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# Effects Of Aqueous And Methanol Extracts Of Vitex Doniana In Carbon Tetrachloride Induced Liver Injury In Rats.

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***Abstract***
*Effect of aqueous and methanol leaf extract of Vitex doniana on carbon tetrachloride ($CCl_4$) induced liver injury in rats was carried out. One hundred and fourteen (114) male rats were randomly assigned into 7 groups. Group 1 with 6 rats served as control which received normal saline (vehicle) daily and olive oil on 7$^{th}$ day. Group 2 with 6 rats served as negative control which received normal saline daily. Group 3 with 6 rats served as positive control which received normal saline and Liv.52 daily. The rest groups (4-7) were administered aqueous and methanol extract of Vitex doniana at the concentration dose of 100, 200, 400 and 800 mg/kg body daily. On the 7$^{th}$ day groups 2-7 were injected intraperitoneally with a single dose of 2.5ml/kg $CCl_4$ in olive oil (1:1 v/v) once, after 2hrs of toxicant administration the rats were starved for 24hrs and then sacrificed for analysis, weight change was examined and organs were excised for histological examination. After treatment periods, the results showed that there was significant ($P<0.05$) decrease in the mean body weights of the rats among the treated group when compared to control rats. The result of histological analysis revealed that, the organs of rats that received $CCl_4$ without treatment were highly distorted when compared with those that received the toxicant and treated with the extract at different doses and Liv.52, the healing increased with increase in concentration of the extract administered, indicating that the extract has hepatoprotective effect on organs and this effect is concentration dependent.*

***Keywords:*** *Aqueous, Methanol, Vitex doniana, Carbon Tetrachloride, Liver, Rats*

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## I. Introduction

Since the beginning of time, man has been dependent on plants for medicinal purposes. Plants have been used in the treatment of variety of diseases and the introduction of the othodox medicine did not affect their use (Chan *et al.,* 2006). Currently, it was estimated that 80% of the population of the world were still dependent on plants for treatment of diseases (McGraw and Eloff, 2008). About 60% of the people in South Africa use plants in conjunction with pharmaceutical (McGraw and Eloff, 2008). In many under-developed and developing countries, rural people are still forced to use traditional medicine due to inaccessibility of healthcare facilities, escalating cost of modern medicine. Traditional patent dealers in Nigeria prepare their concoctions with plant parts plants can be used in various ways: (Baris *et al.,* 2006). Plants are used in production of drugs (Hammer *et al*., 1999). Mixture of plant extracts can be used in the treatment of diseases (Gibbons, 2003). The biological evaluation of plants extract is vital to ensure their efficiency and safety. Hence plant extracts have to be examined to ensure that they are efficient and save for human consumption. And this is important if the extracts are to be used in treatment of diseases (Tanaka *et al*., 2006). Phytochemical constituents are responsible for the medicinal values of plants. Varadarajan *et al.,* (2008) reported that the secondary metabolites (phytochemicals) and other chemical constituents of plant are responsible for their medicinal values.

*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 to 8-18m height with buttress root that can spread up to 5m. This plant can be used in so many ways; it can be used for the treatment of gastroenteritis (Atawodi, 2005), nausea, colic and in epilepsy (Bouquet *et al.*, 1971; Iwu, 1993), hypertensive (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 and Etta, 1984). *Vitex doniana* is also taken to improve fertility and the juice may be squeezed into the eyes to treat eye troubles. The ability of an aqueous and methanol leaf extract of *Vitex doniana* to protect the

liver of albino rats from carbon tetrachloride-induced liver damaged evaluated by measuring the serum levels of Alanine Amino Transferase (ALT), Aspartate Amino Transferase (AST), Alkaline Phosphatase (ALP), bilirubin and total protein has been reported by Ladeji and Okoye (1996).

Carbon tetrachloride is among the chemicals that are capable of causing injury in liver. The mechanism of this is not yet under stood clearly. This study was conducted to evaluate effect of aqueous and methanol leaf extract of *Vitex doniana* on $CCl_4$ induced liver injury in albino rats.

## II. Materials And Method

This study was conducted in March, 2023 in Biochemistry Department, Ebonyi State University, Abakaliki. The leaf of *Vitex donaina* was collected from Outskirt Abakaliki in Ebonyi State and were identified and authenticated by a taxonomist in the Department of Applied Biology, Ebonyi State University, Abakaliki.

**Preparation of plant leaf:** Fresh leaves of *Vitex doniana* was collected, washed and dried at ambient temperature (25ºC) and turning the leaves to avoid fungi growth. After drying, the leaves materials were pulverized using electric blender. Both leaf powders were stored in refrigerator in well labeled, air tight container prior for analysis.

**Extractions:** 500g of powdered leaves was weighed into conical flask and 500ml of distilled water was poured into the flask. The contents of the flask were shaken and the top were covered with aluminum foil and kept at room temperature for 48h after which the extracts were obtained by filtering using a sieve cloth. The extracts were then concentrated by drying in a water bath maintained at a temperature of 45°C until brownish black residues were obtained and these were kept in sealed containers and refrigerated at 2-4°C until required.

