ORIGINAL ARTICLE

Histological Effects of Aqueous Extract of Moringa oleifera on the Liver Tissues of Wistar rats fed with High Fat Diet

ABSTRACT

Moringa oleifera belongs to family of moringaceae and is considered as one of the world's most useful trees, as almost every part of the plant can be used for either as food, or therapeutic purposes. Fatty diet is a significant factor in the pathogenesis of non-alcoholic fatty Liver disease (NAFLD). The study was designed to examine histological effects of aqueous extract of Moringa oleifera on the liver tissues of wistar rat fed with high fat diet. Twenty five adult rats were divided into five groups of five animals each .While group A received distilled water daily only, groups Band C received aqueous extract of Moringa oleifera at doses of 200 mg/kg body weight and fat high diet (30% w/w of the total mash feed) respectively for a duration of seventy days. Others (groups D and E) received aqueous extract of Moringa oleifera at doses of 200 mg/kg body weight and fat high diet for a duration of seventy days. Histology of the liver of the rats fed with high fat diet exhibited significant changes in the architecture of liver tissue. The changes include micro and macro vascular steatosis, increased fatty infiltration, inflammation, sinusoidal dilation, degeneration of veins and vacuolization as compared to normal liver histology. Treatment with 200 mg/kg extract of Moringa oleifera significantly attenuated these effects imposed by high fat diet as compared to the control group. Therefore demonstrated that daily administration of Moringa oleifera leaves extract to rats for a period of 70 days may reverse the formation of hepatic steatosis in non-alcoholic fatty liver disease.

Keywords; Vascular steatosis, non-alcoholic fatty liver disease, Moringa oleifera

Obayuwana E[1]

Ubhenin A. E[2]

Innih, S. O[1]

Iwee R[1]

Eidaghese E.C[1]

[1]Department of Anatomy, School of Basic Medical sciences, College of Medical sciences, University of Benin, Benin City, Nigeria.

[2]Department of Biochemistry, Faculty of Basic Health science, Federal University Lafia, Nasarawa, Nigeria.

[2]corresponding author email:

ehibram@yahoo.co.uk

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INTRODUCTION

Moringa oleifera belongs to family of moringaceae and is commonly called ben oil or drumstick tree. The tree is widely cultivated in Africa, Central and South America, Sri Lanka, India, Malaysia and the Philippines. In Nigeria it is mostly grown in the northern and South-West parts. *Moringa oleifera*is considered one of the world's most useful trees, as almost every part of the tree can be used for either as food, or therapeutic purposes .[1,2,3] The leaves can be eaten fresh cooked or stored as dried powder for several months ,the pods when young can be cooked, eaten like beans.[4] Its oil, leaf extract and micronutrients have been reported to contain anti-microbial, anti- inflammatory, antioxidant, anti-hypotensive, anti-asthmatic, antispasmodic and antidiabetic .[5, 6,7,8,]Other acclaimed medical uses include regulation thyroid status, maintenance of cholesterol levels, anti- tumor, antifertility and anti-ulcer. [9,10,11]. The risk factors such as overweight, visceral adiposity, adipocytokines may increase the flow of free fatty acids (FFAs) to the liver. Other are alterations of intestinal microbial and increased permeation of bacterial endotoxins from the gut which may activate Toll-like receptor signaling cascades and lead to polarization of macrophages. All these events which may include increased FFAs, cytokines from adipocytes, endotoxins, insulin resistance, macrophage polarization may be the underlining mechanism of pathogenesis of NAFLD.[12]The initial stage of NAFLD is liver steatosis characterized by the accumulation of triglycerides as macro- and/or micro vesicular lipid vacuoles in some liver cells.[13] The elevated level of triglycerides in the liver cells arises from imbalance between triglyceride biosynthesis and catabolism, which is due to the result of very high caloric and/or unbalanced diet. An increased de novo synthesis of triglycerides or increased lipolysis in adipose tissue can also account for this imbalance. Excess intake of high fat diet has been implicated in hyperlipidemia, which has been one of the major causes of cardiovascular disease, metabolic syndrome, obesity which presents in fatty liver disease. [14]Alimentary factors, in particular, saturated fats and cholesterol can significantly alter the liver metabolic processes, affect lipid metabolism, biosynthesis of the fatty acids and the lipoprotein formation.[15,16] The degree of saturation of these fatty acids contributes to the development of many diseases such as atherosclerosis, diabetes and steatohepatitis .[17] Intake of high fat diet for a prolong period of time has been the major cause of metabolic syndromes[17].Therefore, the study was designed to examine histological effects of aqueous extract of *Moringa oleifera* on the liver of wistar rat fed with high fat diet.

