

Comparative Hepato-therapeutic Effect of Aqueous and Methanol Leaf extracts of *Vitex doniana* and *Bombax buonopense* in CCl₄ Induced Liver Damage in Male Albino Rats.

O. L. Nweke¹, N. Nwachukwu³ and P. M. Aja² and K. N. Agbafor²

¹ Medical Biochemistry Department Ebonyi State University, P.M.B. 053 Abakaliki, Nigeria.

² Biochemistry Department, Ebonyi State University, P.M.B. 053 Abakaliki, Nigeria.

³ Biochemistry Department, Federal University Technology, P.M.B. 1526 Owerri, Nigeria.

Abstract: Comparative hepato-therapeutic effect of aqueous and methanol leaf extracts of *Vitex doniana* and *Bombax buonopense* in carbon tetrachloride (CCl₄) liver damage in rats were carried out using 114 albino rats. Group A with 6 rats was used as the normal control and it received normal saline and rat feed. Group B to F were given a single dose of 2.5ml/kg of CCl₄ in olive oil (1:1 v/v) via intra-peritoneal cavity before treatment. Group B with 6 rats were given the CCl₄ without treatment and served as negative control. Group C received standard drug (Liv.52) daily for 7 days at the dose of 4ml/kg and served as positive control. Group D and E received aqueous and methanol extract of *Vitex doniana* while groups F and G received aqueous and methanol extract of *Bombax buonopense*. Group D, E, F and G were sub-divided into 4 different groups with 6 rats each by random design method and treated with the extract for 7 days at the doses of 100, 200, 400 and 800 mg/kg body weight. The liver function indices were determined by standard methods. In acute toxicity study no death was recorded even at 1600mg/kg doses but LD₅₀ for the two leaf extracts were recorded at 2000 and 3000mg/kg body weight for *Vitex doniana* and *Bombax buonopense* respectively. The percentage yields of the aqueous and methanol extracts of *Vitex doniana* and *Bombax buonopense* leaves gave 13.57, 19.96, 13.74 and 14.02 % respectively. The result showed significant ($p < 0.05$) reductions in a dose dependent manner in the levels of ALT, AST, ALP, 5'-nucleotidase and bilirubin in rats treated with aqueous and methanol leaf extracts of *Vitex doniana* and *Bombax buonopense*. Significant ($p < 0.05$) increase were observed in albumin, globulin and total protein levels in rats treated with the same extracts. The result of histological analysis revealed that, the liver of rats that received CCl₄ without treatment were highly distorted when compared with those that treated with the extracts. This indicates that the extracts have the potentials to reverse the damaged caused by the administration of CCl₄ in rats as observed in the result.

Keywords: Carbon tetrachloride (CCl₄), Hepato-therapeutic, histology, function indices, *Vitex doniana* and *Bombax buonopense*

I. Introduction

Plant derived substances have recently become of great interest owing to their versatile applications (Baris et al., 2006). Medicinal plants are rich bio-resources of drugs (Hammer et al., 1999). A number of interesting outcomes have been found with the use of a mixture of natural products or plant extracts to treat diseases (Ghosh et al., 2007). The biological evaluation of plants extract is vital to ensure their efficacy and safety. These factors are important if plant extracts are to be accepted as valid medicinal agents for the treatment of infectious diseases (Tanaka et al., 2006).

Vitex doniana belongs to the family of Verbenaceae. The plant is commonly called black plum while is locally known as 'Uchakiri' in Igbo, 'Dinya' in Hausa, 'Oori-nla' in Yoruba, 'Tinya' in Fulani, 'Vabga' in Ghana, 'Mfuru' in Tanzanian, 'Muhonozi' in Uganda (Atawodi et al., 2003). This plant is a shrub which grows into a tree in open wood land and Savannah regions of tropical Africa. This tree grows up to 8-18m height with buttress root that can spread up to 5m. The bark is rough pale brown or greyish white. The leaves are compound and it has five leaflets which is 6-14 cm long (Atawodi et al., 2003). In Nigeria, from information available from the indigenous traditional healers, a decoction of the chopped stem bark part of *Vitex doniana* is prepared and taken orally for treatment of gastroenteritis (Atawodi et al., 2005). The root and leaf are used for treatment of nausea, colic and in epilepsy (Bouquet et al., 1971; Iwu, 1993). Stem bark has anti-hypertensive effect (Olusola et al., 1997). Also stem bark has anti-microbial activity (Atawodi 2005). Bark extract is used in the management of psychiatric cases (Bouquet et al., 1971). It is also used to control bleeding after child birth. Hot aqueous leaf extract is used in the treatment of stomach and rheumatic pains, inflammatory disorder and dysentery (Irvine, 1961; Etta, 1984). *Vitex doniana* is also taken to improve fertility and the juice may be squeezed into the eyes to treat eye troubles. The roots and leave are used for nausea. Chemical constituents of the plant include glycosides, flavonoids, alkaloids and essential fatty acid (Arokiyari et al., 2009).

