Advanced Journal of Microbiology Research Volume 2014
Available online at http://advancedresearchjournals.org/AJMR

Full Length Research Paper

Effect of *Moringa oleifera* seed extract on vital organs and tissue enzymes activities of male albino rats

A. O. Oluduro[1]* and B. I. Aderiye[2]

[1]Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria.
[2]Department of Microbiology, University of Ado-Ekiti, Nigeria.

Accepted 4 August, 2013

The effect of various doses of aqueous extract of *Moringa oleifera* seed on the activities of some internal organs (heart, liver and kidney) and tissue enzymes of male albino rats was investigated. The safe level of the seed extract that would not cause an infarction in the liver of albino rat was less than 2 mg /ml concentration. Daily-dose administration of the *Moringa* seed treated water (1 – 10 mg/ml) to different rat groups for 21 days resulted in significant increase (P > 0.05) in the activities of the following enzymes; aspartate aminotransferase (AST) (162 U/ml), alanine transferase (ALT) (65.32 U/ml), alkaline phosphatase (ALP) (303 U/ml), acid phosphatase (ACP)(499 U/ml) in the serum of the experimental rats compared to a corresponding decreased enzyme activity (AST-131.87 U/ml), (ALT-26.3l U/ml), (ALP-178 U/ml), (ACP-68.48 U/ml) in the liver tissue. Histopathological studies revealed the presence of marked aggregation of bile canaliculi around the portal vein of the liver.

Key words: *Moringa oleifera* seed, toxicity, water, metabolic enzymes, liver.

INTRODUCTION

Focus on plant research has increased in recent times all over the world and results have shown an immense potential of some plants in various traditional water purify-cation system (Amaglah and Benang, 2009). *Moringa oleifera* is a fast growing drought, deciduous tree reaching 3 m in height just after 10 months of cultivation (Valia et al., 1993) . Several biological activities have been reported in the plant including biological coagulation in drinking water by its seed (Jahn, 1988; Oluduro and Aderiye, 2007). The use of *Moringa* seed was once recommended in Java (Jahn, 1988) and later employed for water purification in rural villages in Africa and Asia. Earlier studies have found *M. oleifera* seed to be non-toxic and recommended its use as a coagulant in deve-loping countries (Olsen, 1987) Oral test, acute and chronic toxicity tests on rats with both *Moringa steno-petala* and *M. oleifera* seeds (dosages 50 and 500 mg/kg body weight) have been reported to produce no toxic effects, but, rather increased the weights of the rats (Sattaur, 1983; Jahn, 1988) . On the contrary, the toxicity and mutagenic effects of *M. oleifera* seed extract at 200 mg/l on fish guppies, protozoan and bacteria had been reported (Ndabigengesere, 1995).

However, there remains considerable concern over the toxic effect of *M. oleifera* seed extract in treated drinking water on villagers since the report of Olayemi and Alabi (1994), suggesting apathy to continue the use of the plant for water purification purposes. This study, therefore, sought to investigate the toxicological and histopatho-logical effect of *M. oleifera* seed treated water in rats.

MATERIALS AND METHODS

Source of plant material

M. oleifera seeds were collected from Ibodi Village near Akure in Ondo State and transported in a cool van (20oC) to the Laboratory of the Department of Microbiology, University of Ado-Ekiti, Nigeria. The seed was identified and voucher sample deposited at the herbarium unit of the Department of Plant Science, University of Ado-Ekiti. The sample was later air dried at 100oC for 24 h and ground to a powdery form.

Extraction of *Moringa* seed and water treatment

Five hundred gram of the powdered *M. oleifera* seed was soaked in 200 ml distilled water at ambient temperature for three days and filtered. The bulked filtrate was reduced in vacuo at 400C. The resi-

*Corresponding author. E-mail: Oluduro2003@yahoo.co.uk. Tel: +2348069379885.

due was later stored at -40C until when needed. Varied concentrations (1 – 10 mg/ml) of the extract were used for the treatment of well water.

Source of water

Water sample used was obtained from a well located at No.3, Fiyinfoluwa Street behind Galilee praying ground, Ori-Apata, Adebayo, Ado-Ekiti, Ekiti State, Nigeria. The well about 8 m deep is located approximately 20 m away from a tree whose roots are growing at the bottom of the well. The well is neither concrete ringed nor with a permanent cover.

