EFFECT OF AQUEOUS LEAF EXTRACT OF *Moringa oleifera* ON SOME RENAL FUNCTION INDICES OF RATS

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ABSTRACT

Purpose:The study was designed to investigate the effect of aqueous leaf extract of *M.oleifera* on bile secretion and serum electrolytes in rats. **Methodology**: Eighteen (18) Albino Wistar rats weighing between 180-220g body weights were assigned into three groups (i, ii and iii) of six rats each after acclimatization for seven (7) days. Group 1 (control) received standard feed and water while Groups 11 and 111 (low and high dose groups respectively) received 300mg/kg body weight and 600mg/kg body respectively in addition to food and water daily for 28days.The rats were sacrificed 24hours after an overnight fast using chloroform-ether anesthesia in ratio 1;1. The blood as well as biliary samples was collected for determination of electrolytes. **Result**: The result showed that bile secretion was significantly (P<0.001) increased in the high dose group but was significantly (P<0.001) decreased in the low dose group when compared with the control. The biliary bicarbonate level were significantly (P<0.001) decreased in test groups while there was significant (P<0.001) increased in serum bicarbonate levels compared with control. Serum and biliary phosphate levels were significantly (P<0.001) decreased in test groups compared with control. Sodium levels in serum and bile were significantly (P<0.001) decreased, while serum potassium levels were significantly (P<0.001) decreased in test groups but were significantly (P<0.001) increased test group compared with control. Serum bilirubin levels were significantly lowered in low dose but increased in high dose group compared with control. Uric acid levels in bile and serum were significantly (P<0.001) decreased in test groups. Creatinine was significantly reduced in low dose compared with control following the administration of the extract. Urea and T. cholesterol were significantly (P<0.001) reduced in serum but significantly increased in bile respectively.**Conclusion:** It appears that *M.oleifera* may reduce blood pressure following its marked effect on sodium levels

Keywords: Bile, Uric acid *Moringa oleifera*, Serum Creatinine, Serum Electrolytes.

1. Introduction

M. oleifera leaf preparation have been cited in many scientific works for its physiologic and pharmacologic activities which inform us of its early and present use medicinally in most countries of the world. *M.oleifera* is said to protect the body against ulcers [1].Different constituents' of *M.oleifera* provide the pharmacological basis for its traditional uses in most gastrointestinal disorders [2]. Various parts of *M.oleifera* have been used for treatment of sores, dysentery, pneumonia, cancer [3]. Various studies have shown that isothiocyanate content is responsible for its anticancer activity [4, 5]. The plant also possess antifungal and antibacterial activity antitumor antipyretic, anti-inflammatory antiulcer [6,7].*M.oleifera* leaves are the most commonly used part of the plant. The analysis of the leaves show a rich source of proteins, beta carotene, a pro vitamin A, vitamin C, and minerals such as Ca^{+} and K^{+}, natural antioxidants and carbohydrate compound like sucrose, D-glucose and traces of alkaloids, kaempferat, quercetin, wax, important and essential nutrients to the body [8]. Flavonoids like kaempferitin, kaemphreol, rhamnetin, isoquercetin are also present in the plant. *M.oleifera* has been demonstrated to exhibit anti-cholesterolemic activity [9, 10, 11]. The leaves are reported to contain beta sitosterol, a phytoconstituent with potent cholesterol lowering ability [10] .The antioxidant properties of *M.oleifera* leaves have been evaluated and recommended for use [12], including beta carotene. Also, micronutrients and macronutrients are among other phytoconstituents [11,13,14] present in the plant. [13], 1999 reported that 1g of *M.oleifera* leaves contains 17 times Ca^{++} in milk and 15 times K^{+} in banana. Some of the most common and important problem in clinical medicine arises because of the abnormalities in control systems that maintain constancy of body fluids. For maintenance of homeostasis excretion of water and electrolytes must precisely match intake. If intake exceeds the excretion, the amount of substance in the body will increase. If intake is less than excretion, the amount of substance in the body will decrease [15] .Many foods are known to contain high concentration of electrolytes which when taken may similarly raise extracellular fluid volume

slightly but trigger hormonal changes and other compensatory or adaptive responses. Therefore, this research work is aimed at determining the effect of aqueous leaf extract of *M. oleifera* on some renal function indices of rats.

