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Effect of ethanol extract of *Bryophyllum pinnatum* leaf on lipid profile, renal and hepatic function biomarkers of high salt fed Albino rats

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ABSTRACT

Background & Aim: Salt is an essential electrolyte; however, high salt loading is associated with numerous adverse effects including alterations in many biochemical parameters. This study investigated the effect of ethanol extract of *Bryophyllum pinnatum* leaves on the biochemical indices of high salt-fed albino rats.

Experimental: Twenty-four male healthy albino rats weighing 110-150g were randomly divided into four groups of six rats per group. Group 1 was administered with feed and water, which was the normal control. Group 2 was administered with 10 mL/kg of 18% NaCl only (Negative control), and groups 3 and 4 were administered with 10 mL/kg of 18% NaCl as well as 200 mg/kg and 400 mg/kg of the extract, respectively.

Results: The acute toxicity of the methanol leaves extract of *Bryophyllum pinnatum* in rats recorded no mortality even at a high dose of 5000 mg/kg body weight of the animal, thus LD50 could not be determined. The negative control group was significantly ($P<0.05$) higher in alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST) activities, cholesterol (CHOL), triacylglycerol (TAG), low-density lipoprotein (LDL) and bilirubin level when compared with other groups. There was a significant reduction in the Urea and Creatinine levels in the group administered with 400 mg/kg extract. The administration of high salt (18%) increased serum levels of AST, ALT, ALP, Bilirubin, Urea, creatinine, TAG, Cholesterol, and LDL and reduced the high-density lipoprotein (HDL).

Recommended applications/industries: The result of the high salt-fed untreated rats suggested inflammation of the liver and lipid dysfunction; however, the extract showed a highly potent effect in preventing cell damage that could be caused by high salt intake.

1. Introduction

Dietary salt is ionic compound composed of sodium chloride, which is 40% sodium and 60% chloride (Ayoola *et al.,* 2017). Salt is a vital component of our diets and it is important for proper functioning of different parts of the body (Westphal *et al.,* 2012; Olorunnisola *et al.,* 2021). Dietary salt is essential in maintaining arrays of metabolic and physiological functions such as electrolyte balance, excitation of nerve and muscle functions (Olorunnisola *et al.,* 2021). Reduced salt intake has been reported to partially restore the circadian rhythms of bladder clock genes (Iwamoto *et al.,* 2022). Though salt is an essential electrolyte, high salt loading is associated with numerous adverse effects, including alterations in many biochemical parameters especially lipid profile, kidney and liver parameters, cardiovascular risk and sudden death (Ofen *et al.,* 2015; Cui *et al.,* 2022).

High sodium intake is known to aggravate renal disease (Thomas *et al.,* 2011; Ekinci *et al.,* 2011). High dietary sodium can cause tissue remodeling and a decline in kidney function (Slagman *et al.,* 2011; Keyzer *et al.,* 2015), and also increases blood pressure (Li *et al.,* 2021). High sodium intake decreases renal calcium reabsorption which in turn leads to greater urinary calcium excretions, osteoporosis and kidney stones (Cui *et al.,* 2022). High salt diet has been implicated in the etiopathogenesis of immune derangement and metabolic syndrome (Olorunnisola *et al.,* 2021)

Salt-sensitive individuals respond to a high salt intake with an increase in blood pressure (Vasdev *et al.,* 2017). High salt intake also increases insulin resistance, a condition strongly associated with hypertension (Ertuglu *et al.,* 2021). Scientific literature has shown that normotensive rats develop increased blood pressure and elevated tissue aldehydes when given a high-salt diet (8-18% NaCl). It has been proposed that excess reactive aldehydes contribute to progressive and deleterious changes in hypertensive by increasing cytosolic-free calcium levels, inducing endothelial dysfunction, and changing renal vascular function (Hu *et al.,* 2020).

Medicinal plants have been used for centuries to combat many health challenges and are also useful component in pharmaceutical industries, among these plants is *Bryophyllum pinnatum. Bryophyllum pinnatum* (Lam.) Oken (Crassulaceae) is a perennial herb commonly known as *Zakhm-e-hyat*, *pattharcatta* and *parnabīja* (Kamboj and Saluja, 2019), and widely used in the treatment of several conditions in folklore medicine (Aprioku and Igbe, 2017). Scientific Researchers have reported that *Bryophyllum pinnatum* leaves have antioxidant, anti-inflammatory, antibiotic, antihypertensive antispasmodic, antiulcer, hypoglycemic and hypocholesterolemic activities (Aprioku and Igbe, 2017).