Administration of *M. oleifera* seed treated water and preparation of tissue homogenates

A total of 48 three week old male albino rats weighing between 100 – 150 g were used for the present study. The rats were randomly divided into eleven experimental groups and a control group of 4 rats each. The rats were fed with the commercial feed (Purina Chow).

Water treated with varied concentrations (1 – 10 mg/ml]) of the seed extract was administered orally to different groups of the experimental rats. The control rats were ingested with the equivalent dose of physiological saline. Rat representatives from each group were sacrificed after 7, 14 and 21 days respectively.

Five milliliter of blood from the two jugular veins of the neck of the rat was collected in an ethylene diamine tetra acetic acid anti-coagulant bottle. The liver, heart and kidney were eviscerated into 20% sucrose solution. The tissues were washed clean of blood and dried with clean tissue paper, weighed, cut finely with clean sterile blade and homogenized in ice-cold 20% sucrose solution using sterile pestle and mortar. The homogenates were stored at -200C until prior analysis.

Enzyme assay

The activities of alkaline phosphatase (ALP) and acid phosphatase (ACP) were determined, using the method of Bergemeyer and Brent (1974) and Shi -jun et al. (2004). The spectrophotometric methods described by Kings (1960) and Pavia et al. (2003) were used for the determination of ALT and AST. Alanine transaminase was measured by monitoring the concentration of pyruvate hydrazone at 546 nm while AST was determined by monitoring the concentration of oxaloacetate hydrazone formed with 2, 4-dinitrophenythydrazine at 546 nm.

Tissue processing

Tissue processing was carried out using the method of Baker and Silverton (1998). A portion of the tissue (heart, kidney and liver) preserved in formalin was fixed in 10% normal saline for 48 h and dehydrated sequentially in ascending grades of alcohol for between 2 and 3 h. The tissues were cleared in chloroform overnight and subsequently cleared in xylene for an hour. The cleared tissues were infiltrated and embedded in molten paraffin wax, I, II and III for an hour. The embedded tissues were sectioned, floated on a water bath at 5 - 100C and later dried on the hot plate for 30 min at 600C. The tissue was then stained using haematoxyline stain. The stained tissues were dewaxed in xylene for 30 mins and rehydrated in descending grades of alcohols (absolute alcohol, 95, 90, 80, 70 and 50%). These were finally rinsed in water and re-stained with haematoxyline stain using Baker and Silverton (1998) technique before examining under the light microscope for any pathological change.

Statistical analysis

Data obtained were statistically analyzed using the Student's t-test and analysis of variance. The level of significant difference was determined at $p > 0.05$

RESULTS

The mean values of enzymatic activities in the tissues of the liver, heart and kidney of the experimental rats and the serum of control are presented in Table 1. There were reduced level of the enzymes (AST - 131.8 U/ml, ALT - 26.31 U/ml, ALP - 178 U/ml, ACP - 65.48 U/ml) activities in the liver compared to a marked increased activities (AST - 162 U/ml, ALT - 65.32 U/ml, ALP - 303 U/ml, ACP - 499 U/ml) recorded in the serum of the experimental rats. Meanwhile, the concentration of the markers remain considerably high (AST - 55.78 U/ml, ALT - 243.49 U/ml, ALP - 518 U/ml) in the heart tissues while their levels were low in the serum (AST - 162.11 U/ml, ALT - 65.33 U/ml, ALP - 303 U/ml). However, the concentration of ACP (499 U/ml) was high in the serum but low in the heart tissues (ACP - 243 U/ml).

Similarly in the kidney, there was an increased level of enzyme activities (AST - 287 U/ml, ALT - 126 U/ml, ALP - 476.32 U/ml, ACP - 618 U/ml) while a corresponding decreased levels of activities(AST - 162.11 U/ml, ALT - 65.33 U/ml, ALP - 303 U/ml, ACP-499 U/ml) were recorded in the serum. The concentrations of all the enzymes in the serum of the control rats remained constantly low, but relatively high in all the tissues throughout the period of the experiment (Table 1).

The photographic plates of the liver tissues of the experimental rats fed *ad libitum* with 2 mg/ml concentration of *M. oleifera* seed extract and that of control are

Table 1. Mean values of enzymatic activities (U/ml) in the various organs of albino rats fed with 2 mg/ml of *M. oleifera* seed treated water and control.