2.0 MATERIALS AND METHODS

2.1 Preparation of extract

Fresh mature leaf of *M.oleifera* was collected from Okuku,Yala Local Government, Cross River State in February. The leaves were washed thoroughly to remove debris dust particles and were sun dried for three days. They were later homogenized to powder form and then soaked with distilled water 1:1.5 w/v for 72 hours and filtered using what's man No1 filter paper. The filtrate was subsequently evaporated to dryness in an aerated oven at 45℃. The extract was collected into an air tight container and kept until use.

2.2 Preparation of experimental animals

Eighteen (18) female Albino Wistar rats weighing 180-220g were used for this experiment. They were maintained at the Experimental Animal House of the Faculty of Basic Medical Sciences CRUTECH, Okuku Campus. The animals were housed in ventilated metabolic cages and were fed with normal feed and water; they were also acclimatizing for one week before commencement of the experiment. All experiment protocols and handling were in compliance with the National Institute of Health [NIH]. The rats were randomly assigned into three groups (Group 1), received normal water and feed, the experimental groups represent (Group 2) and (Group 3), (low and high dose groups) received *M .oleifera aqueous leaf* extract of 300mg/kg body weight for low dose and 600mg/kg body weight for high dose. The extracts were administered to the rats orally for 28 days using the orogastric tube.

2.3 Collection of blood and serum samples

The collection of blood samples were done by cardiac puncture. Prior to this the animals were placed inside desiccators which contains cotton wool, soaked with chloroform. The animals lost consciousness of it environment at about 5 minutes and was placed on a dissecting board. Incision was made through the middle mediastinum. The blood was drawn out using a disposable syringe and needle and emptied into non-heparinized bottle, and allowed to clot and then centrifuge to separate the serum and a clean bottle for analysis. Prior to collection of the blood samples, each blood sample bottle was labeled.

2.4 Phytochemical screening (Qualitative analysis)

Standard methods were used evaluate the presence or absence phytoconstituents: Alkaloids, Glycosides. Saponins, Tannin, Reducing compounds, flavonoids [16, 17, 18, 19].

2.5 Statistical analysis

The results were expressed as mean ± sem. P-values of 0.05 was considered significant. The student's t-test and the analysis of variance (ANOVA) were used to compare results. Microsoft Excel package was used to compute the analysis.

3.0 Results

The mean serum chloride level was significantly ($P<0.001$) increased in the low dose group compared with control. The test groups had significantly higher serum HCO_3^- levels than control. The low dose was significantly ($P<0.001$) higher than the high dose. Serum PO_4^- levels in the low dose group were significantly reduced .Mean serum $K^+$and $Na^+$and the low dose bilirubin levels were significantly ($P<0.001$) reduced in test groups compared to the control. But high dose bilirubin levels was significantly higher ($P<0.01$) than control and low dose. The mean serum uric acid urea and cholesterol levels in the test groups were significantly lower ($P<0.001$) than the control.

The mean biliary Cl^- in high dose group and biliary HCO_3^-, PO_4^{2-}, bilirubin levels were significantly decreased ($P<0.001$) in test groups compared to the control (Table 3).Biliary uric acid, urea and biliary levels were significantly ($P<0.001$) increased compared with control. Biliary Na^+ and K^+ were significantly($P<0.001$) decreased in the low dose group while the high dose group biliary Na^+ and K^+ levels were significantly ($P<0.001$) higher compared with control (Table 3, 4).

4.0 Discussion

The phytochemical constituents found in *Moringa oleifera* are alkaloids, glycosides, saponnins, tannins, flavonoids, reducing compounds, polyphenols, phlobatannins, triterpenes, and steroids [12]. These constituents bestow to *M.oleifera* the acclaimed medicinal, physiological, pharmacological, microbial and bactericidal properties. It has been reported that the plant is a rich source of antioxidants, vitamins and minerals [10,11].

There was statistically significant increase in serum concentration of chloride (in low dose group), bicarbonate and phosphate (in high dose), while sodium and potassium were significantly decreased in test groups. Biliary sodium and potassium were significantly increased in high dose group. These effects might be caused by the sun dried leaf extract of *M. Oleifera* but their mechanism is clear.