It is of no doubt that high salt loading may impact negatively on the kidney, liver and lipid profile which may lead to deleterious effect on the body. With limited scientific information on the effect of *Bryophyllum pinnatum* leaf in high salt loading in rats, it is therefore crucial to evaluate the impact of administration of *Bryophyllum pinnatum* leaf extract on lipid profile, kidney and liver function parameters in high salt loaded rats. Hence this study investigated the effect of ethanol extract of *Bryophyllum pinnatum* leaves on the biochemical indices of high salt fed albino rats

2. Materials and Methods

2.1. Materials and chemicals

Ethanol was product of BDH Chemical Company Sule and Arhoghro 164 Ltd, Poole, England. Rat feed was purchased from Pfizer Nigeria Plc. Biochemical kits were products of Randox Diagnostics, Crumlin, UK.

2.2. Collection of plant leaves

Bryophyllum pinnatum leaves were collected from the premises of Federal Polytechnic Nekede Owerri and were identified by a Taxonomist, Dr G. Omosun of the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture, Umudike. Voucher specimens were deposited at the departmental herbarium Voucher No: DPSBH 534. The leaves were washed with distilled water and sun dried for about seven days.

The dried leaves were pulverized into fine powder using Pulverize machine and preserved in cellophane bags until when used.

2.3. Extract preparation

Five hundred gram (500g) of powdered leaves was macerated in 1.5L of 95% ethanol at room temperature for 72h. It was continuously mixed and then filtered using a filter paper (Whatman size No.1). The filtrate

was dried in a water bath at 37ºC, and concentrate was kept in air tight bottle at 4°C until use.

2.4. Phytochemical screening

The qualitative phytochemical screening was carried out using the methods described by Harborne (1973) and Trease and Evans (1989).

2.5. Acute toxicity (LD_{50})

The median lethal dose (LD50) of the ethanol leaf extract of *Bryphyllum pinnatum* was determined using 18 mice (Lorke, 1983). In the first phase, mice were divided into three groups (3 mice per group) and were treated with the extract at doses of 10, 100, and 1000 mg/kg body weight orally. They were observed for 24 h for signs of toxicity. In the second phase, three mice each were treated with the same extract but at doses of 1600, 2900, and 5000 mg/kg body weight orally, respectively. They were also observed for 24 h for signs of toxicity.

2.6. Experimental animals

Twenty four male Albino rats weighing 110 to 150g were obtained from the Animal House of Nnamdi Azikiwe University Awka. The animals were housed in cages and Standard laboratory protocols for animal studies were maintained. Care of experimental animals was taken following the guidelines described by NRC (2011) and approval was obtained from the Ethical Committee of the Department of Physiology and Pharmacology, College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike. The animals were acclimatized for two weeks and maintained at the optimum temperature and relative humidity with 12 h light/dark cycle. The animals were allowed feed and water *ad libitum*.

2.7. Experimental design

Twenty-four (24) Adult male Albino rats of weighing 110 to 150 g were placed randomly into four groups of six animals each.

Group 1 served as the normal rats which was given only feed and water *ad libitum*.

Group 2 served as negative control and was administered with 18% NaCl only.

Group 3 and 4 were administered with 200mg/kg and 400mg/kg b.w of the extract with concomitant administration of 10 mL/kg of 18% NaCl (Bopda *et al.*, 2014) respectively.

The treatment lasted for 14days, after which the animals were sacrificed through cervical dislocation after overnight fasting. Blood was collected through cardiac puncture and transferred into plain sample bottles. The blood samples were centrifuged at 4000 rpm for 10 minutes to obtain the serum.

2.8. Collection of samples

Blood samples were collected in plain bottles through the cardiac puncture and were centrifuged at 2,800 rpm for 10min using the Bran Scientific and Instrument Company England centrifuge. Serum obtained was used for biochemical analysis.

2.9. Biochemical assay

Alanine aminotransamines and aspartate aminotransamines (ALT and AST) were determined according to the method of Reitman and Frankel (1957). ALP was determined by the phenolphthalein monophosphate method (1976). Bilirubin was estimated by colorimetric method as described in the Randox assay kit. Albumin test kit produced by Biosystem kits were used to estimate albumin. Serum Urea and serum creatinine were estimated as described in the commercial reagent assay kits (Randox Diagnostics, Crumlin, UK)

2.10. Lipid profile

The triacylglycerol (TAG) concentration of the serum was determined according to the method of Albers *et al.* (1978) as described in Randox commercial kit, and the total cholesterol and high-density lipoprotein (HDL) concentrations of the serum were determined using the method of Allain *et al.* (1974) and Albers *et al.* (1978), respectively, as described in the respective QCA commercial kits, whereas the serum low-density lipoprotein cholesterol (LDL) and very low-density lipoprotein cholesterol (VLDL) concentrations were calculated using the Friedelwald equation (Friedelwald *et al.*, 1972).

