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Short Communication

Effect of Chronic Administration of Methanol Extract of *Moringa Oleifera* on Some Biochemical Indices in Female Wistar Rats

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Summary: The study was conducted to investigate safety associated with prolonged consumption of *Moringa oleifera* leaves as beverage. Fourteen rats were used in this study. They were divided into 2 groups each containing 7 rats. Rats in group I received 2ml/kg of corn oil (standard vehicle drug). Animals in groups II were administered with 400mg/kg body of methanolic extract of *Moringa oleifera* (MEMO) for five weeks respectively. Serum collected was analyzed for alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein, albumin, globulin, blood urea nitrogen (BUN) and creatinine. There was significant ($P<0.05$) decrease in serum total protein, albumin, globulin and AST activity. The activity of ALT decreased but not significant. Similarly, 400mg/kg body of MEMO led to significant ($P<0.05$) decrease in serum BUN and creatinine. All experimental animals that received 400mg/kb of MEMO had significant ($P<0.05$) decrease in body weight from week to week 4 of the experiment. Taken together, 400mg/kg body of MEMO seemed to be toxic to the liver with apparently no toxicity in the kidney. Hence, prolonged exposure is not advisable as such could portend danger to the liver.

Keywords*: Moringa oleifera*, toxicity, hepatic damage, kidney.

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INTRODUCTION

Moringa oleifera Lam. (drumstick tree, horse-radish tree, synonym: *Moringa pterygosperma* Gaertn.), a member of the family Moringaceae, is a small-medium sized tree, 10–15m high, widely cultivated in East and Southeast Asia, and the West Indies. *Moringa oleifera* is known to tolerate a wide range of rainfall with minimum annual rainfall requirements which has been estimated at 250 mm and maximum at over 3000 mm and a pH of 5.0–9.0 (Palada and Changl, 2003). Different parts of the *Moringa oleifera* tree are reported to possess various pharmacological actions and nutritional qualities (Viera *et al.*, 2010; Promkum *et al*., 2010; Oluduro *et al.*, 2011; Debnath *et al.*, 2011 ; Ogunsina *et al*., 2011;). The leaves and fruits are found to have hypocholesterolaemic activity in Wistar rats and rabbits, respectively (Ghasi *et al*., 2000; Mehta *et al*., 2003). Similarly, the flowers and roots are used in folk remedies, for tumours, the seeds for abdominal tumours, leaves applied as poultice to sores, rubbed on temples for headaches and are said to have purgative properties (Anwar *et al*., 2007). The leaves as well as flowers, roots, gums and fruits are extensively used for treating inflammation (Cheenpracha *et al.*, 2010) and cardiovascular diseases (Chumark *et al*., 2008). Also, its leaves can serve as a rich source of beta-carotene (Nambiar and Seshadri, 2001), vitamin C and E, and polyphenolics (Sreelatha and Padma, 2009). *Moringa oleifera* has been reported for its potent antioxidant and free radical scavenging activities in vitro and in vivo (Singh *et al*., 2009; Sreelatha and, Padma, 2009; Rermal *et al.*, 2009; Atawodi *et al.*, 2010).

The use of computer modelling software package to evaluate safety and risk assessment of phytochemicals in experimental animals has been recently reported (Valerio *et al*., 2010). However, information is lacking on safety and toxicological evaluation of *Moringa oleifera* in animal models. Therefore, this study was designed to elucidate the possible toxicity associated with chronic administration of methanolic extract of Moringa oleifera (MEMO) in female rat model.

MATERIALS AND METHODS

Sodium hydroxide (NaOH), copper sulphate, potassium chloride, sodium- potassium tartarate were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other reagents were of analytical grade and

were obtained from the British Drug Houses (Poole, Dorset, UK) and Randox.

Preparation of plant extract

Fresh leaves of *Moringa oleifera* (MEMO) were harvested from Ajibode extension of the University of Ibadan, Oyo State, Nigeria. The coarse powder (1.2kg) of the leaves was defatted using N-hexane (60-80^{O}C) before being extracted with 95% methanol in a soxhlet extractor at 55 ^{O}C for 6 h. The extract was concentrated under reduced pressure to yield a syrupy mass (40g) and stored in a cool place until the time of use.

Experimental design and animal treatment

Thirty healthy adult female Wistar rats weighing between 210-230g obtained from the Central Animal House, University of Ibadan, Nigeria were randomly assigned to five groups of 6 animals per group. They were housed in plastic suspended cages placed in a well-ventilated rat house, provided rat pellets and water *ad libitum* and subjected to natural photoperiod of 12-hr light : dark cycle. Rats in group I served as control and were administered 2ml/kg body weight of corn oil according to Farombi *et al.* (2007). Animals in group II received 400mg/kg body of methanolic extract of *Moringa oleifera* (MEMO) for five weeks consecutively. The animals were sacrificed at the end of the eight weeks administration by cervical dislocation after an overnight fast. About 5ml of blood was drawn into plain sample bottles and allowed to clot. Clotted blood was later centrifuged at 3,000 revolutions per minutes (RPM) and the clear serum was harvested.

