

Histopathological Effect of Methanolic Leaf Extract of *Maerua angolensis* on Thioacetamide Induced Liver Cirrhosis of Wistar Rats.

ABSTRACT

Introduction: Cirrhosis is defined as the histological development of regenerative nodules surrounded by fibrous bands in response to chronic liver injury that leads to portal hypertension and end stage liver disease. Although the causes are interwoven, some pathological characteristics are common to all cases of liver cirrhosis, including degeneration and necrosis of hepatocytes, and replacement of liver parenchyma by fibrotic tissues and regenerative nodules, and loss of liver function. Presently, effective strategies to treat liver cirrhosis are still lacking, treatment is based on the underlying cause, in advanced cases, a liver transplant may be required.

Maerua angolensis known as bead bean tree is a shrub or small tree, usually growing 5 - 6 metres tall but with reports of some trees up to 10 metres tall. The plant is often rather rambling, with spreading or drooping branches. The bole is rarely straight. A multipurpose tree, harvested from the wild for local use, mainly as a medicine. The tree is attractive, ornamental for garden planting especially in the drier parts. It is commonly found growing in bush and rocky areas but planted on graves in Nupe area of Nigeria.

Aim: This study aims to determine the anti-cirrhotic effect of aqueous extract of the leaves of *Maerua angolensis* on induced cirrhosis on gross appearance of liver of wistar rats.

Methodology This study made use of 25 rats. This was divided into 5 groups, consisting of 5 rats each. Group 1 serve as control administered with normal saline, while other groups were induced with Thioacetamide to establish liver cirrhosis. Group 2 was not treated with anything (positive control). Group 3, 4, and 5 were treated with Low, Moderate and High doses of the extract respectively. At the 8 weeks of the experiment all animals were sacrificed. The livers were excised, washed with normal saline, weighed and Gross pictures were taken.

Results: Macroscopic studies were done which revealed that; Thioacetamide administration induced, marked portal to portal fibrosis and hepatic cells surrounding central vein showed various degenerative changes. The rats had a significantly low food and water intake of medium dose group and high dose group compared to normal controls. Livers of rats treated with thioacetamide and later with the various doses of *Maerua angolensis* leaf extract showed significant restoration at 100mg/kg, 200mg/kg and 400mg/kg. There was histological regeneration of the nodules compared to those rats treated with thioacetamide only.

Conclusion: The results of acute toxicity studies showed LD₅₀ to be greater than 5000mg/kg body weight. The entire groups induced with thioacetamide shows micronodular surface of the liver with significant improvement in the treated groups Food and water intake of each group. Food and water intake was not significantly different from each when compared with control group There was micronodular appearance on the surface of the induced groups which was gradually decrease with treatment with extract.

Key words: Methanol, *Maerua Angolensis*, Thioacetamide and Cirrhosis

1.0 INTRODUCTION

Liver fibrosis is a histological consequence of the wound-healing process resulting from chronic liver diseases such as viral hepatitis, alcoholic liver disease, non-alcoholic fatty liver disease, and other liver disorders. Deposition of excess extracellular matrix that is rich in fibril-forming collagens is a typical finding of liver fibrosis. The excess deposition of the collagen changes the normal architecture of the liver resulting in pathophysiologic damage to the organ. *Mearua angolensis* known as bead bean tree, is a shrub or small tree, usually growing 5 - 6 metres tall but with reports of some trees up to 10 metres tall [1]. The plant is often rather rambling, with spreading or drooping branches. The bole is rarely straight [2]. A multipurpose tree, harvested from the wild for local use, mainly as a medicine. The tree is attractive, ornamental for garden planting especially in the drier parts. It is commonly found growing in bush and rocky areas but planted on graves in Nupe area of Nigeria. *Mearua angolensis* has a long history of use in traditional medicine to manage various painful conditions in Nigeria and other West African countries. Various parts of the plant notably the leaves, roots and stem barks are claimed to reduce pain and are used to manage psychosis, epilepsy, diabetes, peptic ulcer, diarrhoea and arthritis in the traditional medicine. The raw fruit, crushed in water, is sometimes taken to cleanse out the stomach. The demonstration of the antimicrobial activity of the leaf extracts of *M. angolensis* against pathogenic microorganisms is evidence that the extract is a potential source of antibiotics with a broad spectrum of activity. This study validates the use of the plant in traditional phytomedicine to treat diseases caused by the Pathogenic microorganisms.

