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Hydroethanolic Leaves Extract of *Bryophyllum pinnatum* Modulates Kim-1 Expression in Methyl Mercury Induced Kidney Damage

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Abstract **Original Research Article**

Bryophyllum pinnatum is a perennial herb native to Madagascar, it is used locally in the treatment of several conditions in folklore medicine. The aim of this study is to investigate the efficacy of *Bryophyllum pinnatum* leaves aqueous extract in Methyl mercury-induced kidney damage in wistar albino rat model. Methyl mercury, an environmental toxicant has the potential to cause kidney damage when exposed in a large quantity. Twenty five adult male wistar albino rats were given 2 mg/kg of methyl mercury to induce kidney damage. Group B received 2mg/kg of methyl mercury without treatment, animals in Group C received 2mg/kg of methyl mercury and 90 mg/kg succimer, Group D received 2 mg/kg of methyl mercury and 100mg/kg of the plant extract while group E received 2mg/kg of methyl mercury and 200mg/kg. Animals in Group F received 2 mg/kg of methyl mercury and 400mg/kg body weight of the plant extract via oral intubation for 30 days. Kidney injury molecule-1 (Kim-1) and uric acid level of the research animals were analysed to know the effect of the hydroethanolic leave extract of *Bryophyllum pinnatum* in methyl mercury induced kidney damage. A significant change was observed on the levels of Kim-1 and uric acid levels of the wistar albino rats after treatment with Succimer and *Bryophyllum pinnatum* hydroethanolic leaf extract. *Bryophyllum pinnatum* hydroethanolic leaf extract has the potential to reverse the effect of methyl mercury on Kim-1 and uric acid levels of the wistar albino rats.

Keywords: Bryophyllum pinnatum, Kidney injury molecule-1 (Kim-1), Succimer, Methyl Mecury, Uric Acid (UA), ELISA.

INTRODUCTION

Bryophyllum pinnatum, (Lam.) Oken (Crassulaceae), is a widely distributed perennial herb found in temperate, tropical and subtropical areas. It is originally found in most parts of Madagascar, and several other regions of the world including Asia, Australia, and New Zealand (Aprioku & Igbe, 2017). It is used traditionally in ethnomedicinal practices in the treatment of different kinds of diseases (Yadav, 2016). *Bryophyllum pinnatum* is commonly known as *Zakhm-e-hyat*, *pattharcaṭṭa* and *parṇabīja* Yadav 2016. It is grown around houses and in gardens for both ornamental and medicinal purposes. The plant can grow to about 1.5 meter in height with leaves arranged in opposite direction. *Due to its numerous medicinal benefits, B. pinnatum* is known as life plant, air plant, maternity plant, love plant, miracle leaf, cathedral bells, mother of thousands, leaf of resurrection plant, and *Lao di Sheng gen*. In Nigeria, it is generally called "Never Die", different tribes in Nigeria equally have different local names for *Bryophyllum pinnatum*. It is called Ododuk mmong (in Efik), Abamoda (in Yoruba), Ugwoba (in Igbo) and Karan (in Hausa) (Aprioku & Igbe, 2017; Bassey *et al.*, 2021). The leaves of *Bryophyllum pinnatum* are widely used in traditional and ayuverda medicine for treatment of urinary insufficiency, stone disorders, rheumatism, body pain, arthritis, heartburn, skin ulcers, peptic ulcer, diabetes mellitus, microbial infections, and hypertension

(Gulkari & Wanjari, 2016; Ojewole, 2005; Ojewole, 2002).

Other Pharmacological studies on *B. pinnatum* reported several biological activities some of which could authenticate the plant's traditional uses including, immunomodulatory, CNS depressant, analgesic, antiinflammatory, antimicrobial, antitumor, antiulcer, insecticidal, anticonvulsant, antioxidant, and antihypertensive properties (Supratman *et al.,* 2000; Okwu & Josiah, 2006; Supratman *et al.,* 2001). Research has shown that the leaves of *Bryophyllum pinnatum* have a wide spectrum of therapeutic potentials attributed to the rich phytochemicals content such as such as flavonoids, triterpenes, alkaloids, steroids, saponins, glycosides, tannins, bufadienolides (Bassey *et al.,* 2021). Methyl mercury is a toxic chemical that is of interest with regard to environmental health harzard, inorganic mercury circulating in the general environment is dissolved into freshwater and seawater, condensed through the food chain, ingested by humans, and consequently affects human health (Hong *et al.,* 2012).

