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Comparative Study of the Effect of Methanol Fruit Extracts of Morinda Citrifolia and Morinda lucida on the liver of Wistar Albino Rats.

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Abstract:- Crude herbal preparations of different Morinda plant species had been used in the treatment of ailments in Nigerian folk medicine with little or no attention directed to their toxicities. The aim of this study is to compare the effect of methanol fruit extracts of Morinda citrifolia (MCE) and Morinda lucida (MLE) on the liver. The active components in the fruits of these Morinda plant species were extracted with 90% methanol. Medium lethal doses of the extracts (LD_{50}) were investigated using Lorke method. The extracts were administered to groups of rats (groups 1- 7) at the doses of 100, 200, and 400mg/kg b.w respectively by intubation, while group 1 received normal saline (control). Test and control animals were fed with normal rats' feed and water. Two rats from each group were sacrificed every 7 days to collect blood for liver function tests using automatic analyzer Mind ray (BS - 120) Chemistry Analyzer. Also, the livers of rats were excised for histopathology using eosin and haematoxylin method of staining. The median lethal dose (LD_{50}), revealed no death of mice even at 5000mg/kg b.w for both species of Morinda indicating that MCE and MLE were relatively safe. The alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) activity decreased non-significantly ($p>0.05$) in rats fed with both extracts showing that they could not have caused liver injury. Also there was non- significant decrease ($p>0.05$) in the total and conjugated bilirubin concentrations in the rats fed with both extracts when compare with the control rats. These results were supported with the histopathological results of the rats fed with both MCE and MLE which showed no significant difference ($p>0.05$) in the livers of test rats when compared with that of the control rats. The results from this study could suggest that MCE and MLE have no toxic effect on the liver. This could support the use of MCE and MLE in Nigeria folk medicine.

Keywords:- *Histopathological, Injury, Median lethal dose.*

I. INTRODUCTION

Plants parts contain bioactive substance that can be extracted used as medications. These plants are known as medicinal plants. They are administered to man in order to promote health and for the treatment of various diseases. Many people considered medicinal plants to be relaively safe, cheaper and more potent than synthetic drugs in disease management. Immense benefits have been derived by man from using medicinal herbs in disease management (Oyewole and Akigbala, 2011).

Morinda is one of these medicinal plants. It is in a genus of flowering plants in the madder family, Rubiaceae. Its generic name is derived from the Latin words morus "mulberry", from the appearance of the fruits, and indica, meaning "of India". There are many species of Morinda trees although the species of interest in this work are Morinda citrifolia and Morinda lucida. Morinda citrifolia Linn Rubiaceae is one of the most important traditional Polynesian medicinal plant commercially known as Noni, cheese fruit and indigenously found in open coast region at sea level and in forest areas about 1,300 feet above sea level.

It is a small tropical evergreen shrub or tree, three to twelve meters high. It has straight trunk, large green leaves and yellow fruit. The size of the fruit is about 12cm. It has a foul taste and a soapy smell when ripe. The fruit of this plant has been used as food, drink, medicine, colourful dye, cosmetics purpose and has a high demand in medicines for different kinds of illnesses for example, diabetes, high blood pressure, AIDS, arthritis, cancer, gastric ulcer, sprains, mental depression, senility, poor digestion, atherosclerosis, blood vessel problem etc., (Kochuthressia and Jaseentha, 2015). Then, M. lucida is also an evergreen shrub or small to medium-sized tree bearing a dense crown of slender, crooked branches. It can grow from 2.4 - 18 metres tall. The branches are often crooked or gnarled and it is a multipurpose species yield dyes, timber, fuel and traditional medicines.