Bombax buonopense is a large tree that grows to 40m height with large buttress roots that can spread up to 6m. The bark is covered with large conical spines, especially when young, and sheds them with age to some degree. The branches are arranged in whorls; the leaves are compound and have 5 to 6 leaflets and 5 to 25 secondary veins. The individual leaflets have entire margins and are also large. The underside of the leaflets may be glabrous or puberulous (Beentje and Smith, 2001). *Bombax buonopense* is widely distributed in Africa. The plant belong to the family of Malvaceae and its Common vernacular name include 'Vabga' in Ghana, 'Kurya' in Hausa, 'Akpu' in Igbo and 'Ogbolo' in Yoruba. Traditional patent dealers in Nigeria prepare their concoctions with plant parts. This practice remains a public health problem of enormous magnitude affecting over 500 million people yearly (Atawodi et al., 2003).

There is paucity of documented information on the hepato-therapeutic effect of these local medicinal plants. Since there is high cost of available drugs for the treatment of liver damage /liver related diseases, therefore result of this research work would contribute immensely in solving the health problem in both developed and developing countries of the world, hence the global research has turned toward the use of medicinal plants with little or no side effect in the prevention/treatment of diseases/ailments. This study therefore evaluated the comparative hepato-therapeutic effect of *Vitex doniana* and *Bombax buonopense* leaves on toxic effect of CCl₄ in albino rats.

II. Materials and Methods

Collection of *Vitex doniana* and *Bombax buonopense* Leaves

This study was conducted in March, 2014 in Biochemistry Department, Ebonyi State University, Abakaliki. The leaf of *Vitex doniana* and *Bombax buonopense* were collected from Abakaliki in Abakaliki Local Government Area of Ebonyi State, Nigeria. The plants were identified and authenticated by a taxonomist in the Department of Applied Biology, Ebonyi State University, Abakaliki.

Preparation of *Vitex doniana* and *Bombax buonopense* leaves in powdered form: Fresh leaves of *Vitex donian* and *Bombax buonopense* were collected, washed and dried at ambient temperature (25°C) and the leaves were turned severally to avoid fungi growth. After drying, the leaves were pulverized using electric blender. The leaf powders were stored in refrigerator in well labelled and air tight container prior for the extraction.

Preparation of *Vitex doniana* and *Bombax buonopense* leaves Extracts: Exactly 100g of powdered leaves of *Vitex doniana* and *Bombax buonopense* were weighed and soaked 1500 ml into conical flasks and allowed for 24 hours. After 24 hours of soaking the aqueous extracts were obtained by filtering the mixtures repeatedly with muslin cloth to obtain the filtrates and residues. The aqueous extracts were gotten by subjecting the filtrates into rotary evaporator. The same steps were taken to obtain the methanol extracts. The extracts were kept in sealed containers and stored in refrigerator at 2-4°C until required.

Experimental Animals: A total of 114 male albino rats weighing (200-300g) were used. They were acclimatized for two weeks and maintained at normal room temperature (25°C) in the animal house of Department of Biochemistry, Ebonyi state university, Abakaliki. Before starting the experiment all the animal were weighed weekly. They were kept in cages, fed on commercial rats feed and allowed free access to clean water.

III. Animal grouping and treatment:

Acute Toxicity Test:

Acute toxicity of aqueous and methanol extracts of *Vitex doniana* and *Bombax buonopense* leaves were carried out according to the method of Lorke, (1983).