Liver cirrhosis is an advanced stage of liver fibrosis with distortion of the hepatic vasculature and architecture.

Histologically, regenerative nodules with fibrous tissues form in response to chronic injury and lead to Liver cirrhosis [3]. Histologically, Liver Cirrhosis is characterized by diffuse nodular regeneration surrounded by dense fibrotic septa with subsequent collapse of liver structures which then causes pronounced distortion of vascular architecture in the liver. [4]. It is often an indolent disease; most patients remain asymptomatic until the occurrence of decompensation, characterized by ascites, spontaneous bacterial peritonitis, hepatic encephalopathy or variceal bleeding from portal hypertension [5]. cirrhosis is often a consequence of fatty liver disease due to alcoholism or other causes, but can also be caused by hepatitis B and hepatitis C. Cirrhosis is more common in overweight persons and smokers. [6]. It is generally accepted that cirrhosis due to any cause may predispose to liver cancer, and that this condition is more frequent in geographic area of high incidence of liver cancer. [7]. Liver transplantation remains the only curative option for a selected group of patients, but pharmacological treatments that can halt progression to decompensated cirrhosis or even reverse cirrhosis are currently being developed. [8].

Although the causes of liver cirrhosis are multifactorial, there are some pathological characteristics that are common to all cases of liver cirrhosis, including degeneration and necrosis of hepatocytes, and replacement of liver parenchyma by fibrotic tissues and regenerative nodules, and loss of liver function. Fibrosis as a precursor of cirrhosis is a pivotal pathological process in the evolution of all chronic liver diseases to cirrhosis. [9], macroscopically, the liver is initially enlarged, but with the progression of the disease, it becomes smaller. Its surface is irregular, the consistency is firm, and the colour is often yellow (if associated with steatosis). Depending on the size of the

nodules, there are three macroscopic types: micronodular, macronodular and mixed nodular. Microscopically there is loss of normal architecture—that is, loss of normal central–portal relationships and an irregular pattern of central veins may suggest a cirrhotic process, especially if regenerative cell plates ("twin plates," two cells thick) are present or if the fragments have rounded edges, suggestive of nodularity. *Mearua angolensis* known as bead bean tree, is a shrub or small tree, usually growing 5 - 6 metres tall but with reports of some trees up to 10 metres tall [10].

The plant is often rather rambling, with spreading or drooping branches. The bowl is rarely straight. A multipurpose tree, harvested from the wild for local use, mainly as a medicine. The tree is attractive, ornamental for garden planting especially in the drier parts. It is commonly found growing in bush and rocky areas but planted on graves in Nupe area of Nigeria. . *Mearua angolensis* has a long history of use in traditional medicine to manage various painful conditions in Nigeria and other West African countries. Various parts of the plant notably the leaves, roots and stem barks are claimed to reduce pain and are used to manage psychosis, epilepsy, diabetes, peptic ulcer, diarrhoea and arthritis in the traditional medicine . [11], the raw fruit, crushed in water, is sometimes taken to cleanse out the stomach. In a study carried out by Ayo *et al.* [12].the demonstration of the antimicrobial activity of the leaf extracts of *M. angolensis* against pathogenic microorganisms is evidence that the extract is a potential source of antibiotics with a broad spectrum of activity. This study validates the use of the plant in traditional phytomedicine to treat diseases caused by the Pathogenic microorganisms.

METHODOLOGY

3.1 STUDY AREA

The study was conducted at Laboratory of Histopathology department, school of medical laboratory science, Usmanu Danfodiyo University, Sokoto and Service Laboratory.of Histopathology department, , Usmanu Danfodiyo University Teaching Hospital, Sokoto.

3.2 EXPERIMENTAL ANIMALS

The wistar rats were purchased from the Animal house of Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto. They were kept in a well-ventilated room with optimum environmental conditions of temperature, relative humidity, dark/light cycle and were fed standard feed pellets and tap water. They were acclimatized for two weeks prior to the experiment.