The liver is the largest organ in the body. Its gross anatomical divisions comprise the right, left, caudate and quadrate lobe (Ellis, 2011). The liver performs different kinds of biochemical, synthetic and excretory functions. Examples of the functions includes bilirubin metabolism, porphyrin metabolism, bile acid metabolism, amino acid and protein metabolism carbohydrate metabolism, lipid and lipoprotein metabolism, Hormone metabolism, vitamin metabolism, biotransformation and detoxification function, alcohol degradation etc (Ellis, 2011). No single biochemical test can detect the global functions of the liver (Thapa and Walia, 2007). "Liver function tests" are used to detect and manage liver diseases although they are of little value in assessing the liver function per se. The various uses of liver function tests include screening, checking disease pattern, assess disease severity and for follow up (Thapa and Walia, 2007). The aminotransferases and AP are markers of hepatocyte injury while total bilirubin is the tests of liver metabolism (Tinsay *et al.*, 2014).

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Alanine Aminotransferases (ALT) and Aspartate Aminotransferases (AST) participate in gluconeogenesis by catalyzing the transfer of amino groups from aspartic acid or alanine to ketoglutaric acid to produce oxaloacetic acid and pyruvic acid respectively. Aspartate Aminotransferases (AST) is present in cytosolic and mitochondrial isoenzymes and is found in the liver, cardiac muscle, skeletal muscle, kidneys, brain, pancreas, lungs, leucocytes, and red cells. It is less sensitive and specific for the liver. Alanine Aminotransferases (ALT), a cytosolic enzyme is found in its highest concentrations in the liver and is more specific to the liver. Hepatocellular injury triggers the release of these enzymes into the circulation. Serum increase in aminotransferases concentration is triggered by alcohol and medication (Robert and Thomas, 2011). Very high levels should prompt further evaluation without delay. Alkaline phosphatase is a hydrolytic enzyme which acts optimally at alkaline pH. It is formed in the liver and it's existed in almost all tissues of the body. Under some conditions, such as gestation, budding children, high alkaline phosphatase activities are normal physiological phenomenon. Pathologic high alkaline phosphatase activity may exist in hepatobilliary diseases, bone diseases, bone metastases and hyperthyroidism (Limdi and Hyde, 2003). Decrease activity occurs uncommonly and is observed in about 0.2% old people.

The concentration of bilirubin in the serum is determined by the balance between bilirubin production and clearance by hepatocytes. Elevated serum bilirubin levels may be due to excessive bilirubin production, which occurs in states of increased red blood cell turnover, such as hemolytic anemias or hematoma resorption; impaired uptake, conjugation, or excretion of bilirubin; and release of unconjugated or conjugated bilirubin from injured hepatocytes or bile ducts (Longo, et al., 2010). Almost all of serum bilirubin in healthy persons is unconjugated. Furthermore, the fraction of conjugated bilirubin that is covalently bound to albumin (Weiss, *et al.* 1983); (Limdi and Hyde, 2003) increases in patients with cholestasis and hepatobiliary disorders, when the excretion of conjugated bilirubin is impaired, resulting in increased serum concentration of conjugated bilirubin. This explains the prolonged elevation in bilirubin seen in patients recovering from hepatobiliary injury (Tinsay et al., 2014).

Thus, this work will compare the effects of methanol fruit extracts of *Morinda citrifolia* and *Morinda lucida* on the liver to determine the specie that is toxic or preferable in traditional medicine.

II. PLANT MATERIALS SAMPLE (MORINDACITRIFOLIA)

The *M. citrifolia* and *M. lucida* fruits were obtained from Hezekiah Dike Street, Obinagu, Awka and Nnamdi Azikiwe University Awka plantation. These plants were identified in the Botany laboratory of Nnamdi Azikiwe University. A voucher specimen was deposited in the herbarium of the University and was authenticate by Dr. B. O. Aziagba with the herbarium numbers, page 14, cabinet 2 and shelf 35. The fruits were dried in an oven at 50^{o}C, soaked for 48 hours and extracted with 90% Lobachem methanol.

III. PREPARATION OF FRUIT EXTRACT

Morinda fruits were rinsed with distilled water and dried in an oven at 50^{o}C. The dried fruits were ground into powder with a manual blender. About four hundred and fifty grams (450 gms) of the dried fruit powder was macerated in 900ml of 99% Lobachem methanol, agitated for 5 minutes with an electric blender and left for 48 hours at room temperature. The mixture was pressed with a muslin cloth, filtered with Whatmann No.1 filter paper and then concentrated in an oven at 50^{o}C. Different concentrations of the extracts were then used to feed the animals.

