family Crassulaceae [13]. It is a weed of gardens, parks, roadsides, railways lines, waste areas, coastal environs, creek-banks, open woodlands, forest and forest margins that is naturalized in tropical, sub-tropical and warmer temperate regions [14]. The plant is commonly known as air plant, love plant, miracle leaf, life plant. It is a perennial herb that has been used in folklore medicine in several

countries. This herb contains a wide range of chemical compounds such as alkaloids, flavonoids, steroids, lipids, and organic acids [15]. It contains bufadienolide, cardiac glycosides [16,17]. The *Bryophyllum pinnatum* plant leaf extracts have analgesic effect [18], antimicrobial, anti-inflammatory effect [19,16] and sedative effects [20]. The effectiveness of *B. pinnatum* in the prevention and treatment of ethylene glycol-induced urolithiasis has also been reported [21]. They observed significant reduction of urine oxalate levels with reduction in kidney calcium-oxalate deposition on administration of aqueous leaves extracts of *B. pinnatum* leaf.

In the South East of Nigeria the Igbos use the plant in folkoric medicine to treat and manage several illness including cough, skin diseases, stomach disturbance, nervous problems, hypertension and kidney problems [15]. In the South West of Nigeria, the plant is considered sedative, wound-healing, diuretic, anti-inflammatory, and cough suppressant. Leaf juice of plant is used to treat boils and skin ulcers, intestinal parasites, bronchitis, pneumonia [22,23,24].

The present study is therefore aimed at investigating the renal protective properties of aqueous leaf extract of *B. pinnatum* from Nigeria against petrol vapour- induced toxicity on male albino rats. In Nigeria petrol pump workers are exposed to the petrol vapour at least 5 to 6hr daily, hence we thought it necessary to evaluate the renal-protective properties of the aqueous leaf extract of *B. pinnatum* from Nigeria in male albino rats.

2. MATERIALS AND METHODS

2.1 Plant Leaves

Bryophyllum pinnatum leaves were collected from Abia State University, Uturu campus and were identified at the Department of Plant Science and Biotechnology, Abia State University, Uturu. Voucher specimens were deposited at the departmental herbarium Voucher No: DPSBH 268. The leaves were washed with distilled water to remove dirt, sun dried for about eight days to a constant weight. The dried leaves were milled into fine powder using Arthur Miller milling machine and preserved in cellophane bags until when used.

2.2 Preparation of Leaf Extract

Ten grams (10 g) of the powdered samples of *B. pinnatum* leaf were dissolved in 300 ml of distilled water and extracted by cold maceration for 24 hours. The mixtures were then filtered using a muslin bag. Volume of extract recovered was 256 ml, and the concentration of extract was determined to be 26.4 mg/ml. The sample was then placed in airtight container and refrigerated until used.

2.3 Animal Studies and Experimental Design

A total of fifty healthy male Albino rats of eight weeks old and weighing between 165 g – 185 g were placed randomly into five groups of ten animals each. Group 1 served as positive control and were not treated with the plant extract or exposed to petrol vapour. Group 2 served as negative control and were exposed to the petrol vapour and not treated with the plant extract. Groups 3, 4 and 5 were treated with the plant extract at 20 mg/kg body weight, 40 mg/kg body weight and 60 mg/kg body weight respectively and exposed to the petrol vapour for 5 hrs daily for 21 days. The animals were allowed feed and water *ad libitum*. Standard laboratory protocols for animal studies were maintained. Approval for animal studies was obtained from the Animal Ethics Committee of the College of Medicine and Health Sciences, Abia State University, Uturu, Nigeria.

2.4 Treatment of Animals

The animals were starved overnight prior to the commencement of the administration of the *B. pinnatum* extract. The extract was reconstituted in physiological saline (0.9%) to give the desired concentration of 20 mg, 40 mg and 60 mg. The administration of the extract was by oral intubation using a gavage intubation at 20 mg/kg body weight, 40 mg/kg body weight and 60 mg/kg body weight for groups 3, 4 and 5 animals respectively before exposure to petrol vapour 5 hr daily for 21 days. During the 5 hr daily exposure, the petrol was placed in plates (without cover) and stationed very close to the cages of the animals constantly and also occasionally sprayed around the environment where the animals were stationed. (Occasional spray around the environment was to accommodate for spill out of petrol in car tanks and containers around petrol stations).

2.5 Collection of Blood Samples and Serum Preparation

At the 22nd day the animals were sacrificed after overnight starving. Incisions were made into their thoracic cavity. Blood samples were collected by heart aorta puncture using a 10 mL hypodermic syringe and allowed to clot in sample vials. The samples were centrifuged at 3000 rpm for 5 min. using the Bran Scientific and Instrument Company England centrifuge. The supernatant was harvested by simple aspiration with Pasteur pipette and stored in clean tubes at - 4℃ until analysis.

2.6 Estimation of Parameters

2.6.1 Determination of total protein

Total protein test kits produced by Randox laboratories were used. This test involves formation of colored complex between cupric ions in alkaline medium with peptide bonds. The intensity of the color formed is proportional to the concentration of the protein.