Inorganic mercury discharged from various contaminants flows into seas, rivers, and streams, is converted to methyl mercury by bacteria and plankton in the seas and oceans. This methyl mercury accumulates in fish and shellfish, humans become contaminated by this toxicant through consumption of these fishes (Hong *et al.,* 2012). Methyl mercury is highly poisonous and the toxicity varies according to its form, exposure and individual susceptibility (Mahaffey *et al.,* 2004). Research has shown that methyl mercury toxicity can cause different damages to the body including kidneys damage. The kidney is the major route of excretion hence they are more vulnerable to heavy metal toxicity, oxidative stress is one the major effect of this toxicity (Rana *et al.,* 2018).

The kidneys are two bean-shaped organs in the renal system which helps the body pass waste as urine. They also help filter blood before sending it back to the heart. Recently, there has been increased cases of kidney diseases. The kidney damage refers to pathologic abnormalities documented by biopsy or imaging, alterations in urinary sediment or proteinuria (proteinuria/creatinuria > 200 mg/g, albuminuria/creatinuria > 30 mg/g). Damage usually preceeds alterations in functions (Lopez-Giacoman & Madero, 2015). When the kidneys are damaged, waste products and fluid build-up in the body. This causes swelling in the ankles, nausea, weakness, poor sleep, and shortness of breath. Without treatment, the damage can get worse and the kidneys may eventually stop working (Munger *et al.,* 2007). If the kidneys are not able to function properly, the body becomes overloaded with toxins which can lead to kidney failure if left untreated (Lopez-Giacoman & Madero, 2015). Renal failure occurs when the kidneys fail to function effectively. Renal failure is mostly classified as Acute renal failure (acute kidney injury [AKI]) and chronic kidney disease (CKD). Acute renal failure (acute kidney injury [AKI]) is characterized by the rapid loss of the kidney excretory function. AKI is induced by a change in systemic blood flow resulting in reduced kidney perfusion and glomerular filtration rate (van Duijl *et al.,* 2019).

Acute renal failure (acute kidney injury [AKI]) is one of the most important complications among hospitalized patients. It accounts for a high rate of in-hospital deaths (Lo *et al.,* 2009) and occurs within 2–7 days (Han & Bonventre, (2004). Chronic kidney disease (CKD) is characterized by primary renal failure and irreversible renal structural lesions that have been present for months to years (Sinkala *et al.,* 2017). Chronic kidney disease (CKD) affects between 8% and 16% of the population worldwide and is often underrecognized by patients and clinicians (Chen *et al.,* 2019). Chronic kidney disease is typically identified through routine screening with serum chemistry profile and urine studies. Less commonly, patients may present with symptoms such as gross hematuria, "foamy urine" (a sign of albuminuria), nocturia, flank pain, or decreased urine output. If CKD is advanced, patients may report fatigue, poor appetite, nausea, vomiting, metallic taste, unintentional weight loss, pruritus, changes in mental status, dyspnea, or peripheral edema (Chen *et al.,* 2019).

Renal failure of any origin is associated with a high mortality in the critically ill patient, despite significant advancement in therapeutics including renal replacement therapy (RRT) such as dialysis (Urbschat *et al.,* 2011). Current assessments of renal function make use of serum creatinine (SCr), blood urea nitrogen (BUN) and urinary output which has remained unchanged for several decades Urbschat *et al.,* (2011). Serum creatinine distinguishes acute kidney injury (AKI) from other acute illnesses including sepsis, myocardial infarction (Siew *et al.,* 2011; Simmons *et al.,* 2004). Generally, these biomarkers commonly used shows poor sensitivity and specificity for indicating early, acute changes in kidney function and do not differentiate between the renal function itself represented by the functional nephron number and the extent of active lesion as indicator of active kidney damage (Urbschat *et al.,* 2011; Mori and Nakao, 2007).