Chemicals: All chemicals used were of analytical grade.

IV. METHODS

Acute toxicity was studied in two phases according to the method of Lorke (1983). Feed and water were given to the mice *ad libitum.*

The automatic analyzer Mind ray (BS - 120) Chemistry Analyzer, was used to determine the different concentrations of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total and unconjugated (direct) bilirubin.

Histopathology was done using eosin and haematoxylin method of staining by Baker and Silverton (1989).

V. STATISTICAL ANALYSIS

One-way analysis of variance (ANOVA) was used to analyze the research data using Statistical Packages for Social Sciences (SPSS) version 16. Group means obtained after each treatment were compared with control measurements and differences were considered significant when $p< 0.05$.

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VI. RESULTS

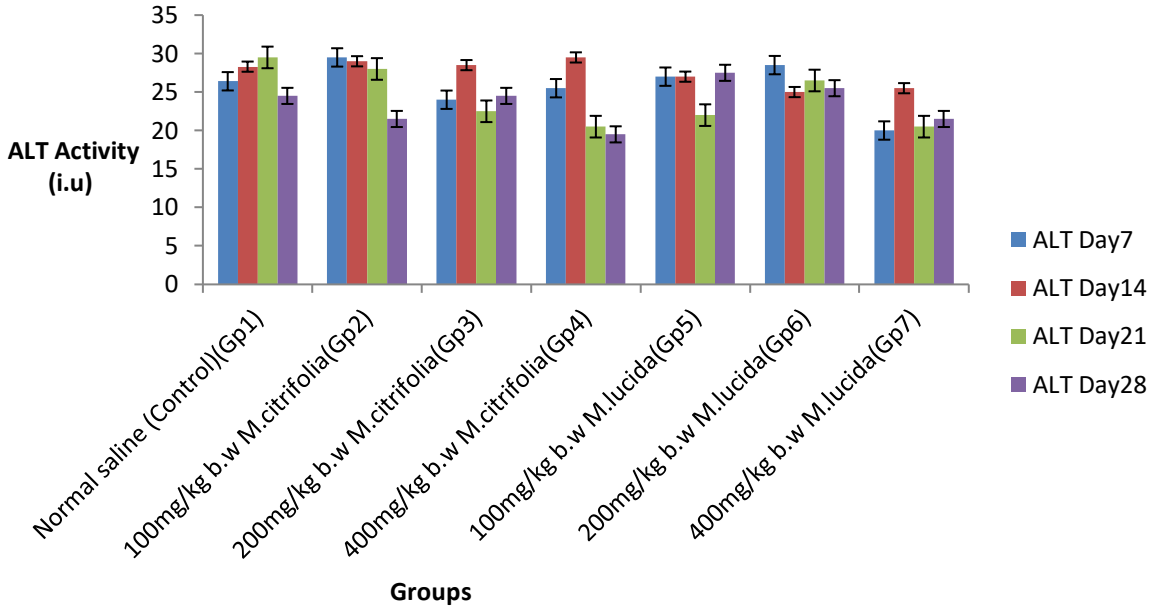

Fig 1:- showed the serum alanine aminotransferase (ALT) activity in rats fed with MCE and MLE at seven days intervals.