Sub-Acute Toxicity Test:

In this study, 114 male rats were randomly assigned into 7 groups (i.e. Group A, B, C, D, E, F and G). Group A with 6 rats was used as the control and it received normal saline (vehicle) and normal rat feed. Group B to F were given a single dose of 2.5ml/kg of CCl₄ in olive oil (1:1 v/v) via intra-peritoneal cavity before treatment. Group B with 6 rats were given the CCl₄ without treatment and served as positive control. Group C received standard drug (Liv.52) daily for 7 days at the concentration of 4ml/kg. Group D, E, F and G received aqueous and methanol extract of *Vitex doniana* and *Bombax buonopense*. Group D, E, F and G were subdivided into 4 different groups with 6 rats each by random design method and treated with the extract for 7 days at the doses of 100, 200, 400 and 800 mg/kg body weight. The rats were starved for 24 hrs and then sacrificed for analysis. Also, the animals were weighed on the first day and on the last day of the study.

Collection of samples from animals: After the treatment period, the rats were anaesthetized using chloroform and dissected using dissecting tools. The bloods were drawn directly from the heart using syringe. Blood was stored in bottles without anticoagulant. The blood specimens were centrifuged at 3000rpm for 10 min to

separate the serum from the red cells. After centrifugation, the serum were separated for biochemical assays and stored in specimen bottles ready for analysis.

Biochemical Analysis: The method of (Ghosh et al., 2007) was used to determine the level of AST, ALP and ALT. Raymond and David, 1980; Reinhold, 1953; Malloy and Evelyn, 1937 were used to determine the level of 5'-nucleotidase, total protein and total bilirubin respectively. The livers of the rats were also dissected out, fixed in 10% normal saline, dehydrated using different grades of absolute alcohol ranging from 50%, 70% and 90% at 30mins interval. The tissue was de-alcoholized with xylene, impregnated with wax in a hot ovum at temperature of 600C, the impregnated tissue was embedded with molten paraffin and allowed to solidify, the block of tissue was trimmed using rotary microtom at 10 micron to obtain the cutting surface of the tissue, and the sectioned tissue was dried in a hot plate and allowed to cool. The tissue was stained with heamatoxylin and Eosin method of staining. The photomicrographs were observed under the microscope with magnifications of X400.

Statistical Analysis: Data obtained were subjected to one way analysis of variance (ANOVA). Means were compared for significance using Duncan's multiple range test ($p < 0.05$)

IV. Results

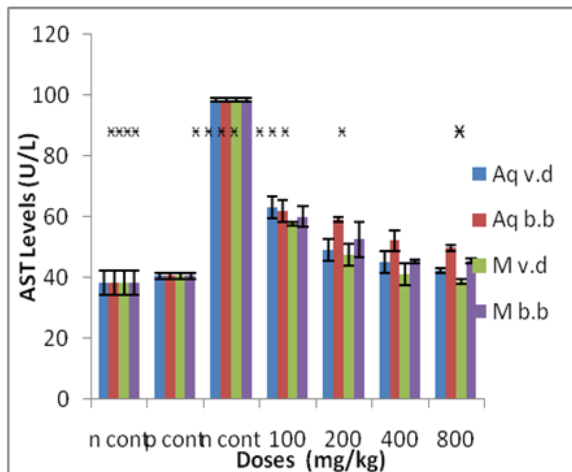


Figure 1: AST Level in CCl_4 induced Liver damage in Albino Rats treated with aqueous and methanol extracts of *Vitex doniana* and *Bombax buonopense* leaf.

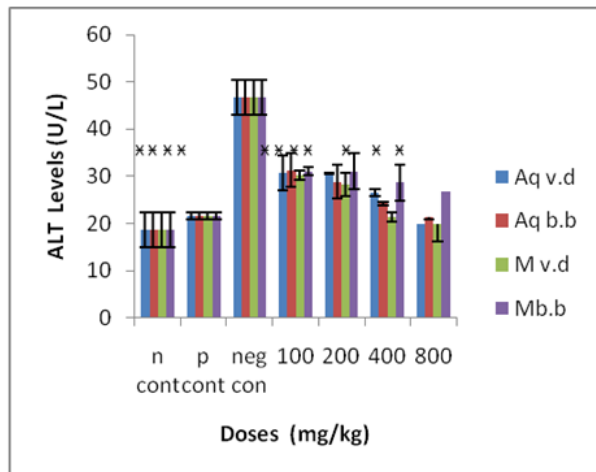


Figure 2: ALT Level in CCl_4 induced Liver damage in Albino Rats treated with aqueous and methanol extracts of *Vitex doniana* and *Bombax buonopense* leaf.