MATERIALS AND METHODS

Plant Collection and Identification

Moringa oleifera leaves were harvested from area around Owan east local government area of Edo state, Benin City. They were identified in the Department of plant biology and biotechnology, faculty of life sciences, University of Benin. The leaves were dried in a shaded room for one week and grinded to powdered form using mortar and pestle (mechanical form).
The powdered extract was put in a dried container and was kept in a cool dry place**.**

Preparation of Plant Extract

The powdered extract was soaked in distilled water for forty-eight hours at room temperature. The mixture was filtered using a Buchner funnel and Whatman No.1 filter paper. Dried aqueous extracts were obtained after removing the solvent by evaporation under reduced pressure using Rotary evaporator[18].The extract was stored in an air-tight container and kept in the refrigerator at 4°C until use

Experimental Animals

Twenty-four (25) Adult Wistar rats of both sexes, weighing 150-300 g were randomly separated into four (4) groups; Control, A, B, & C, of six rats each. The rats were procured from the Animal House, Department of Pharmacology, University of Benin, Benin City. The rats were allowed two weeks to acclimatize to Anatomy Departmental Animal House, where they were housed in standard animal cages. They were allowed free access to drinking water and standard livestock feed (vital grower's feed, livestock feed company, Benin City).All animals were treated in accordance with the Guide for the care and use of laboratory Animals prepared by the National Academy of Sciences and Published by the National Institute of Health Guide for the use of Laboratory Animal (NIN, 2002 Production, No. 83-23), Revised 1978 and also according to our previous published article.[19]

Preparation of High Fat Diet

A mixture containing 50%w/w of egg yolk (boiled) and saturated fat were mixed with in mash feed (growers mash (30%w/w) to give high fat diet. The resulting mash containing (30%w/w) egg yolk saturated fat mixture and 70%mash feed were then fed to rats for a periods of seventy days (ten weeks).

Experimental Design

The design consisted of 25 rats, divided in five groups of five rats each.

Group A: (Control) received1ml distilled daily.
Group B: Treated with 200mg/kg of the aqueous extract *Moringa oleifera* only.
Group C: fed with high fat diet only.
Group D: fed with high fat diet and 200 mg/kg of the aqueous extract *Moringa oleifera* simultaneously.
Group E: fed with high fat diet for ten weeks and thereafter treated 200 mg/kg of the aqueous extract *Moringa oleifera*.
To ensure accuracy of treatment, administration of the aqueous leaf extract of *Moringa oleifera*was done using orogastic tube for seventy consecutive days (10weeks).