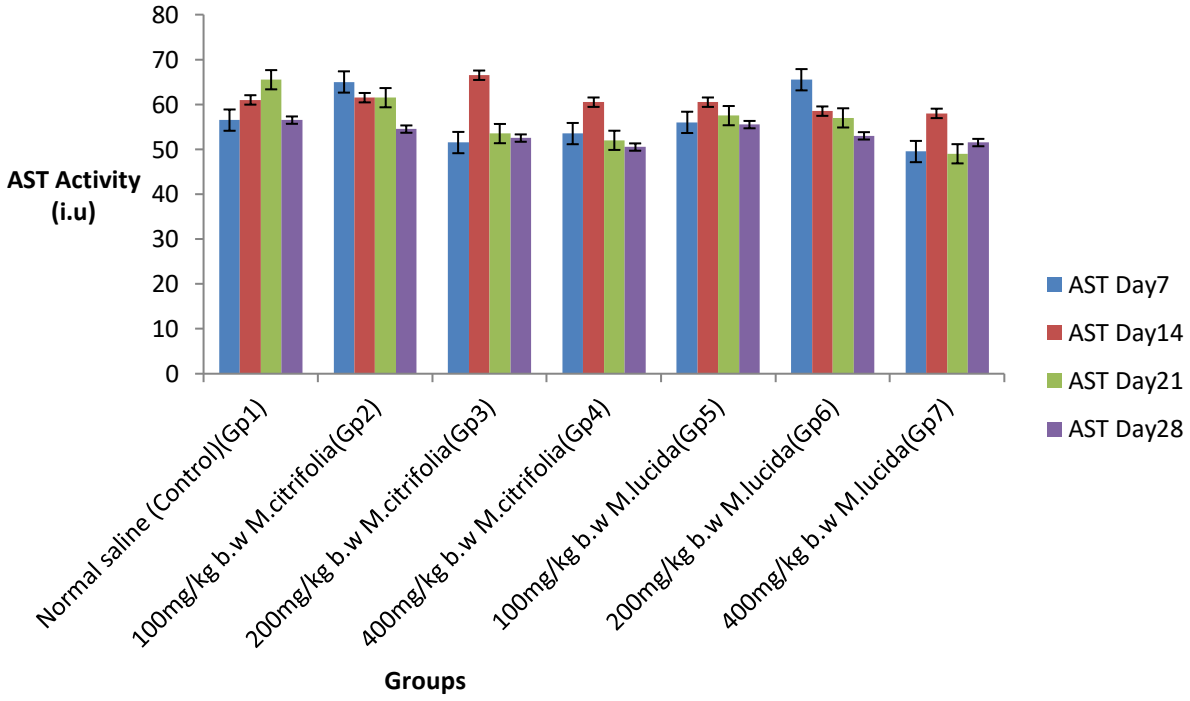

Fig 2:- showed the serum aspartate aminotransferase (AST) activity in rats fed with MCE and MLE at seven days intervals.

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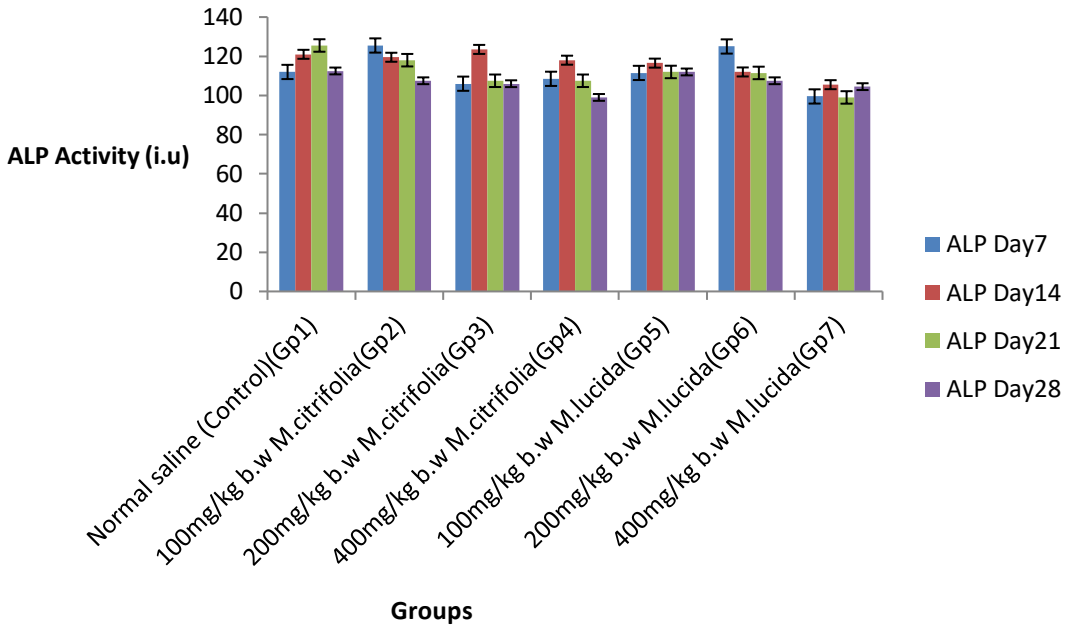