2.11. 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 and discussion

3.1. Acute toxicity of the extract

Researchers have directed most of their efforts towards providing empirical proof to back up the use of medicinal plants for tradomedical practices (Casmir *et al.*, 2017). In this study, the acute toxicity of the ethanol leave extract of *Bryphyllum pinnatum* in mice recorded no mortality even at a high dose of 5000 mg/kg body weight of the animal, thus LD_{50} could not be determined which implies that the ethanol extract of *Bryphyllum pinnatum* leaf was not toxic to the animals. Thus, the non-mortality at 5000 mg/kg is suggestive of a high degree of safety of the extract of *Bryphyllum pinnatum* leaf at the dose evaluated in this study.

3.2.Phytochemicals content of Bryophyllum pinnatum leaf

The results obtained from qualitative Phytochemicals analysis showed that *Bryophyllum pinnatum* leaf contained flavonoids, tannins, saponins, alkaloids, cardiac glycoside, steroids and phenols with flavonoids and phenols been more abundant (Table 1). Plant possess vital phytochemicals that have either defensive or disease protective properties (Temitope and Oluwafemi, 2012). These phytochemicals, either alone and/or in combination, have tremendous therapeutic potential in curing various ailments such as cancers, coronary heart disease, diabetes, high blood pressure, inflammation, microbial, viral and parasitic infections, psychotic diseases, spasmodic conditions, ulcers (Temitope and Oluwafemi, 2012). Flavonoids possess strong antioxidant activity and free radical-scavenging properties and inhibit protein glycation (Hag *et al.*, 2018). Tannins as polyphenolic compounds have several biological activities such as anti-inflammatory, antioxidant, free radical-scavenging, and anti-mutagenic activities (Hag *et al.*, 2018). The possession of phytochemicals by *Bryphyllum pinnatum* explained the appreciable outcome of the *in vivo* study.

Table 1. Preliminary phytochemicals content of the extract.

Phytochemicals	Inference
Flavonoids	++
Tannins	+
Saponin	+
Alkaloids	+
Cardiac glycoside	+
Steroid	+
Phenols	++

+ mean presence

3.3. Effect of ethanol extract on liver function biomarkers in high salt fed albino rats.

The rats that were treated with the extract at doses of 200 and 400 mg/kg displayed significant ($P<0.05$) reductions in the AST, ALT, ALP activities and Bilirubin level compared with the negative control. In the albumin level, the negative control had significant (<0.05) decrease compared to the extract treated groups. A non-significant ($P>0.05$) decrease in the albumin level was recorded in the groups treated with 200 and 400 mg/kg of the extract when compared to the normal control (Table 2). These biochemical parameters were used as indices for assessing organ dysfunction or damage. The liver plays an important role in many metabolic processes; any disturbance in the liver would affect the normal level of measurable biochemical parameters in this organ. AST, ALT, and ALP are marker enzymes present in high concentrations in the liver, when liver cells are inflamed or damaged, these enzymes leak into the blood stream leading to a rise in the plasma level of these enzymes (Ndrepepa, 2021). ALT is selectively a liver parenchymal enzyme than AST and a sensitive indicator of acute liver damage (Leoni *et al.*, 2018). A marked elevation of ALT levels is observed most often in persons with diseases that affect primarily hepatocytes such as viral hepatitis, ischemic liver injury (shock liver) and toxin-induced liver damage (Diana, 2007). Elevated mitochondrial AST is seen in extensive tissue necrosis during myocardial infarction and also in chronic liver diseases like liver tissue

degeneration and necrosis (Thapa and Anuj, 2007). In this study, the bio-markers of the liver showed reduction in the extract treated rats compared to the untreated (negative control). There was significant increase in the liver biomarkers (ALT, AST, ALP) activities in the rats treated with 10 mL/kg of 18% NaCl alone compared to the normal control rats.

Table 2. Result of liver function assay of test and control animals.