2.6.2 Determination of albumin

Albumin test kit produced by Biosystem kits were used to estimate albumin. The principle of this test is based on albumin quantitatively binding to bromocresol green (BCG). The complex of albumin-BCG so formed absorbs maximally at 578 nm, and the absorbance is directly proportional to the concentration of albumin in the sample.

2.6.3 Hematological parameters

Hemoglobin was determined according to the method of Alexander and Griffths, [25]. White blood cell, red blood cell count and pack cell volume was determined as described by Dacie and Lewis [26]. using Biosystem kits. Total bilirubin was assayed as described by Tietz [27], using Biosystem kits.

2.7 Renal Function Tests

The serum concentrations of Creatinine and Urea were determined using auto-analizer (Biosystem A25 Random Access Analyzer). Serum concentrations of Sodium, Potassium, Calcium, Magnesium, Hydrogen carbonate, Hydrogen phosphate, Sulphate and Chloride were determined using auto-analizer (EasyLyte Plus Analyzer). The manufacturer's instructions for the entire biochemical test were strictly adhered to.

2.8 Statistical Analysis

Values were represented as Mean ± SD. Data obtained were subjected to one way Analysis of Variance (ANOVA) and group means were compared using Duncan's new multiple range tests. Differences were considered to be significant at ($p \leq 0.05$).

3. RESULTS

The effect of the *B. pinnatum* leaf extract on the mean body weight of the experimental animals is as reported in Table 1. The mean body weights of the treated animals were significantly increased ($p \leq 0.05$) compared to the negative control.

Table 2 shows the effect of the extract on hematological parameters. The plant extract treated animals showed significant increase in the hematological parameters assayed. The effect of the plant extract is dose dependent.

The plant extract significantly reduced the kidney function biomarkers assayed (Table 3) in the experimental treated with the plant extract before exposure to the petrol vapour. The effect is dose dependent.

Table 1. Effect of *B. pinnatum* aqueous leaf extract on mean body weight (g) of albino rats after 21 days

Group 1 (positive control)	Group 2 (Negative control)	Group 3 (20 mg/kg)	Group 4 (40 mg/kg)	Group5 (60 mg/kg)
181.45±4.83	174.86±3.42	176.48±8.63*	178.54±4.64*	179.33±5.21*

**Values are mean ± SD of triplicate determinations (n=10)*
*Values with * are statistically significant ($p \leq 0.05$) compared with the negative control*

Table 2. Effect of *B. pinnatum* aqueous leaf extract on some hematological parameters

Parameters	Group1 (positive control)	Group 2 (negative control)	Group 3 (20 mg/kg)	Group 4 (40 mg/kg)	Group 5 (60 mg/kg)
PCV (L/L)%	41.68 ± 0.58	31.62 ± 2.24*	38.26 ± 2.62*	38.86 ± 1.75*	40.12±3.25*
Hb (g/dl)	16.56 ± 1.42	13.56 ± 1.14	14.36 ± 0.75	14.97 ± 2.65*	15.24±3.63*
Bilirubin (mg/dl)	0.36 ± 0.02	0.48 ± 0.21*	0.45 ± 1.13*	0.41 ± 0.09*	0.39 ± 0.05*
RBC (µ/l)	257.45 ± 6.23	236.45 ± 4.34	242.78±5.16*	248.55±21.52*	253.14±2.66*
WBC (µ/l)	149.64 ± 2.15	128.22 ± 4.62.	130.57 ±4.52	138.22 ± 7.42*	142.52±4.12*

**Values are mean ± SD of triplicate determinations (n=10)*
*Values with * are statistically significant (p≤0.05) compared with the control*

Table 3. Effect of *B. pinnatum* aqueous leaf extract on Kidney function biomarkers of albino rats

Parameters	Group 1 (positive control)	Group 2 (negative control)	Group 3 (20 mg/kg)	Group 4 (40 mg/kg)	Group 5 (60 mg/kg)
Urea (mg/dl)	27.34 ± 3.45	21.87 ± 2.64*	25.76 ± 3.57*	24.89±4.65*	23.64 ± 2.63
Creatinine (mg/dl)	1.26 ± 0.15	0.86 ± 0.05*	1.10 ± 0.62*	0.98 ± 0.13*	0.95 ± 0.23
Albumin (mg/dl)	0.94 ± 0.03	0.76 ± 0.10*	0.90 ± 0.04*	0.88 ± 0.05*	0.85 ± 0.11
Protein (mg/dl)	5.56 ± 1.21	4.53 ± 2.03*	5.23 ± 1.05*	5.12 ± 2.06*	4.97 ± 1.73
Na^+ (mEq/L)	25.78 ± 0.24	10.67 ± 0.39*	21.56 ± 1.67*	15.59 ±4.67*	13.72 ±1.05*
K^+ (mEq/L)	1.89 ± 1.27	4.79 ± 1.64*	3.46 ± 1.08	3.18 ± 2.35*	2.17 ± 2.52*
Ca^{2+} (mEq/L)	4.79 ± 0.56	1.89 ± 1.62*	2.15 ± 1.23*	1.98 ± 0.76	1.92 ± 0.12
Mg^{2+} (mEq/L)	2.28 ± 0.55	1.76 ± 0.62*	2.06 ± 0.68*	1.91 ± 0.43*	1.90 ± 0.22
Cl^- (mEq/L)	100.46 ± 3.57	87.86 ± 5.64*	98.73 ± 3.65*	91.24 ±6.32*	90.10 ±4.53*
HCO_3^{2-} (mMol/L)	25.68 ± 1.54	21.57 ± 2.65*	23.47 ± 3.21*	23.16 ±1.76*	22.05 ±2.15*
HPO_4^{2-} (mMol/L)	0.75 ± 0.01	0.27 ± 0.11*	0.57 ± 0.08*	0.44 ± 1.05*	0.36 ± 1.21
SO_4^{2-} (mMol/L)	0.59 ± 0.03	0.26 ± 0.54*	0.42 ± 0.02*	0.40 ± 0.42*	0.34 ± 0.15*