Serum/plasma sodium concentration depends on intake of water in response of thirst, as stimulated or suppressed by the plasma osmolality; excretion of water largely affected by Arginine Vasopresin (AVP) release in response to changes in blood volume or osmolality; and the blood volume status which affects sodium excretion through aldosterone, angiotensin 11, and Atrial Natriuretic Peptide (ANP) The extract caused decreased serum Na^+. Reduced serum sodium concentration is a common electrolyte abnormality [15] caused usually by a fall in plasma osmolality. The kidneys have ability to conserve or excrete large amount of sodium depending on the sodium content of the extracellular fluid (ECF) and the blood volume. Decreased serum concentrations might be due to increased sodium loss, water retention or imbalance .In the other hand, increased serum sodium concentration may also occur due to excessive loss of water relative to sodium loss, decreased water intake or sodium intake or retention of H^+, as well as gastro intestinal loss. These need further investigations.

Potassium uptake in the ECF into the cells is important in normalizing an acute rise in the plasma. Serum K^+ decreased (Table 2) may occur in gastrointestinal or urinary loss or with increase cellular up take, in stool and large doses of diuretics [15]. Potassium loss through the gut is usually associated with renal potassium retention resulting in lowered urinary potassium. It has been reported that the movement of potassium from blood to lumen is dependent upon active uptake across the basal cell membrane by Na^+- K^+, ATPase followed by diffusion of K^+ through the luminal membrane K^+ channel into the tubular fluid. Agents that interfere with the generation of negative luminal potential impair secretion of K^+. This maybe the basis of reduced serum K^+ concentrations in the test animals [20].

The body has complex systems for monitoring concentrations of different solutes in the intracellular and extracellular fluid. Although water intake was not measured, plasma sodium ion concentration may sometimes be within the normal range if loses of salt and water proceed in parallel. Some abnormalities may be expected in a number of other parameters reflecting approximate renal, hormonal and hemodynamic responses [20]. Typically plasma urea concentration rises (table 2) as urea concentration is affected by both glomerular filtration and urine flow rate.

Abnormal acid base balance occurs in a wide range of disorders. A variety of physiological mechanism act to prevent wide swings in pH of the extracellular fluid. The first of these reactions is the blood and tissue buffers, of which the most important is the acid/bicarbonate buffer system. The bicarbonate system is important because HCO^-_3 is present in relatively high concentration in the extracellular fluid and two of its key components are under physiological control: CO_2 by the lungs and the bicarbonate by the kidneys. Respiratory compensation for acid base disturbance can occur quickly due to alterations in ventilatory drive mediated through pH change in the brain stem [21]. In response to acid accumulation ventilation is increase, serving to reduce pCO_2. Conversely systemic alkalosis leads to inhibition of ventilation. The kidney provides another line of defense against disturbances. of arterial pH, when acid accumulates due to chronic respiratory or metabolic (non- renal) causes, the kidney has long term capacity to enhance urinary excretion of acid effectively increasing bicarbonate [21].

Decreased phosphate levels may be caused by redistribution into cells in periods of increase energy utilization. Clinical manifestation of PO_4^{2-} depletion reflects widespread involvement of PO_4^{2-} in tissue metabolism which defects appear in blood. On other hand, increase PO_4^{2-}concentration is usually the result of decrease renal function in acute chronic renal failure. Redistribution of phosphate into the plasma can be the contributing factor to increase PO_4^{2-}tumor lysis syndrome and in catabolic state. Phosphate accumulation will be aggravated in any of the condition when PO_4^{2-} containing preparations are taken.

Excess bilirubin in blood gives rise to jaundice. The common causes are increase destruction of red cells with rapid release of bilirubin into blood. Obstruction of bile duct cause damage to liver cells. Therefore extract of high dose may predispose one to jaundice.

M.oleifera has been shown to reduce cholesterol in the low dose group fed 300mg/kg body weight. The decrease in serum cholesterol in the low dose group is beneficial and is in agreement with the report of [10]; but the high dose 600mg/kg body weight treated rat increased cholesterol, a probable to health hazard.