GROUP	AST (U/L)	ALT (U/L)	ALP (U/L)	Total Bilirubin (mg/dL)	Albumin (g/dL)
Normal rats	67.50±0.70^{b}	62.93±1.41^{b}	88.45±1.36^{b}	0.77±0.02^{b}	33.67±0.84^{a}
Negative control	87.00±2.12^{c}	79.98±1.67^{c}	103.36±2.99^{c}	0.97±0.06^{c}	26.49±2.69^{b}
200 mg/kg	64.00±1.41^{b}	54.42±6.15^{a}	86.01±1.43^{b}	0.71±0.00^{ab}	33.06±0.91^{a}
400 mg/kg	59.50±2.12^{a}	57.87±2.58^{a}	77.08±1.56^{a}	0.68±0.07^{a}	30.34±3.26^{a}

(n = 6), and values with different superscripts are significantly (P<0.05) different from any paired mean within each of the columns.

3.4. Effect of ethanol extract on creatinine and urea level in high salt fed albino rats.

In Table 3, the creatinine and urea level was significantly (P<0.05) higher in the negative control when compared with other groups. The 400 mg/kg test group showed significant reduction (P<0.05) in the urea and creatinine level compared to the controls. A non-significant (P>0.05) increase in the urea and creatinine were recorded in the 200 mg/kg of the extract when compared to the normal control.

Table 3. Effect of the extract on creatinine and urea level.

GROUP	Urea (mg/dL)	Creatinine (mg/dL)
Normal rats	31.36±1.86^{b}	1.12±0.07^{b}
Negative control	56.35±2.98^{c}	1.44±0.24^{c}
200 mg/kg	36.88±1.76^{b}	1.12±0.10^{b}
400 mg/kg	24.83±1.15^{a}	0.98±0.06^{a}

(n = 6), and values with different superscripts are significantly (P<0.05) different from any paired mean within each of the columns.

3.5. Effect of ethanol extract on lipid profile in high salt fed albino rats

The levels of cholesterol, triacylglycerol (TAG), high density lipoprotein (HDL), low density lipoprotein (LDL) and very low density lipoprotein (VLDL) of normal and experimental groups of rats are shown in Table 4. High salt fed group (negative control) caused a significant (P<0.05) rise in the levels of cholesterol, TAG, LDL and VLDL as compared with normal rats. The group administered 400 mg/kg of the extract had significant (P<0.05) decrease in cholesterol, TAG, LDL and VLDL compared to control. The HDL level in the groups administered 200 and 400 mg/kg of the extract were significantly (P<0.05) increased compared to the negative control. A non-significant (P>0.05) decrease in the HDL level was recorded in the 200 mg/kg of the extract when compared to the normal control.

Lipid profile is a panel of blood tests that serves as an initial screening tool for abnormalities in lipids, such as cholesterol and triglycerides. Lipid profiles are commonly used in the routine evaluation of cardiovascular risk, given the high correlations of hypercholesterolemia and hyperglyceridemia and cardiovascular risk (Hedayatnia *et al.*, 2020). There was reduction in the HDL and increased TAG, LDL, and Cholesterol in the negative control group, unlike the extract treated group that produced a reduction in the total cholesterol, low density lipoprotein and triacylglycerol concentration, while conversely, high density lipoprotein level increased. The decrease in cholesterol and LDL levels achieved by the administration of *B. pinnatum* leaf extract indicates a possible protection against hypercholesterolemia with its complications. Alteration of serum lipid profile is known to occur when there is high intake of sodium chloride and this is likely to increase the risk of coronary heart diseases. A reduction in serum lipids, particularly, the LDL, total cholesterol and triacylglycerol concentrations accomplished through the administration of the extract was measured as being lucrative for the treatment of many diseases.

Table 4. Effect on lipid profile.

GROUP	CHOL (mg/dL)	TAG (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	VLDL
Normal rats	121.66±4.88^{c}	149.96±2.95^{c}	30.24±1.42^{b}	61.43±3.86^{c}	29.99±0.98^{c}
Negative control	177.96±3.12^{d}	165.90±1.90^{d}	17.79±2.14^{c}	126.98±1.86^{d}	33.18±1.10^{d}
200 mg/kg	109.96±2.32^{b}	130.31±3.65^{b}	29.48±1.32^{b}	54.42±2.80^{b}	26.06±1.04^{b}
400 mg/kg	104.09±1.81^{a}	117.13±1.95^{a}	37.79±2.42^{a}	42.87±1.28^{a}	23.42±1.20^{a}

Values with different superscripts are significantly (P<0.05) different from any paired mean within each of the columns (n = 6).

4. Conclusion

Excess dietary salt induces adverse cardiovascular, hepatic and renal effects according to epidemiological and experimental studies. It is concluded from this study that the ethanol extract of *Bryophyllum pinnatum* leaf could serve as a potential agent in the prevention of hepatotoxicity, nephrotoxicity and lipid dysfunction that could be resulting from high intake of salt.

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