Due to uncertainities associated with this commonly used diagnosis, several biomarkers for kidney injury have been evaluated in clinical trials in the past. Such biomarkers include, kidney injury molecule-1 (KIM-1) (Kim-1 in animal models), neutrophil gelatinase-associated lipocalin (NGAL), interleukin-18 (IL-18), liver-type fatty acid-binding protein (L-FABP), N-acetyl-β-D-glucosaminidase (NAG), tissue inhibitor of metalloproteinase-2 (TIMP-2) and insulin-like growth factor-binding protein 7

(IGFBP7) (van Duijl *et al.*, 2019). Kidney injury molecule-1 (KIM-1) is a type 1 transmembrane glycoprotein with an immunoglobulin and mucin domain (Prozialeck *et al.*, 2009; Urbschat *et al.*, 2011). KIM-1 also known as hepatitis A virus cellular receptor 1 (Havcr1) (Prozialeck *et al.*, 2009) is not detectable in normal kidney tissue or urine, but is expressed at very high levels in dedifferentiated proximal tubule epithelial cells in human and rodent kidneys after ischemic or toxic injury (Urbschat *et al.*, 2011; Prozialeck *et al.*, 2009). KIM-1 has been used recently in the quantification of urinary excretion of renal tubular proteins which are overexpressed in response to acute kidney injury (van Duijl *et al.*, 2019).

MATERIALS AND METHODS

Plant Material

Fresh leaves of *Bryophyllum pinnatum* were obtained from the Botanical garden of University of Port Harcourt, Rivers State, Nigeria and were confirmed by a plant Taxonomist, in the Department of Plant Science and Biotechnology, University of Port Harcourt, Rivers State, Nigeria. The plant leaves specimens were deposited at the departmental herbarium and voucher number assigned as UPH/PSB/2022/034.

Experimental Animals

Thirty (30) male wistar albino rats aged 8-10 weeks; weighing between 200 to 250g were purchased from and housed in the animal house of Department of Physiology, University of Port Harcourt, Nigeria. The rats were allowed to acclimatize to laboratory conditions for one week in a well-ventilated cage with 12-hours light and dark cycle at room temperature (23± 2ºC). The animals were maintained on a standard feed and water ad libitum. Approval for the study was obtained from the Animal Research Ethics Committee of the Faculty of Allied Medical Sciences of the University of Port Harcourt, Rivers State, Nigeria. The guidelines on the Care and Handling of Research Animals (NIS, 1985) as well as other procedures following the approval for the study were strictly adhered to.

Extract Preparation

The fresh leaves of *Bryophyllum pinnatum* were detached from the stems, cleaned and air dried for eight days and then pulverized into powder using an electric blender. The leaf powder was weighed and extracted by maceration in hydroethanolic (70% ethanol) medium at room temperature. The extract was decanted and filtered using whatman filter paper of 24.0cm. The filtarate was macerated twice using the same volume of solvent to exhaustively extract the leaves. The ethanol was removed under pressure using rotary evaporator at a temperature of 53^0C. The crude hydroethanolic leaves extract of *Bryophyllum pinnatum* obtained in a paste form was stored in a desiccator and reconstituted as at when needed for the experiment using distilled water.

Experimental Design

The rats were randomized into six groups (A-F) of five rats each cage. Animals in group A served as control and were given distilled water and normal feed only. Animals in group B received 2mg/kg of methyl mercury without treatment, this served as disease control. Animals in group C received 2mg/kg of methyl mercury and 90 mg/kg succimer, group D received 2 mg/kg of methyl mercury and 100mg/kg of the plant extract while group E received 2mg/kg of methyl mercury and 200mg/kg. Animals in Group F received 2 mg/kg of methyl mercury and 400mg/kg body weight of the plant extract via oral intubation for 30 days. After the last administration of the extract on the 30th day, the rats were fasted overnight but allowed access to water ad libitum. The rats were sacrificed by cervical dislocation under deep diethyl ether anaesthesia. Blood samples were collected separately into labelled plain and EDTA bottles, for measurement of serum Kim-1 and Uric acid levels.

Biochemical Estimation

The serum Kidney injury Molecule 1 (Kim-1) level of the wistar albino rats were analysed Using ELISA kits From Melsin Diagnostic from China. Uric Acid. The serum Uric acid levels of the wistar albino rats were analysed Using Fortress Kits from United Kingdom.

Statistical Analysis

Statistical analysis was done using statistical package from SPSS Inc, Chicago, USA.

RESULT

The results were expressed as mean ± standard deviation SD. Groups were compared using one way analysis of variance (ANOVA) and Post Hoc analysis using least significance difference (LSD).

Effect of Crude Aqueous Leaves Extract of *Bryophyllum pinnatum* on Kim-1 and Uric acid

Table One below shows the effect *Bryophyllum pinnatum* hydroethanolic leaves extract on Kim-1 and Uric acid levels.