Method of Sacrifice and Tissue Collection

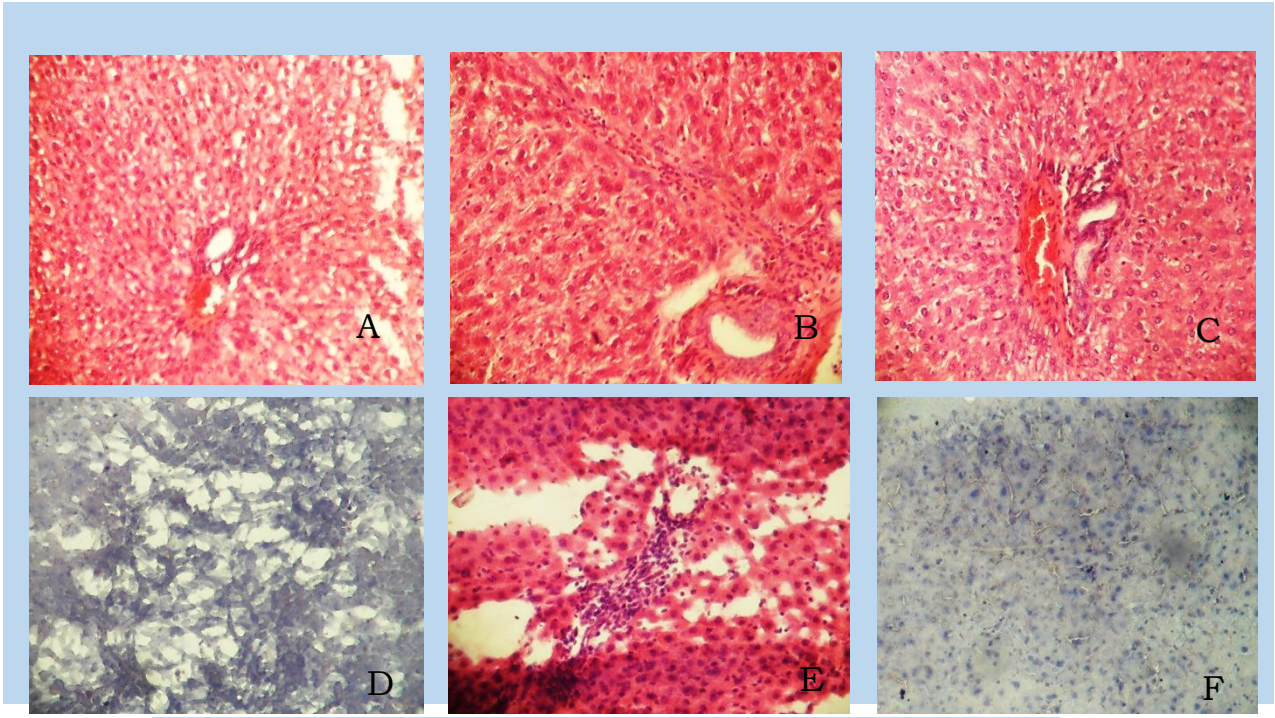
At the end of the experiment, the animals were sacrificed under chloroform anesthesia. They were dissected and the liver tissues were excised, weighed and placed in specimen bottles containing buffered formalin for fixation.

Histopathological analysis

The liver were examined grossly in all the dissected rats. The portions of the liver was immediately fixed in 10% neutral formalin for a period of 24 hours, dehydrated in several grades (70-100%) alcohol embedded in paraffin (58-60°C) and sectioned at 5 µm thickness . The sections were stained with hematoxylin, eosin and also Sudan black B. [20]

Photomicrography:

The figures below show photomicrographs representing the histological results of the processed tissues.