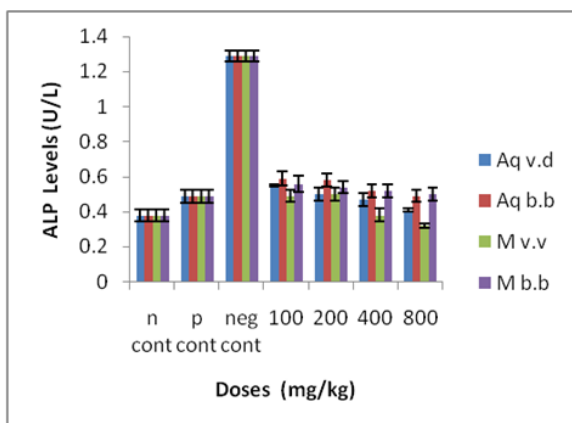


Figure3: ALPL Level in CCl_4 induced Liver damage in Albino Rats treated with aqueous and methanol extracts of *Vitex doniana* and *Bombax buonopense* leaf.

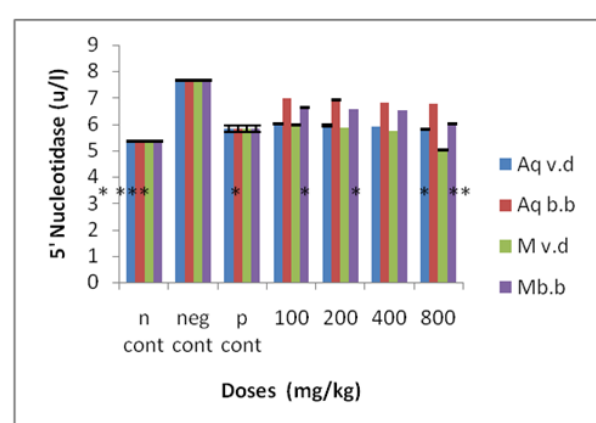


Figure 4: 5' Nucleotidase Level in CCl_4 induced Liver damage in Albino Rats treated with aqueous and methanol extracts of *Vitex doniana* and *Bombax buonopense* leaf.

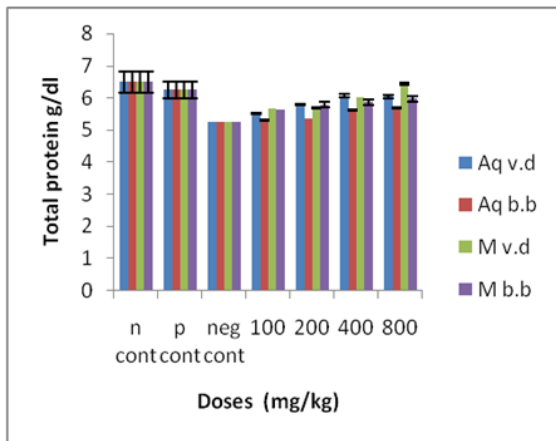


Figure 5: Total Protein Level in CCl₄ induced Liver damage in Albino Rats treated with aqueous and methanol extracts of *Vitex doniana* and *Bombax buonopense* leaf).

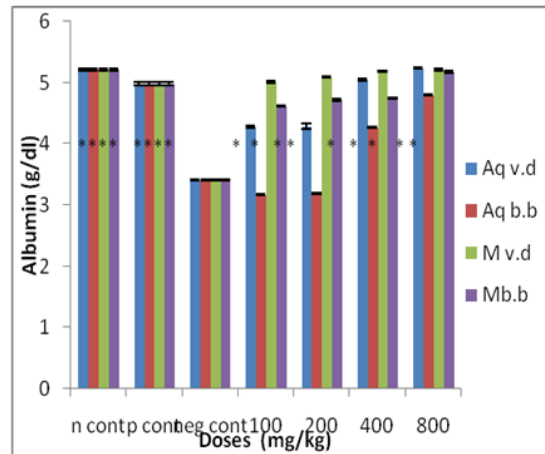


Figure 6: Albumin Level in CCl₄ induced Liver damage in Albino Rats treated with Aqueous and methanol extracts of *Vitex doniana* and *Bombax buonopense* leaf.