3.3 PLANT COLLECTION

The leaves of *Maerua angolensis* for this study were purchased from Central Market of Yola, Adamawa state, Nigeria. The plant was authenticated at the Botany unit of Usmanu Danfodiyo University, Sokoto.

3.4 EXTRACT PREPARATION

The leaves were cleaned and air-dried at room temperature for 7 day and ground to fine powder using mortar and pestle. One hundred grams of the powdered material was macerated in 250 ml methanol for three days with periodic shaking. Then, the extract was filtrated and the filtrate was collected. The filtered liquid extracts were subjected to rotary evaporation and subsequently concentrated under reduced pressure (in vacuum at 40°C). Then, the extract was evaporated to dryness and stored at 4°C in an air tight bottle.

3.5 ACUTE TOXICITY TESTING

Acute toxicity testing was conducted using Lorke's Method (. [13].

Phase I, nine (9) rats were used and randomly assigned into 3 groups of 3 rats each. The 1st group was administered 10mg/kg body weight of the extract using

an oral cannula, the 2nd and 3rd group received 100mg/kg and 1000mg/kg body weight respectively. The animals were then observed for 24 hours to monitor their behaviour for signs of toxicity as well as mortality.

Phase II, three (3) rats were used and randomly placed into 3 groups of an animal each. The animals were administered high doses of 1600mg/kg, 2900mg/kg and 5000mg/kg respectively. They were then observed for 24 hours for signs of toxicity and mortality.

3.6 INDUCTION OF CIRRHOSIS

Cirrhosis was induced in wistar rats by intraperitoneal injection of thioacetamide (TTA) 200mg/kg body weight. Thioacetamide was dissolved in distilled water and was given for 3 days per week for 8 weeks following the method of

Kantah *et al.* [14]; Al-attar and Shawish. [15] and Li *et al.* [16]. Study has demonstrated that regenerative nodules and liver surface in rats are more prominent in the cirrhotic model induced by thioacetamide not treated.

3.7 EXPERIMENTAL DESIGN

This study made use of 25 rats. This was divided into 5 groups, consisting of 5 rats each. Group 1 serve as control administered with normal saline, while other groups were induced with Thioacetamide to establish liver cirrhosis. Group 2 was not treated with anything (positive control). Group 3, 4, and 5 were treated with Low, Moderate and High doses of the extract respectively. At the 8 weeks of the experiment all animals were sacrificed. The livers were excised, washed with normal saline, weighed and Gross pictures were taken.

TABLE 1 SUMMARY OF EXPERIMENTAL DESIGN

EXPERIMENTAL GROUPS OF WISTAR RATS	TREATMENT GIVEN	NO. OF ANIMALS
GROUP 1(Control)	Normal Saline	5
GROUP 2 (Induction of cirrhosis)	TTA + No Treatment	5
GROUP 3 (Induction of cirrhosis + Treatment [Extract])	TTA + Low Dose	5
GROUP 4(Induction of cirrhosis + Treatment [Extract])	TTA + Moderate dose	5
GROUP 5 (Induction of cirrhosis + Treatment [Extract])	TTA + High dose	5

3.8.1 LABORATORY ANALYSIS

After the sacrifice of the wistar rats with chloroform vapour in an enclosed transparent jar Liver was carefully removed with surgical blade washed with normal saline, gross examination was made and gross pictures were taken and presented alongside with control groups.

3.9 DATA AND STATISTICAL ANALYSIS

All the results were expressed as mean $\pm$ S.D. Data analysis was performed using GraphPad Prism 6.0 software (GraphPad, San Diego, USA). A value of $p < 0.05$ was considered to be statistically significant

RESULTS

The methanolic extraction procedure yielded percentage of 10.8g of the extract. The extract was greenish black in colour; it was gummy and had a slightly sweet smell.

Using Lorke Method, all the graded doses of the aqueous extract of *Mearua*

angolensis administered to the rats showed no sign of toxicity or behavioural change. After 24 hours' observation, no deaths were recorded in both Phase I and Phase II of the experiment. The results of acute toxicity studies showed LD₅₀ to be greater than 5000mg/kg body weight.