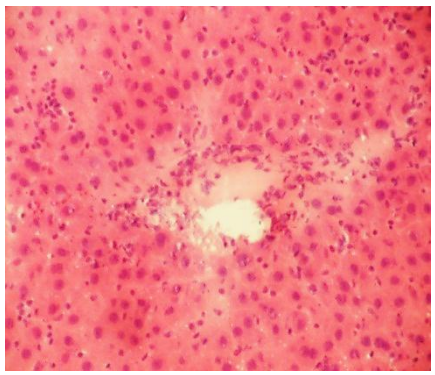
G H

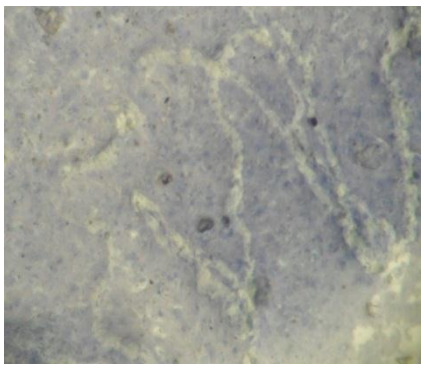
FIGURES: A: Control: Rat liver composed of hepatocytes, sinusoids, central vein and bile duct (H&E x 100), **B**: Rat liver given Moringa only showing mild periportal lymphocytosis A (H&E x 100), **C**: Rat liver given high fat diet only showing focal intimal erosion and mild periportal lymphocytosis (H&E x 400), **D**: Rat liver given high fat diet showing macrovesicular steasis (positive for sudan black B) (S.B x 100), **E**: Rat liver given fat and Moringa simultaneously showing unremarkable hepatocytes and moderate periportal lymphocytosis (H&E x 100), **F**: Rat liver given fat and Moringa oleifera simultaneously showing negative sudan black Hepatocytes (S.B x 100), **G**: rat liver given high fat diet, then Moringa, showing focal perinuclear fat vacuolation (H&E x 400), **H**: Rat liver given high fat diet, then moringa oleifera showing hepatocytes negative for sudan black B.A (S.B x400).

RESULTS AND DISCUSSION

Histopathological examination is one of methods used to evaluate the effect of medicinal plants on experimental induced steatosis in rats .Nonalcoholic fatty liver disease (NAFLD) is a common health problems characterized by high lipid accumulation in the hepatocytes of the liver parenchyma. The pathological manifestation mimic to that of alcohol-induced liver injury in human, but it occurs in individuals without a significant history of alcohol ingestion. NAFLD comprises a wide spectrum of liver damage, ranging from simple macrovesicular steatosis to steatohepatitis, advanced fibrosis, and cirrhosis.[20,21] Fatty liver is classified as either macrovesicular or microvesicular steatosis according to the size of the lipid vacuoles.

The most common form is macrovesicular steatosis and is generally the more severe disease though is often reversible[20,21]. Macrovesicular steatosis are more abundant than the microvesicular as was observed above. The findings is in consonant with that of human and animal forms of non-alcoholic fatty liver disease NAFLD which is characterized by a mixed macro and microvesicular type of hepatic steatosis [22-28] .The histology of the liver tissue of rats fed with high fat diet exhibited significant alteration in the architecture of the liver tissue. The alteration include micro and macro vascular steatosis, increased fatty infiltration, inflammation , sinusoidal dilation, degeneration of veins and vacuolization as compared to normal liver histology in figure A,C. Treatment with the extract at dose of 200 mg/kg significantly attenuated these effects imposed by high fat diet as compared to high fat diet control figure B,D and E.





These damages were as result of up-regulation of the key genes responsible for lipid biosynthesis in the liver and also the down-regulation of the gene for fatty acid oxidation[29,30] . The effect of *Moringa oleifera* in attenuating these damages is possibly due to up-regulation of gene for fatty acid oxidation and or the down-regulation of hepatic biosynthetic genes[31,32]. The mechanism of action of the extract might also due to free radical scavenging effect which is primarily attributed to some

phytochemicals present in the extract. The development of the histopathological changes in the groups fed with high fat diet can be explained in terms of the fact that some metabolites from lipid biosynthetic pathways are suitable precursor for many pathological processes especially inflammation and proliferation.[33,34]

Therefore, this extract seems to reverse fatty liver disease in the groups fed with high fat diet. This agrees well with the finding that medicinal plants as well as some food supplements ameliorated or reversed hepatic steatosis in animal models or patients with non-alcoholic fatty liver disease .[35-38]

Therefore we demonstrated that daily administration of *Moringa oleifera*leaves extract to rats for a period of 70 days may reverse the formation of hepatic steatosis in non-alcoholic fatty liver disease.

COMPETING INTERESTS

The author's affirm that there is no competing interests.

CONTRIBUTION OF AUTHORS

The authors declare that this work was done by the author's affirmed in this article and all liabilities to claims relating to the content of this article.

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