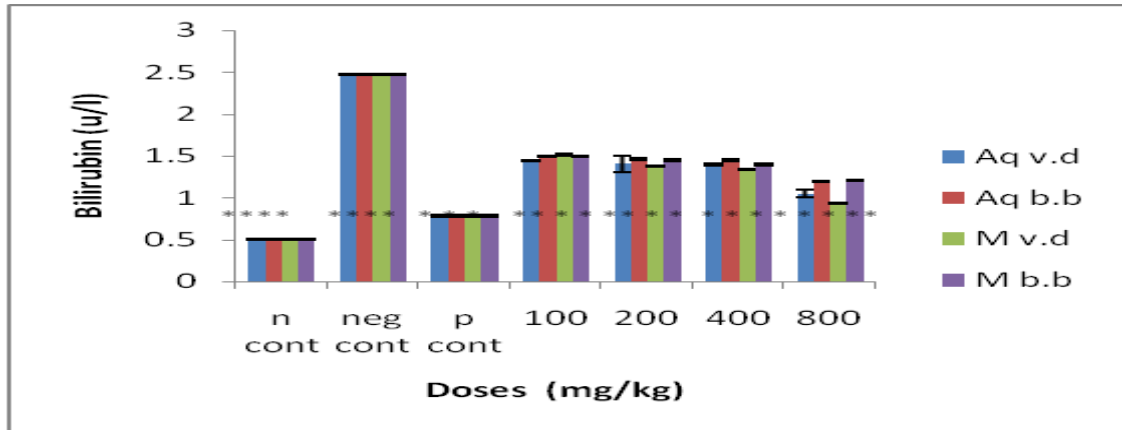


Figure 7: Bilirubin Level in CCl₄ induced Liver damage in Albino Rats treated with aqueous and methanol extracts of *Vitex doniana* and *Bombax buonopense* leaf.

Note: Data are shown as mean \pm S.D (n=6). Mean values in bars with (*) have significant differences ($p < 0.05$) when compared with normal control. Where N-control is normal control, p- control is positive control and neg cont is negative control (those induced without treatment).

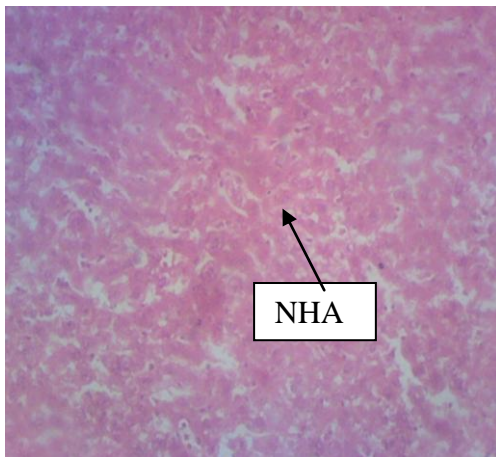


Plate 1: Photomicrogram of control rats liver stained with H/E showed normal hepatic architecture (NHA).

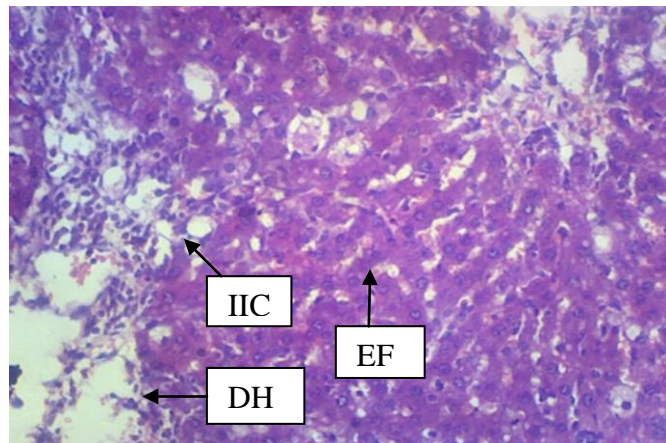


Plate 2: Photomicrogram of liver in CCl₄ induced liver damage in albino rats without treatment stained with H/E showed distortion of hepatic architecture (DHA), extensive fatty change (EFC), infiltration of inflammatory cell (IIC) around portal tract otherwise portal hepatitis.

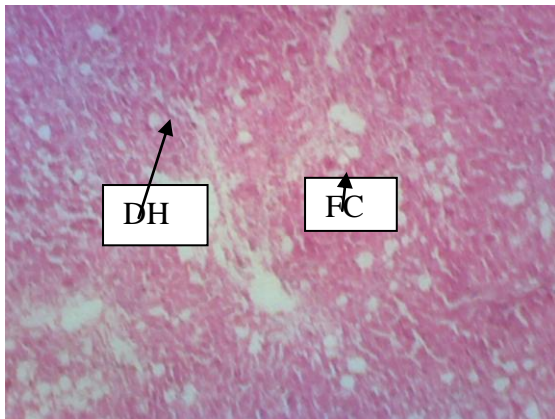


Plate 3: Photomicrogram of liver in CCl_4 induced liver damage in albino rats treated with standard drug. Stained with H/E showed distortion of hepatic architecture (DHA), mild fatty change (MFC).