Table 4 Shows the macroscopic changes on the surface of the Liver smooth and shining in group 1 which is control while in the thioacetamide induced liver cirrhosis in wistar rats. Similarly in other groups shows marked micronodular surface in group 2 while group 3, 4 and 5 there decreased in severity of the micronodular appearance due to increase in concentration of extract doses given.

Table 5 shows the statistical value of food and water intake of each group. The was monitored daily for 1 week at pre intervention and 1 week at post intervention. The food intake of animals that received the different concentrations of the extract was not significantly different from the previous phase. However, their food intake was not as high as the normal control. A *p* value of 0.049 which is statistically significant; therefore further test (Dunnett's multiple comparisons test) was carried out to compare the pairs of mean with significant difference. This test revealed a statistically significant difference in the food and water intake between control + medium dose (*P*=0.0315) and control+ High dose (*P*=0.0352). However, there was no statistically significant difference between control + thioacetamide and control+ low dose.

Table 2 Physical Properties of *Mearua angolensis* extract

Plant Part	Extract Type	% Yield	Texture	Colour	Smell
Leaves	Methanollic	10.8	Gummy	Greenish- black	Sweet

Table 3 LD₅₀ of methanolic extract of the leaves of *Mearua angolensis* in rats.

Table 6 Shows the statistical values of weights due to the food was provided for each group, after 24 hours the amount of food remaining, including any that has spilled out of the stainless steel container was recorded, intake was calculated as the weight of food provided. There statistical significant different across all the groups.

Figure 1: Macroscopic appearances of control and cirrhotic wistar rat livers. (A) Normal external livers surface of rats injected with saline (smooth). (B) External surface of livers of rats injected with thioacetamide for 8 weeks not treated, severe Micronodular surface of the Liver

Figure 2: Macroscopic appearance of control and cirrhotic wistar rat livers. (A) Normal smooth external livers surface of rats injected with saline. (B) External surface of livers of rats injected with thioacetamide for 8 weeks and treated with low dose of extract shows less severe Micronodular surface of the liver

Figure 3 Macroscopic appearance of control and cirrhotic wistar rat livers. (A) Normal external smooth livers of rats injected with saline. (B) External surface of livers of rats injected with thioacetamide for 8 weeks and treated with moderate dose of the extract shows moderate Micronodular surface

Figure 4 Macroscopic appearance of control and cirrhotic wistar rat livers. (A) Normal external smooth livers of rats injected with saline. (B) External surface of livers of rats injected with thioacetamide for 8 weeks and treated with high dose of extract to induced liver damage and mild Micronodular surface of the Liver

EXTRACT	OBSERVED CHANGES / MORTALITY	
DOSAGE (mg/kg)	PHASE I	PHASE II
10	0/3	-
100	0/3	-
1000	0/3	-
1600	-	0/1
2900	-	0/1
5000	-	0/1

Table 4 Macroscopic and Microscopic features of Thioacetamide induced in liver of wistar rats administered with low, moderate and high dose of Methanolic Extract of Leaf of *Maerua angolensis*.

Experimental Groups of Rats	Macroscopy (Liver)	Microscopy (Central vein)	Microscopy (Portal triad)	Microscopy (Hepatocytes)
Negative control	Smooth surface	Normal	Normal	Normal
Positive control	Micronodular surface	Marked portal to central fibrosis (i.e. Porto central)	Marked portal to central fibrosis (i.e.Porto central)	Ballooning degeneration with areas of macro and micro vesicular steatosis.
Low dose	Micronodular surface	Marked portal to central fibrosis (i.e. Porto central)	portal-central Fibrosis	regeneration and focal areas of ballooning degeneration.
Moderate dose	Micronodular surface	Marked portal to central fibrosis (i.e. Porto central)	portal-central Fibrosis	regeneration and focal areas of ballooning degeneration.
High dose	Micronodular surface	Marked portal to central fibrosis (i.e. Porto central)	portal-central Fibrosis	regeneration and focal areas of ballooning degeneration.