Fig. 3:- showed the serum alkaline phosphatase (ALP) activity in rats fed with MCE and MLE at seven days intervals.

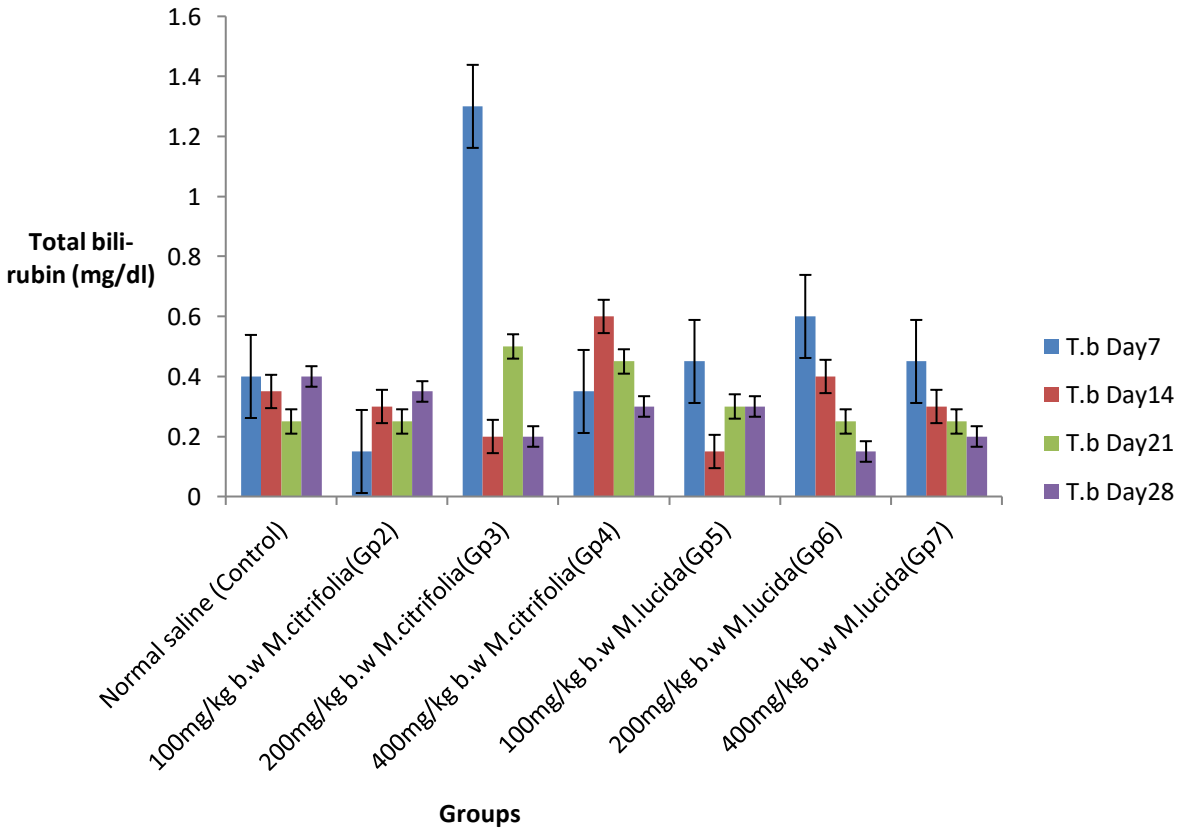

Fig. 4:- showed the serum total bilirubin concentrations in rats fed with MCE and MLE at seven days intervals.