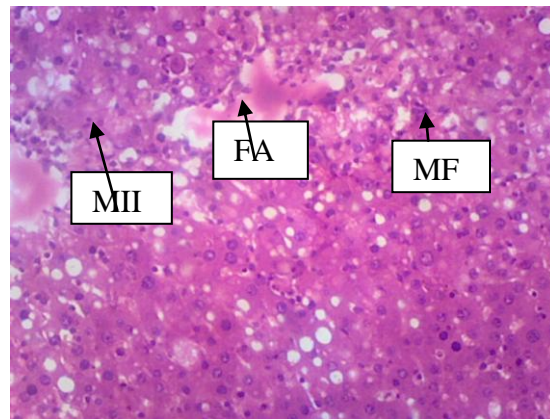


Plate 4: Photomicrogram of liver in CCl_4 induced liver damage in albino rats treated with 100 mg/kg of aqueous leaf extract of *Vitex doniana* stained with H/E showed focal area of necrosis (FAN), moderate fatty change (MFC), mild infiltration of inflammatory cells (MIIC).

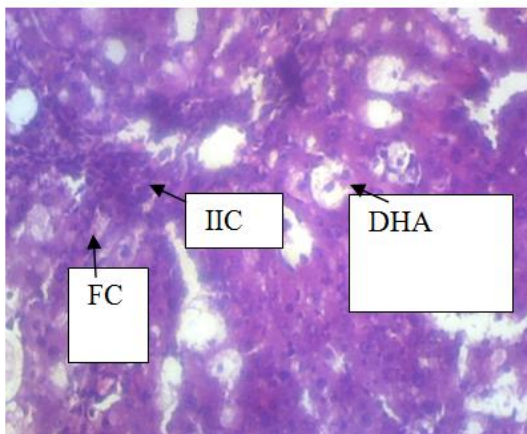


Plate 5: Photomicrogram of liver in CCl_4 induced liver damage in albino rats treated with 200 mg/kg of aqueous leaf extract of *Vitex doniana* stained with H/E showed fatty change (FC), infiltration of inflammatory cells at the portal tract (IIC), distortion of hepatic architecture (DHA).

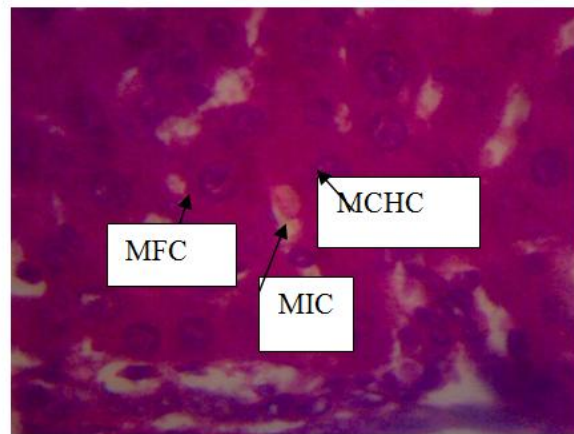


Plate 6: Photomicrogram of liver in CCl_4 induced liver damage in albino rats treated with 400 mg/kg of aqueous leaf extract of *Vitex doniana* stained with H/E showed moderate congestion of hepatic cells (MCHC), mild inflammatory cell (MIC), mild fatty change (MFC).

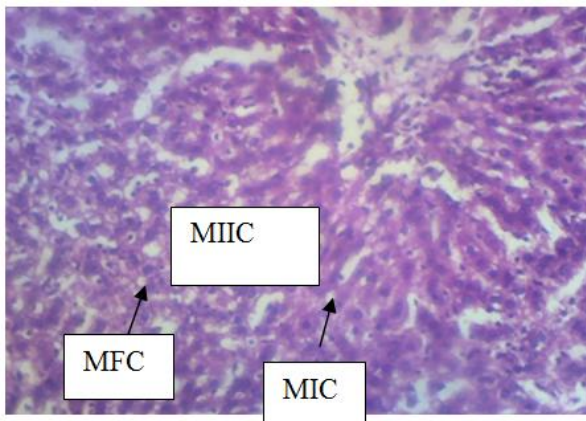


Plate 7: Photomicrogram of liver in CCl₄ induced liver damage in albino rats treated with 800 mg/kg of aqueous leaf extract of *Vitex doniana* stained with H/E showed moderate fatty change (MFC), mild inflammatory cells (MIC), mild infiltration of inflammatory cells (MIIC).