Organ	Enzymatic activities(U/ml Mean values)							
	Experimental rat				Control rat			
	AST	ALT	ALP	ACP	AST	ALT	ALP	ACP
Kidney	287.OO	126.00	476.32	618.00	232.00	110.50	387.00	580.00
Liver	131.87	26.31	178.00	68.48	197.00	180.00	399.10	603.00
Heart	55.78	243.49	518.00	243.49	152.68	101.80	402.00	508.00
Serum	162.11	65.33	303.00	499.00	139.67	59.00	285.00	410.00

shown in Figures 1 and 2 respectively. There was marked aggregation of many bile canaliculi around the portal vein of the liver of the experimental rats (Figure 1) and the rate of aggregation corresponded to the concen-tration of the *M. oleifera* seeds extract used. The hepatic cells also appeared shrunk. However, in the control rats, the hepatic cells appeared comparatively normal and the central vein appeared normal in the hepatic lobule (Figure 2). Meanwhile, there were no visible pathological changes observed in the heart and kidney of the experi-mental rats.

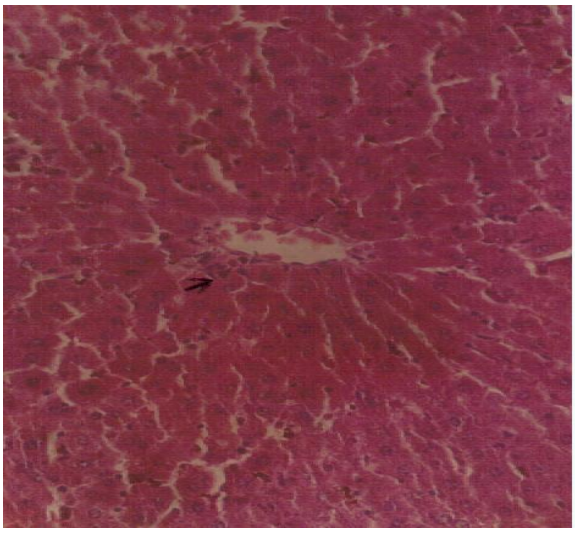

Figure 1. Liver tissue of albino rats fed *ad libitum* with 2 mg [ml] of *M. oleifera* seed extract. Legend : Aggregation of bile canaliculi.

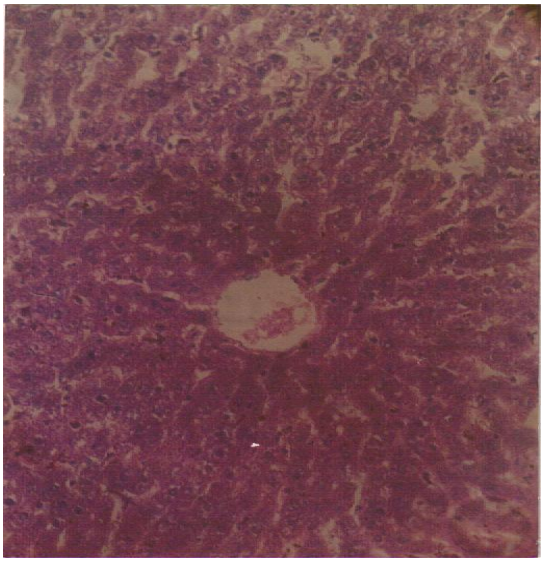

Figure 2. Liver tissue of the control rat.

RESULTS AND DISCUSSION

M. oleifera seed – treated well water administered at concentrations 2 mg[ml] to the rats lowered the activities of the enzymes AST, ALT, ALP and ACP in only the liver tissues but, considerably increased the activities of these enzymes in the serum. This might be due to the damage of the liver tissue due to the alteration of the membrane components in the tissue, resulting in the release of these enzymes into the blood hence, increased level of serum enzyme activities. There were significant changes in the activities of the ALT, AST, ALP and ACP in the serum and liver tissue of the rats at $P > 0.05$. An increased ALP activity in the serum suggests tissue damage which is often used in the diagnosis of liver and bone marrow disease (Osteomalacia, bone cancer) and hepatitis (Nelson and Cox, 2000; Anderson, 2002). Moreover, an elevated serum ACP may suggest an increased rate of tissue destruction which is rarely elevated in patients with liver and kidney diseases thus, useful in diagnosis of metastatic carcinoma of the prostate (Rodwell and Kennelly, 2000). Increased serum AST and ALT levels are important in the diagnosis of the heart and liver damage caused by drug toxicity, infection or heart attack (Nelson and Cox, 2000).