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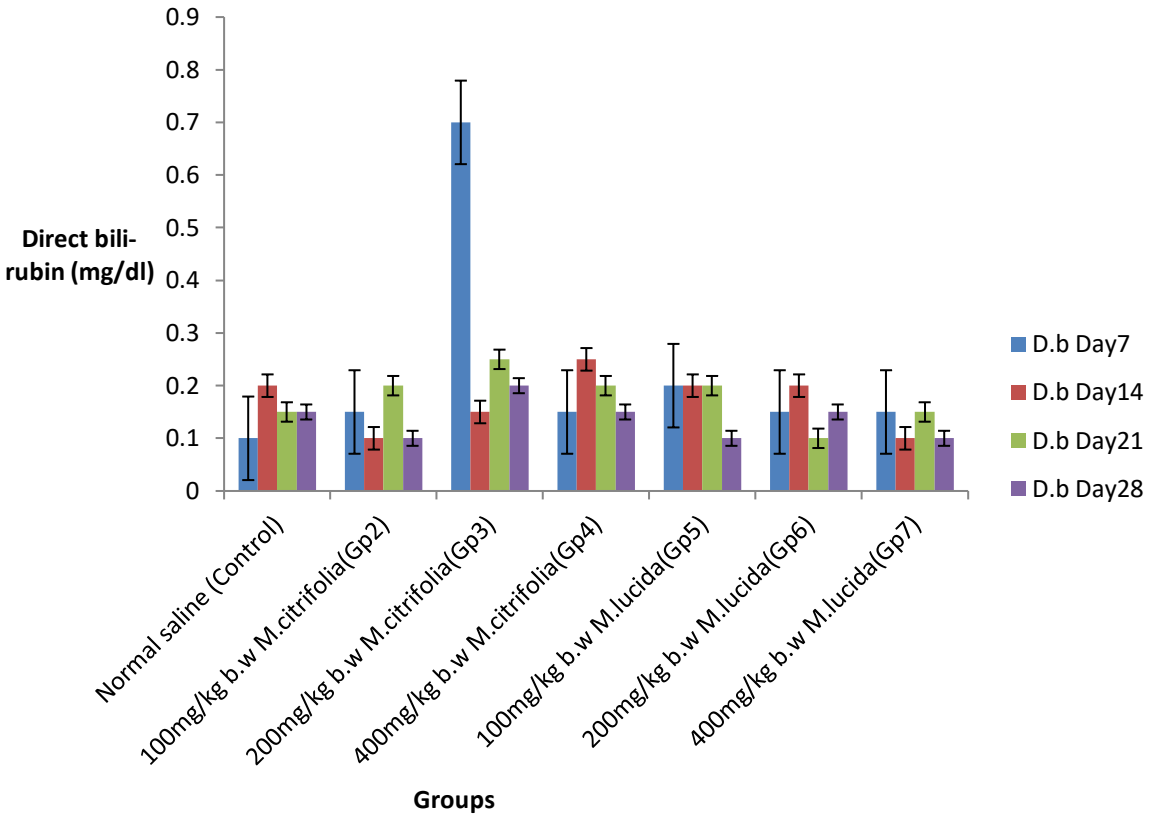

Fig. 5:- showed the serum direct bilirubin concentrations in rats fed with MCE and MLE at seven days intervals.

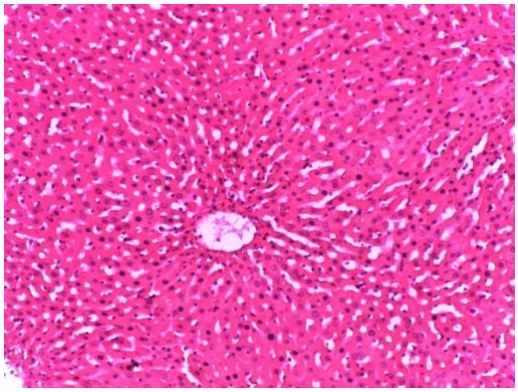

Plate 1 represents the liver of albino rats fed with normal saline (group 1) showing tissue with normal tissue disposition.