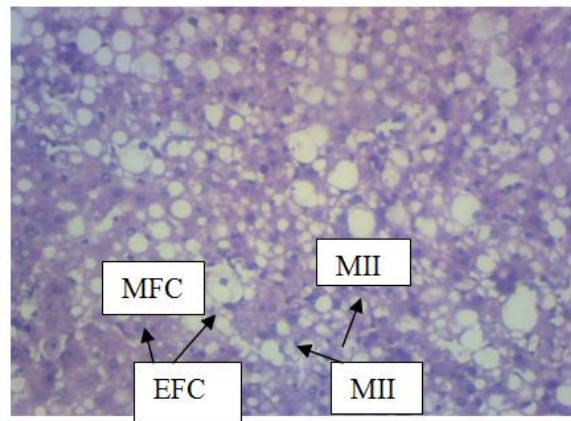


Plate 8: Photomicrogram of liver in CCl₄ induced liver damage in albino rats treated with 100 mg/kg aqueous leaf extract of *Bombax buonopense* stained with H/E showed extensive fatty change (EFC), mild fatty change (MFC), mild infiltration of inflammatory cell (MIIC), mild infiltration of inflammatory cell (MII).

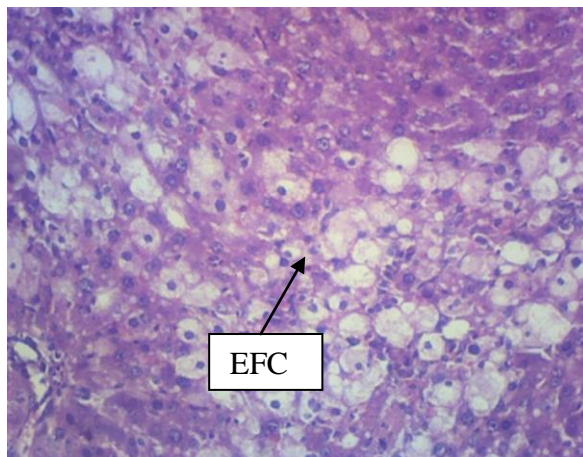


Plate 9: Photomicrogram of liver in CCl₄ induced liver damage in albino rats treated with 200 mg/kg of aqueous leaf extract of *Bombax buonopense* stained with H/E showed mild infiltration of inflammatory cells (MIIC), extensive fatty change (EFC), well perfused tissue.

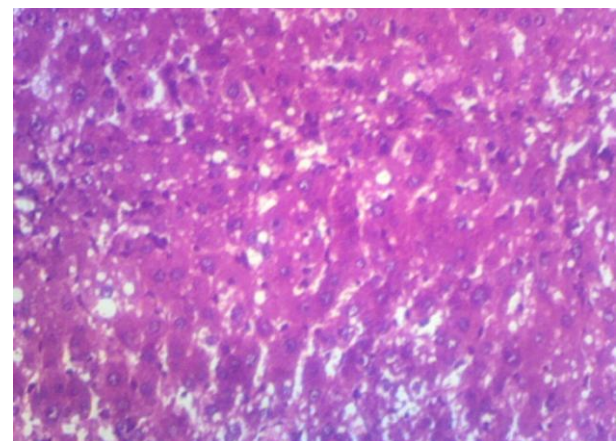


Plate 10: Photomicrogram of liver in CCl₄ induced liver damage in albino rats treated with 400 mg/kg of aqueous leaf extract of *Bombax buonopense* stained with H/E showed well perfused tissue, mild fatty change (MFC), mild infiltration of inflammatory cell (MIIC).