The high level of activities of AST, ALT and ALP in the kidney and heart tissues of the rats and their correspond-ing decreased activities in the serum is indicative of non toxicity of the seed extracts in these organs. The high se-

rum ACP activity may not be of any toxicological significance. According to Nelson and Cox (2000) acid phosphatase is classified as a non functional enzyme because it performs no known physiologic function in the blood and could be found at levels up to a million fold in the blood of normal individuals.

The aggregation of bile canaliculi around the portal vein of the liver (Figure 1) may be due to the attachment of cations (sodium and magnesium ions) to the portal veins resulting in dilation of the hepatic vein, which might have enhanced osmoregulation (Worobetz et al., 2005). This is suggestive of induced effect occasioned by the seed and may likely justify the observed elevated levels of serum enzymes activities and their corresponding decrease in the liver tissue. This study therefore revealed the potentials of prolonged consumption of water treated with 2 mg /ml of *M. oleifera* seed to constitute liver infarction.

REFERENCES

Anderson A (2002). Effect of anti-inflammatory drugs on the enzyme activity of the rat liver of glandular fraction which increases vascular permeability J. Pharmacol., 17: 2253-2254.

Amaglah FK, Benanng A (2009). Effectiveness of *Moringa oleifera* seed as coagulant for water purification. Afr. J. Agric. Res. 4(1):119-123.

Baker FJ, Silverton RE (1998). Manual of Tissue Processing. 6th Edition. Butter Worth and Co. New York, Pp. 191-199.

Bergemeyer HU, Brent E (1974). LDU-UV assay with pyruvate and NADH. In: Method of Enzymatic Analysis (Ed): H.U. Bergemeyer. Academic press, New York. pp. 574-579.

Jahn SAA (1988). Using *Moringa* seeds as coagulants in developing countries. J. Am. Water Works Assoc. 80: 45-50.

Kings EJ (1960). Transaminases Assay. J. Canad. Med. Assoc., 31:376-380.

Ndabigengesere A, Narasiah KS, Talbot BE (1995). Active agents and mechanism of coagulation of turbid waters using *Moringa oleifera.* Water Res., 29(2): 703-710.

Nelson DL, Cox MM (2000). Enzymes, Bart H: structures and catalysis, Lehninger *Principles of Biochemistry*, 4th edition, W. H. Freeman and Company, New York. pp. 190-237.

Olayemi AB, Alabi RO (1994). Studies on Traditional water purification using *Moringa oleifera* seeds. Afr. Study Monogr. 15*(*3): 135-142.

Olsen A (1987). Low technology water purification by bentonite clay and *Moringa oleifera* seeds flocculation as performed in Sudanese village: Effects of *Schistosoma mansoni cericariae*. Water Res., 21: 81-92.

Oluduro AO, Aderiye BI (2007). Efficacy of *Moringa oleifera* seed extract on the Microflora or Surface and Underground Water. J. Plant Sci., 2(4): 453-458.

Pavia CN, Pryrho A, Lannes-Vieira J, Vaccluo M, Soares MBP, Gattass cerli R (2003). *Trypanosoma cruzi* sensitizes mice to fulminant seb-induced shock, over release of inflammatory Cytokines and independence of Chagas' Diseases or TCR V (beta)-Usage. Shock J., 19(2): 163-168.

Rodwell VW, Kennelly PJ (2000). Enzymes: General Properties. *Harpers Biochemistry*. McGraw-Hill, New York. 25: 74-85.

Sattaur O (1983). The light of the village. New Scientist, 100: 830-831.

Shi-Jun ZP, Wang GT, Youhai HC (2004). Critical roles of Trail in hepatic cell death and hepatic inflammation. J. Clin. Investigation, 113(1): 58-64.

Valia RV, Patil VK, Patel ZN, Kapadia PK (1993). Physiological responses of drum stick (*Moringa oleifera*) land to varying levels of ESP. Indian J. Plant Physiol., 36(4): 261-26.

Worobetz V, Hilsden RJ, Shaffer EA, Simon JB, Pare P, Bain VG, Wong M, Blendis L, Adams P, Healthcote, J, Lee SS, Lil LB, Hemming AW, Levy GA (2005). First Principle of Gastroenterology: The Basis of Disease and an Approach to Management. Chapter 14:462-465.