**Values are mean ± SD of triplicate determinations (n=10)*
*Values with * are statistically significant (p≤0.05) compared with the negative control*

4. DISCUSSION

Mean body weight of experimental animals (negative control) decreased (p≤0.05) significantly (p≤0.05) compared to positive control (Table 1). Animals treated with the extract before exposure to the petrol fumes showed significant increase (p≤0.05) in body weight compared to the negative control which was not treated. The effect of the extract on mean body is dose dependent as shown in (Table 1), mean body weight increased as dose of administered extract increased. Shazid et al. [28] had reported the high protein content of *B. pinatum* leaves; hence the increase in mean body weight may be due to the high content of protein in the extract and the phytochemicals which could elicit increase in protein synthesis.

Hematological parameters assayed showed significant (p≤0.05) decrease in the negative control group compared to the positive control group (Table 2). Experimental animals treated with the plant extract before exposure to the petrol fumes showed significant (p≤0.05) increase in the hematological parameters assayed. PVC, Hb, Bilirubin, RBC and WBC increased significantly (p≤0.05) in all the treated groups. The effect of the extract is dose dependent. *Bryophyllum pinatum* leaves are rich in phytochemicals; alkaloids, flavonoids, steroids, lipids, and organic acids [15]. The flavonoids may have induced the detoxification enzymes through up-regulation of their genes by interacting with the antioxidant response system [29,30,31]. Flavonoids posses electrophilic centers that are capable of reacting with sulfhydryl groups through oxido-reduction or alkylation [32]. The significant increase in assayed hematological parameters further confirms the use of this plant as a blood tonic [23,33,34]. Flavonoids are the largest group of polyphenols that have been identified in vegetables, fruits and other plant parts and linked to reducing the risk of degenerative diseases [35].

Renal function biomarkers (Table 3) shows the negative control group had significant increase

(p≤0.05) in the parameters assayed. Experimental animals treated with the extract before exposure to petrol fumes showed significant increase in the assayed parameters compared to the negative control. Urea, creatinine, albumin, and serum protein increased significantly (p≤0.05) compared to negative control. Marked plasma protein depletion, especially of the albumin fraction results in decreased plasma volume [36]. The increase may be as a result of the protective effect of the plant extract on the kidney, hence increased kidney tubular reabsorption [37,38]. Oxidative stress produced by reactive oxygen species (ROS) constitutes the mechanism of production and progression of many renal diseases. The effect of ROS on mesangial and endothelial cells leads to oxidative injury which may alter the structure and function of the glomerulus [13]. The rich antioxidant phytochemical like flavonoids in the *B. pinnatum* leaves may have protected the kidney from the ROS produced by the petrol fumes [39]. The anti-urolithiatic effect of aqueous extract of *B. pinnatum* (Lam.) leaves using ethylene-glycol-induced renal calculi has been reported [24]. The kidney is a major organ for excretion and its stability is estimated by the creatinine clearance, which is a function of the glomerular filtration rate [2].

In the renal function test, a significant decrease in the results of urea, creatinine, sodium ion, potassium ion, magnesium ion, chloride ion, hydrogen phosphate, sulphate and bicarbonate ion was observed across the treated test groups when compared with the negative control group. The balance of sodium, potassium, chloride and bicarbonate in the blood is a good indicator of how well the kidneys and heart are functioning [36]. The main electrolytes in the body: sodium, potassium, chloride and bicarbonate (CO_2) can be used to evaluate symptoms of heart disease and monitor the effectiveness of treatments for high blood pressure, heart failure and liver and kidney disease [40]. *Bryophyllum pinnatun* leaf is rich in phytochemicals; alkaloids, flavonoids, steroids, lipids, and organic acids [15]. These phytochemicals may have contributed to the significant increase in these electrolytes [21].

5. CONCLUSION

This study has lent credence to the claim by ethno-medicine practitioners that the plant leaves may be used for the treatment and management of renal dysfunction.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The experimental protocols were approved by the Ethical Committee for Animal care and use of the College of Medicine, Abia State University, Uturu.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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