Animal ethics

All of the animals received humane care according to the criteria outline in the Guide for the Care and the Use of Laboratory Animals prepared by the National Academy Science and published by the National Institute of Health (PHS, 1986). The experiment was carried out at Biochemistry Laboratory, Department of Veterinary Physiology, Biochemistry and Pharmacology, Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria.

Serum Biochemistry

Protein concentration was determined by the method of Lowry *et al*. 1951. Serum sample (1ml) was dissolved in 39ml of 0.9% saline to give a 1 in 40 dilution. This was followed by the addition of 3ml of Biuret reagent to the sample.

The mixture was incubated at room temperature for 30 minutes after which the absorbance was read at 540nm. And the protein content of sample was thereafter calculated from the standard using Bovine Serum Albumin (BSA). AST (EC 2.6.1.1) activity was assessed according to Reitma and Frankel (1957). Briefly, 0.1ml of diluted serum was mixed with phosphate buffer (100mmol/L, pH 7.4), L-aspartate (100mmol/L), α-oxoglutarate (2mmol/L) and the mixture was incubated for exactly 30min at 37^{o}C. 0.5ml of 2, 4-dinitrphenylhydrazine (2mmol/L) was added to the reaction mixture and allowed to stand for exactly 20min at 25^{0}C. Then 5.0ml of NaOH (0.4mol/L) was added and the absorbance read against the reagent blank after 5 min at 546nm. AST was measured by monitoring the concentration of oxaloacetate-hydrazone formed with 2, 4-dinitrophenylhydrazine. ALT (EC 2.6.1.2) activity was determined following the principle described by Reitman and Frankel (1957), 0.1ml of diluted serum was mixed with phosphate buffer (100mmol/L, pH 7.4), L-alanine (100mmol/L), and α- oxoglutarate (2mmol/L) and the mixture was incubated for exactly 30 mins at 37^{o}C. 0.5ml of 2, 4-dinitrophenylhydrazine (2mmol/L) was then added to the reaction mixture and allowed to stand for exactly 20 mins at 25^{o}C. Then 0.5ml of NaOH (0.4mol/L) was added and the absorbance was read against reagent blank after 5 mins. Reagent blank was prepared as described above replacing sample with 0.1ml of distilled water. ALT was measured by monitoring the concentration of pyruvate hydrazone formed with 2, 4-dinitrophenylhydrazine at 546nm. Albumin was measured with Bromocresol green, a dye at an acid pH of 3.8 preferentially bound albumin to produce a shade of green colour which was measured with a spectrophotometer at 630nm (Gustafsson, 1976). Serum blood urea nitrogen (BUN) and creatinine levels were assesses using Randox kits according to manufacturer's instruction. The blood urea nitrogen (BUN) and creatinine levels in all the sample sera were estimated by modified methods based on diacetylmonoxime reaction (Marsh *et al.*, 1965) and Jaffe's reaction (Biod and Sirota, 1948), respectively, on standard diagnostic test kits (Randox Laboratories, Crumlin, U.K.).

Statistical analysis

All values are expressed as mean±S.D. The test of significance between two groups was estimated by Student's t test. "One-way ANOVA with Dunnett's post-test was also performed using GraphPad Prism version 4.00.

RESULTS

There was significant ($P<0.05$) decrease in weight gain in rats that received 400mg/kg of MEMO weeks 1 and 2 of administration of MEMO compared with the control values (fig 1). At 5 weeks of administration, rats that received the extract had significant increase in weight gain (230.00±10.00) compared with the control (218.00±12.41) respectively. Serum total protein (3.62±0.20), albumin (1.10±0.03) and globulin (2.50±0.22) obtained in animals that were administered with the

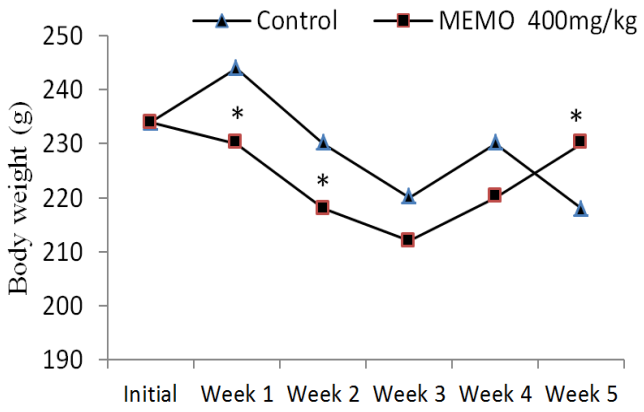

Figure 1: Effect of prolonged administration of MEMO on weekly weight gain. n=8, *p<0.05.

extract reduced significantly at p<0.05, p<0.001 and p<0.001 respectively compared with the control total protein (5.58±0.22), albumin (2.18±0.08) and globulin (3.88±0.22) respectively (Table 1). The increase in ALT obtained in rats administered with 400mg/kg of MEMO was not significantly (p>0.05) different from the control. Similarly, serum AST activity in rats administered with the extract fell (p<0.001) significantly when compared with the control. Serum creatinine and blood urea nitrogen (BUN) which are markers of kidney damage reduced (p<0.05) significantly in animals exposed to MEMO compared with control (table 1) respectively.