5.0 Conclusion:

It can be inferred that the aqueous leaf extract of *M .oleifera* at doses of 300mg and 600mg/kg body weight showed marked effects on serum sodium and potassium levels in the rat. Decreased serum Na^+ may precipitate retention of water. While increased bilirubin levels in the high dose treated group suggest that the extract at high dose may predispose to jaundice.

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Table 1: Results of phytochemical screening of air-dried leaf extracts of *M .oleifera*

Chemical constituent	Ethanol extract	Aqueous extract
Alkaloids	++	+
Glycosides	++	+
Saponins	+	++
Flavonoids	+++	++
Tannins	++	+
Reducing Compouds	++	+
Polyphenols	++	+++
Phlobatanins	-	-
Triterpenes	++	+
Steroids	+	+
Anthraquinones	-	-
Hydroxymethylathraquinone	-	-

Key: + = present; ++ = present in excess; +++ = present in much excess; - = absent

Table 2: Effects of *Aqueous leaf extract of M.oleifera* on serum electrolyte of albino wistar rats

Parameter	Control	Low dose	High dose
Clmmol/L	109.00±0.01	121.00±0.01^{c,c}	105.01±0.01^{c}
HCO_3^-mmol/L	21.00±0.00	31.01±0.01^{c,c}	28.01±0.01^{c}
PO_4^{2-}mmol/L	9.22±0.01	7.46±0.01^{c,c}	9.08±0.01
K^+mmol/L	4.56±0.05	3.49±0.01^{c,c}	3.80±0.01^{c}
Na^+mmol/L	140.00±0.00	135.01±00^{c}	136.01±0.01^{c}

Values are mean±SEM. a=p<0.05,b=p<0.01, c=p<0.001, n=6

Table 3 Effect of Aqueous leaf of *M. oleifera* on serum physiological parameters in albino wistar rats

	Control	Low dose	High dose
Bilirubin (mmol/L)	15.57±0.06	9.26±0.00^{c,c}	15.94±0.03
Uric acid (mmoL)	5.88±0.00	2.88±0.1^{c,c}	5.80±0.01
Creatinine (mmol/L)	0.24±0.01^{a}	0.20±0.01^{a}	0.024±0.00^{a}
Uric(mmoI/L)	21.01±0.1	15.00±0.01^{c}	15.01±0.01^{c}
Cholesterol (mmoI/L)	1.46±0.00	0.88±0.01^{c,c}	0.97±0.1^{c}

Value are Mean± SEM.a=p<0.05, b=p<0.01, c=p<0.001,n=6

Table 4 Effect of Aqueous leaf Extract of *M. oleifera* on Bile Electrolytes on Albino Wistar Rats

	Control	Low dose	High dose
Cl^-(mmol/L)	102.01±0.01	102.07±0.10^{c,c}	90.03±0.05
HCO_3^- mmol/L)	30.06±0.05	13.01±0.01^{C,C}	15.54±0.8^{C}
PO_4^- (mmol/L)	11.81±0.1	6.34±0.1^{C,C}	4.26±0.01^{C}
K^+(mmol/L)	6.80±0.1	6.30±0.01^{C,C}	7.61±0.7^{C}
Na^+(mmol/L)	142.95±0.12	142.66±0.051^{C,C}	144.96±0.08

Value are Mean± SEM. a=p<0.05, b=p<0.01, c=p<0.001, n=6

Table 5: Effect of Aqueous Leaf Extract *M. oleifera* on Bile physiological parameters on Albino wistar rats

	Control	Low dose	High dose
Billirubin	35.04±0.07	23.86±0.05^{c,c}	26.31±0.01^{c}
Uric acid(mmol/L)	5.92±0.03	12.23±0.02^{c,c}	4.83±02^{c}
Creatinine(mmol/L)	0.005±0.00	0.005±0.00	0.004±0.00
Urea(mmol/L)	24.01±0.01	25.20±0.47^{a,b}	26.93±0.10^{c}
Cholesterol(mmoI/L)	0.42±0.01	0.67±0.00^{b}	0.58±0.01^{c}
Bile(ml)	0.68±0.29	0.06±0.01^{c,c}	0.90±0.01^{c}

Value are Mean± SEM. a=p<0.05, b=p<0.01, c=p<0.001, n=6