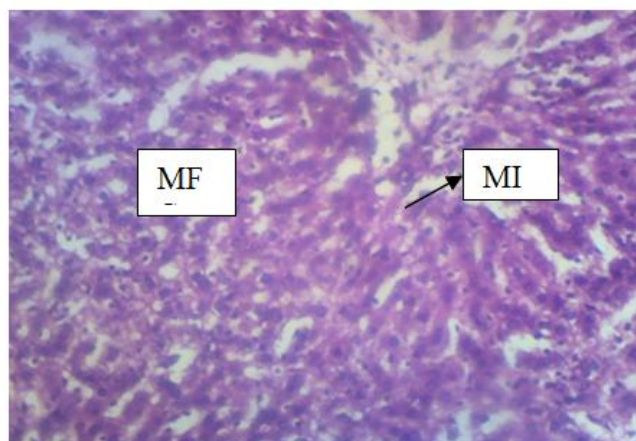


Plate 11: Photomicrogram of liver in CCl₄ induced liver damage in albino rats treated with 800 mg/kg of aqueous leaf extract of *Bombax buonopense* stained with H/E showed moderate fatty change (MF), mild inflammatory cells (MI).

V. Discussion:

The result of the percentage yields of the aqueous and methanol extracts of *Vitex doniana* and *Bombax buonopense* leaves were shown to be 13.57, 19.96, 13.74 and 14.02 % respectively. Results obtained showed that methanol is a better solvent for the extraction of bioactive compound. The LD₅₀ of aqueous and methanol extract of *Vitex doniana* and *Bombax buonopense* following oral administration was 2,000mg/kg and 3,000mg/kg using the arithmetic method of (Lorke, 1983). The calculated LD₅₀ showed that the extract is not very toxic. Clark and Clark, (1977) reported that any substance that the LD₅₀ in rats falls between 50-500mg/kg should be regarded as very toxic, while substances with LD₅₀ above 500mg/kg but below 1000mg/kg are classified as being moderately toxic. The signs of toxicity observed before death following the administration of the two extracts are loss of appetite, paralysis of hind limbs which progress to fore limbs. The toxicity observed may have resulted from the various organic chemicals like saponin, tannin, glycosides and phenolic compound present in the extracts (Abdulrahman, 2004).

The result showed significant ($p < 0.05$) reductions in a dose dependent manner in the levels of ALT, AST, ALP, 5'-nucleotidase and bilirubin in rats treated with aqueous and methanol leaf extracts of *Vitex doniana* and *Bombax buonopense*. Significant ($p < 0.05$) increase were observed in albumin, globulin and total protein levels in rats treated with the same extracts as shown in figure 1-4. Aja et al. (2014) reported that administration of the aqueous, ethanolic and methanolic extracts of *Moringa oleifera* seeds at 100,200,400, and 800 mg/kg body weight significantly decreased ($p < 0.05$) the levels of aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) in a dose dependent manner. Aja et al. (2015) also reported showed significant ($P < 0.05$) decrease in ALT, AST and ALP levels in alloxan induced diabetic albino rats treated with *Cajanus cajan* and *Moringa oleifera* ethanol leaf extracts in dose dependent manner. The finding of this study correlates with the report of Aja et al. (2015) that reported significant ($p < 0.05$) increase in albumin, total bilirubin and conjugated bilirubin levels in rats treated with *Moringa oleifera* and *Cajanus cajan* ethanol leaf extracts. Aja and Onu, (2010) also reported that the administration of *Ocimum basilicum* ethanol extract in albino rats significantly ($p < 0.05$) increased the activities of AST and ALT at doses of 200 and 400 mg/kg body weight. According to Khan et al. (2008) AST is found both in cytoplasm and mitochondria while ALT is a cytoplasmic enzyme. Raised levels of transaminases observed in the study have serious implications for the animals administered with the ethanolic extract. AST are widely used to assess liver function. The elevation of AST alongside the ALT makes the liver a target of suspicion as this is the usual pattern in case of hepato-toxicity caused by toxic agents (Ezeonu and Ezejiolor, 1998).

The result of histological analysis revealed that, the liver of rats that received CCl₄ without treatment were highly distorted when compared with those that treated with the extracts as shown in Plate 1-11. Aja et al. (2014) reported that histological evaluation of the liver tissues showed intact architecture and the results suggest that aqueous or ethanolic or methanolic seed extract of *Moringa oleifera* has no adverse effect on the liver status of the rats. The result of the histological analysis conforms to the result of the liver function indices as shown in Figure 1-4.

VI. Conclusion

This study has revealed that administration of aqueous and methanol leaf extracts of *Vitex doniana* and *Bombax buonopense* in rats have potentials to protect the liver function, integrity and architectures from hepato-toxins.

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