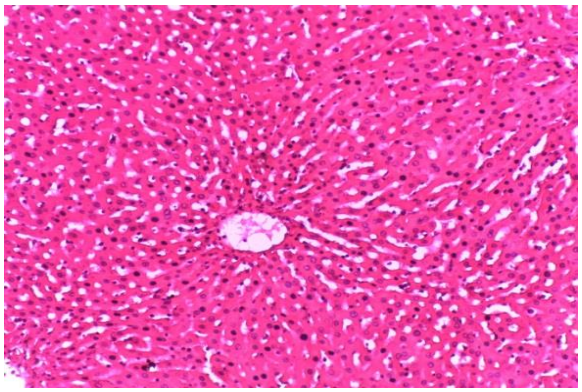

Plate 3 represents the liver of albino rats fed with MCE at the 28th day of extracts administration showing tissue with normal tissue disposition.

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Plate 4 represents the liver of albino rats fed with MLE at the 28th day of extracts administration showing tissues with normal tissue disposition.

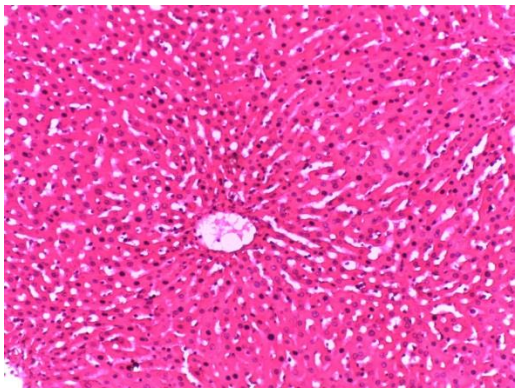

Fig. 6:- showed the histopathology results of liver of albino rat fed with normal saline and different doses of MCE and MLE.

VII. DISCUSSION AND RESULTS

The acute toxicity test of methanol extracts of MCE and MLE recorded no sign of toxicity or death of mice even at the dose of 5000mg/kg body weight for both species indicating that both extracts could be relatively safe (Lorke, 1983).

The result of liver function test (LFT) showed non-significant decrease ($p>0.05$) in the serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) activity in the rats fed with graded concentrations of MCE and MLE when compared with the control rats fed with normal saline. The reduction in this case may indicate that the extracts impaired the over-production of these enzymes. The lowering effect of these extracts on liver enzymes (ALT, AST and ALP) might imply that MCE and MLE could not have caused liver injury, (Kim *et al.*, 2008) also this supported the claims that *Morinda* fruit extract was used in the treatment of carbon tetrachloride (CCL_4) induced hepatic injury (Adejo *et al.*, 2014). The total and direct bilirubin level was non-significantly lower ($p>0.05$) in rats treated with MCE and MLE when compared with the control rats treated with normal saline.This could indicate that the conjugating ability of the liver was not compromised from the total and direct (conjugated) bilirubin concentrations (Muchova *et al.*, 2011). The synthetic ability of the liver could also be maintained. This result supported the report that the serum bilirubin (conjugated and total) concentrations that were raised by CCl_4-induced liver damage was lowered by MLE showing the hepatoprotective and ameliorative potency of the two extracts (Adejo *et al.*, 2014).

All the photomicrographs in the histological results revealed no pathological changes on the liver. This confirmed the LFT results which indicated that MCE and MLE could not have caused harm to the liver.

VIII. CONCLUSION

Acute toxicity (LD_{50}) test of Morinda citrifolia and Morinda lucida extracts indicated that the plants were relatively safe.

Both MCE and MLE reduced liver enzymes; implying that they could not cause liver damage. Morinda citrifolia and Morinda lucida extracts also lowered the total and conjugated bilirubin level. This could imply that both extracts could not have affected the liver adversely. Also the histopathology results revealed no harmful effects to the liver.

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