	Kim-1 (pg/ml)	UA (µmol/L)
Control	153.33±8.06	58.37±2.81
Methyl Mecury	815.36±9.63	513.98±7.86
Methyl Mecury,SUC	423.93±10.48	200.9±1.81
MM+ 100mg	381.92±9.44	182.64±1.64

MM+ 200mg	329.63±9.53	186.54±2.09
MM+ 400mg	248±4.7	106.71±4.82

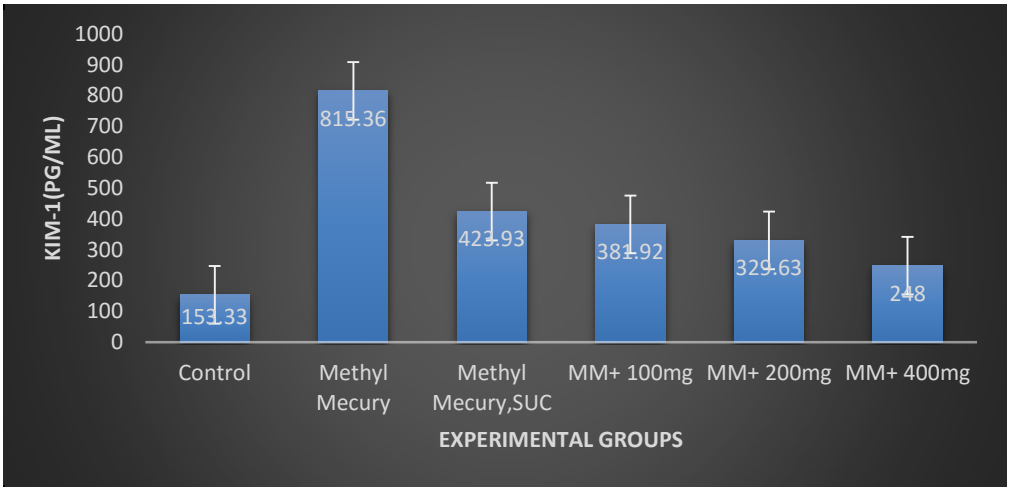

Figure 1

In the present studies, there was a significant ($p < 0.01$) increase in Kim-1 levels (815.36±9.63 (pg/ml)) after treatment with 2 mg/kg methyl mercury which indicates an expression of kidney injury molecules-type 1. Treatment with 90 mg/kg of succimer and 100, 200, 400 mg/kg of *Bryophyllum pinnatum* respectively led to a decrease in the expression of Kim-1 levels of the rats. A similar effect was observed in the Uric acid levels of the rats. A significant decrease ($p < 0.01$) was observed in the serum uric acid levels of the rats 200.9±1.81, 182.64±1.64, 186.54±2.09 and 106.71±4.82 after treatment with 100, 200 and 400 mg/kg of *Bryophyllum pinnatum* respectively. Overall, these results indicates that *B. pinnatum* could be considered to have modulatory effect on methyl mercury induced kidney damage.

CONCLUSION

Bryophyllum pinnatum leaves showed preventive effect against kidney damage induced by methyl mercury an environmental toxicant. Different researchers have also demonstrated that methyl mercury can induce kidney damage. Research has shown that methyl mercury releases oxygen radicals at decomposition and the released oxygen radical causes severe damage to cells by activating the chain of lipid peroxidation of the cell membrane which in turn increases the expression of KIM-1. In the present studies, the modulative effect of *Bryophyllum pinnatum* on methyl mercury induced kidney damage could be as a result of its phytochemical content. Research has shown that *Bryophyllum pinnatum* is rich in flavonoids, saponins, tannins, glycosides, terpenoids, steroids and alkaloids Aprioku & Igbe, (2017), Yadav *et al.*, (2016). In another study, the antioxidant activities of *Bryophyllum pinnatum* was demonstrated, the administration of *Bryophyllum pinnatum* increases the glutathione (GSH) and catalase activities in damaged kidney tissues Yadav *et al.*, (2016). This shows that the effect hydrothanolic extract of *Bryophyllum pinnatum* on the Kim-1 expression may be due to phytochemicals and antioxidant properties of *B. pinnatum.* The result of this research validates the ethnomedicinal use of *Bryophyllum pinnatum* in the treatment of kidney disorders in previous studies.

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Conflict interests

The authors declare that there is no conflict of interest.

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