Table 5 Food and water intake of induced Thioacetamide induced liver cirrhosis and Therapeutic potentials of methanolic leaf extract of *Maerua angolensis* on liver of wistar rats

Parameters	Control	Thioacetamide	Low dose	Medium dose	High dose	Test statistic and p value
Food intake	14.42±1.66	8.16±1.26	8.55±0.919	7.05±2.616	5.16±0.472	F=5.22 P=0.049 Df=4,5
water intake	11.54±1.26	8.26±1.10	7.35±1.202	6.7±0.989	8.93±1.19	

*P= Analysis of variance.

Table 6: Effect of weight on Thioacetamide induced liver cirrhosis in wistar rats.

	Control	Thioacetamide	Low Dose	Medium Dose	High Dose	Test Statistic And P Value
Week 1	133.32±11.29	145.48±7.71	145.3±43.69	144.3±29.27	138.9±15.10	F=35.71 P=0.0009
Week 2	132.78±11.99	145.78±7.430	142.95±42.77	144.15±31.60	137.63±14.653	
Week 4	133.48±10.08	141.98±6.71	142.2±44.40	142.6±28.14	136.1±13.19	
Week 6	133.76±10.368	140.72±6.803	141.3±41.43	140.9±29.83	134.36±14.79	
Week 8	134.22±9.401	138.9±5.468	138.75±41.79	138.4±28.99	132.6±13.79	
Week 10	134.9±8.602	138.34±5.849	138.8±40.58	138.4±30.97	133.3±11.22	

*P= Analysis of variance.

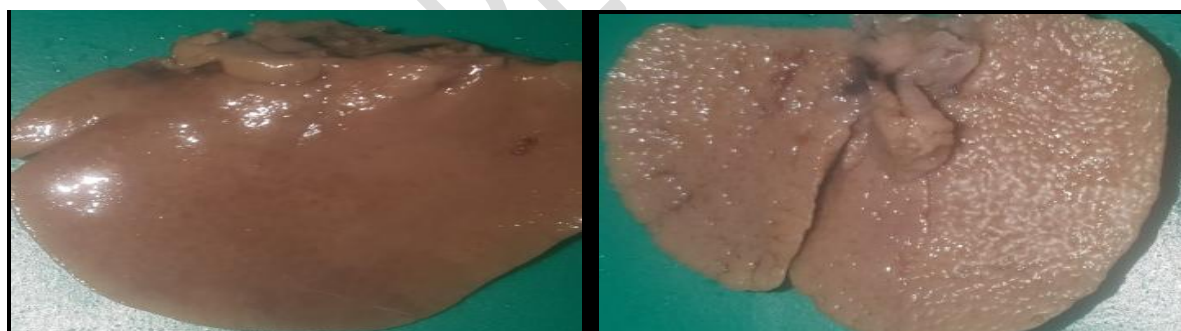


Figure 1 GROUP 1 A (Control) administered with normal saline and GROUP 2 B (Induction of cirrhosis) administered with TTA + No Treatment



Figure 2 GROUP 1 A (Control) administered with normal saline and GROUP 3 B (Induction of cirrhosis + Treatment [Extract]) administered with TTA + Low Dose of the Methanolic leaf extract of *Maerua angolensis*)

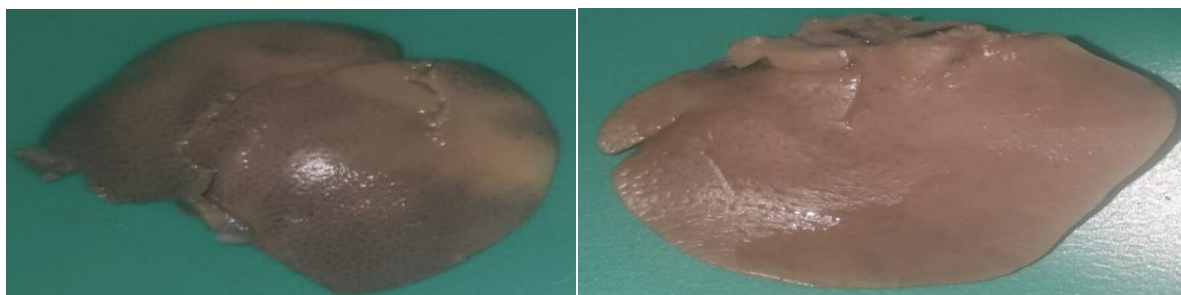


Figure 3 GROUP 1 A (Control) administered with normal saline and GROUP 4 B (Induction of cirrhosis + Treatment (extract) administered with TTA + Moderate dose of the Methanolic leaf extract of *Maerua angolensis*)