DISCUSSION

In our study, total protein, albumin and globulin decreased significantly in animals exposed to 400mg/kg of MEMO for five weeks. The decrease observed in these serum proteins was a good pointer to hepatic damage due to reduced capability of the hepatocytes to synthesize enough serum proteins. Normal albumin in the bloodstream is important for maintaining many physiologic functions in the body. One of these functions is critical for maintenance of fluid pressure in the arteries and veins. When the protein level falls below a certain point, the fluid in these vessels can leak out and pool in the abdominal or thoracic cavities. Assay of serum albumin level is often considered as a test for liver function. The hepatic synthesis of albumin has been reported to decrease significantly in end-stage liver disease (Sherwin *et al*., 1996). In this study, 400mg/kg of MEMEO caused significant decrease in serum albumin level. Our findings are in agreement with the reports of other authors that reduced serum/plasma albumin was associated with hepatic damage (Shin *et al*., 2010; Yousef *et al*., 2010). According to our study, we also observed a significant decrease in serum total protein in rats that were administered with 400mg/kg of MEMO. Decrease in serum total protein is known to be associated with defective capability of the hepatocytes to synthesize proteins. Protein synthesizing potentials of the hepatocytes have been extensively documented (Sathesh *et al*., 2009; Ahmed and Urooj, 2010; Najmi *et al*., 2010) to crash significantly following hepatic injury/damage.

Cellular leakage of enzymes occurs often when the cell architecture and integrity is damage. Accordingly, the presence of enzymes in the serum above their normal level is a pointer to clinical diagnosis of variety of pathological conditions. Serum aspartate aminotransferase (AST, EC 2.6.1.1) and alanine aminotransferase (ALT, EC 2.6.1.2) have been reported by numerous authors as markers for acute and chronic hepatocellular damage (Dufour *et al*., 2000a; Dufour *et al*., 2000b). Pyridoxal-5-phosphate (PLP) which is the active form of vitamin B6 is the coenzyme for both ALT and AST respectively (Rej, 1977). Significant decrease in serum AST might be due to metabolic, drug-induced or iatrogenic (unknown cause). Pathophysiological conditions associated with deficiency of vitamin B6 might lead to decrease in serum AST and ALT activities or underestimation of their activities if optimized methods are not used (Lum, 1995). Decrease in the serum/plasma activities of aspartate aminotransferase has been shown to correlate with pyridoxal-5-phosphate deficiency (Waner and Nyska, 1991; Evans and Whitehorn, 1995; Hall, 2001; Saori *et al*., 2003). Different authors have found significantly lower in vivo serum concentrations of PLP in epileptic patients treated with anticonvulsant drugs (Young, 2000; Apeland *et al*., 2002; Apeland *et al*., 2003). The animals administered with MEMO had significant reduction of body weight. This effect might be potentially beneficial for the obese individual addicted to high calorie intake. The mechanism(s) of loss of body weight could be through loss of body fat (Palit *et al*., 1999) or decrease in serum cortisol level (Garrison and Chambliss, 2006).

Blood urea nitrogen (BUN) produced in the liver is derived from the diet or tissue sources and is excreted in the urine via the kidney. Serum urea accumulates in the serum in renal disease when the

Table 1: Effect of prolonged administration of MEMO on serum liver and kidney function tests

Treatment	Total protein (mg/dl)	Albumin (mg/dl)	Globulin (mg/dl)	ALT (U/L)	AST (U/L)	BUN (mg/DL)	Creatinine (mg/DL)
Control	5.58±0.22	2.18±0.08	3.88±0.22	58.60±0.75	17.33±1.63	1.25±0.01	1.23±0.02
MEMO	3.62±0.20*	1.00±0.03$^{\#}$	2.50±0.22$^{\#}$	58.67±1.33	10.75±0.95$^{\#}$	1.08±0.03*	1.07±0.06*

Values expressed as mean±S.D, n=8, *p<0.05, $^{\#}$p<0.001

rate of production exceeds that of excretion (Mayne, 1994). Serum creatinine is basically derived from endogenous sources by tissue creatinine breakdown (Mayne, 1994). Therefore, Elevation of urea and creatinine levels in the serum had been taken as the index of nephrotoxicity (Ali *et al.,* 2001; Flaoyen *et al*., 2001). In our study, there was a clear significant reduction in both serum creatinine and urea in rats exposed to prolonged administration of MEMO. This shows that MEMO is not toxic to the kidney. Interestingly, the results obtained on the liver function tests were opposite to that of the metabolites associated with kidney function test. According to this study, prolonged use of MEMO was toxic to the liver and not to the kidney. Our previous report showed that MEMO at 400mg/kg was toxic both to the liver and the kidney in male Wistar rats (Oyagbemi et al., 2013). Surprisingly, this was the opposite in female Wistar rats. However, the use of *Moringa oleifera* leaves as beverages in sub-Saharan Africa should be minimized as prolonged exposure to this medicinal plant could probably precipitate liver damage.

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