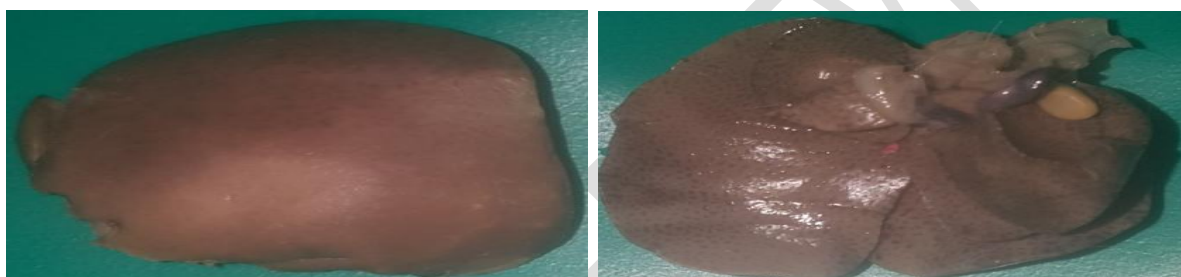


Figure 4 GROUP 1 A (Control) administered with normal saline and GROUP 5 B (Induction of cirrhosis + Treatment [Extract]) administered with TTA + High dose of the Methanolic leaf extract of *Maerua angolensis*)

5.1 DISCUSSION

The methanolic extraction of the leaves of *Maerua angolensis* procedure produced a percentage yield of 10.8g% . The extract was greenish brown in colour,.

There was no mortality observed in mice after oral administration of the methanol extract of *Maerua angolensis* leaf extract even at doses as high as 5000 mg/kg signifying that the oral LD50 was more than 5 000 mg/kg which is in line with median lethal dose gotten by Talluri., *et al* [17]. Thus the experimental doses used (100, 200 and 400 mg/kg) were within safe margin. However the Median lethal dose obtained differs from the median lethal dose observed by 3500mg/kg and 3800mg/kg gotten by Malami *et al.* [18], from the extraction of the stem bark which

indicates more safety of the leaf extract than the stem bark.

Livers of rats treated with thioacetamide and later with the various doses of *Maerua angolensis* leaf extract showed significant restoration at 100mg/kg, 200mg/kg and 400mg/kg . There was histological regeneration of the nodules compared to those rats treated with thioacetamide only. They showed a reduced extent and development of fibrous septa. It could be concluded by the above results that the leaf extract of *Maerua angolensis* leaf extracts have the capacity in reduce the damage caused by injury in the liver.

Loss of appetite is one of the symptoms of cirrhosis and is measured by reduction in food intake. It is currently attributed to the presence of cytokines such as tumor

necrosis factor α (TNF- α) (Bémeur *et al.*, [19]. The majority of cirrhotic patients unintentionally follow a low caloric diet, a fact that is attributed to various side-effects observed in cirrhosis. Early satiety due to impaired gastric accommodation and impaired expansion capacity of the stomach due to the presence of ascites often leads to an inadequate nutrient intake Tsiaousi *et al.*, [20]. Thioacetamide induced cirrhosis had similar effect on food intake. The rats had a significantly low food and water intake of medium dose group and high dose group compared to normal controls. However, Animals receiving the extract showed no improvement in both food and water intake which is probably due to the time frame designed for this experiment.

Weight loss is experienced during any type of liver disease independent of age and sex (Anastácio *et al.*, [21]. In this study, there is no statistically significant difference between the mean weight of rats administered with thioacetamide and normal rats.

6.1 CONCLUSION

The results of acute toxicity studies showed LD₅₀ to be greater than 5000mg/kg body weight. The entire groups induced with thioacetamide shows micronodular surface of the liver with significant improvement in the treated groups Food and water intake of each group was not significantly different from each when compared with control group there was micronodular appearance on the surface of the induced groups which gradually decrease with treatment with extract.

Ethical Approval:

As per international standard or university standard ethical approval has been collected and preserved by the authors.

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