**Lethal dose 50 ($LD_{50}$):** Lethal dose 50 test involves the administration of a substance to a group of animals at increasing doses in order to determine the dose that kills 50% of the test animals within a set time frame. The administration of the leaf extracts was orally. The animal used for $LD_{50}$ was grouped into 2 phases. Phase 1 received lower doses of the extracts while phase 2 received higher doses of the same extract.

**Animal grouping:** Healthy Westar male albino rats weighing (200-300g) were purchased from the Department of Animal Science, University of Nigeria Nsukka. They were acclimatized for two weeks and maintained at normal room temperature (25ºC) in the animal house of faculty of biological sciences, Ebonyi state university, Abakaliki. They were kept in cages, fed on commercial rats feed and allowed free access to clean water. One hundred and fourteen (114) male rats were randomly assigned into 7 groups. Group 1 with 6 rats served as control which received normal saline (vehicle) and normal rat feed. The rest groups were injected intraperitoneally with a single dose of 2.5ml/kg $CCl_4$ in olive oil (1:1 v/v) once on the $7^{th}$ day, after 2hrs of toxicant administration. The rats were starved for 24hrs and then sacrificed for analysis. Also, the animals were weighed on the first day and on the last day of the study.

Group 1: Rats given normal saline and olive oil
Group 2: Rats given $CCl_4$ Without treatment
Group 3: Rats given $CCl_4$ and treated with Liv.52
Group 4: Rats given $CCl_4$ and treated with 100 aqueous and methanol extracts of *Vitex doniana*
Group 5: Rats given $CCl_4$ and treated with 200 aqueous and methanol extracts of *Vitex doniana*
Group 6: Rats given $CCl_4$ and treated with 400 aqueous and methanol extracts of *Vitex doniana*
Group 7: Rats given $CCl_4$ and treated with 800 aqueous and methanol extracts of *Vitex doniana*

**Liver tissue examination:** After the treatment period, the rats were anaesthetized using chloroform and dissected using dissecting tools. The liver was excised and preserved in 10% formol acetic acid for histological analysis.

## III. Result And Discussion

Albino rats were weighed on the first day and on last day of treatment in order to examine the effect of $CCl_4$ induced changes in body weight (Figure 1-2). After treatment there was a tremendous decrease in the body weights during these periods of treatment; the results showed that there was significant ($P<0.05$) decrease in the mean body weights of the rats among the treated group as shown in figure 1 and 2 when compared to control rats.

The percentage change in body weight was significantly ($p<0.01$) higher in rats treated with $CCl_4$ alone without any extracts administration when compared with control rats. The rats treated with methanol extract of the plants also showed high decrease in body weight when compared with rats treated with aqueous extract of the plant and those treated with Liv.52.

Photomicrogram of liver in $CCl_4$ induced liver damage in albino rats with or without treatment stained with H/E in plate 1-11

Plate 1 represents the normal architecture of hepatic cells and plate 2-11 represent microscopic appearance of rat Liver after $CCl_4$ treatment.

Plate 2 showed distortion of hepatic architecture, extensive fatty change, infiltration of inflammatory cells around portal trad while plate 3 showed distortion of hepatic architecture and mild fatty change

Plate 4 showed focal area of necrosis, moderate fatty change, mild infiltration of inflammatory cells while plate 5 showed distortion of hepatic architecture, fatty change, infiltration of inflammatory cells

Plate 6 showed moderate congestion of hepatic cells, mild fatty change, mild inflammatory cells while plate 7 showed moderate fatty change, mild inflammatory cells

Plate 8 showed extensive fatty change, mild infiltration of inflammatory cells while plate 9 showed extensive fatty change, mild infiltration of inflammatory cells, well per fused tissue

Plate 10 showed mild fatty change, mild infiltration of inflammatory cells, well per fused tissue while plate 11: Moderate fatty change, mild inflammatory cells

These results showed that the toxicity of $CCl_4$ is related to weight loss in rats, there was a significant ($p<0.05$) reduction in percentage change in weight in group treated with $CCl_4$ without extract treatment when compared to the control rats. Also, group administered with Liv.52 showed low percentage change in weight when compared to control rats and higher percentage change in weight when compared to extract treated groups. These reduction of weight in extract treated group may be attributed to the presence of phytochemical constituent present in the sample for instance tannin depress voluntary feed intake, and because they bind to compound such as fiber, protein and prevents their digestibility and subsequently reduce iron growth rate of the animals (Huang *et al.*, 2010). They also cause decrease palatability and reduced growth rate (Roeder, 1995).

Histological section (plate 1-11) of the organs showed that the level of hepatic damaged were higher in $CCl_4$ treated rats than those rats supplemented with aqueous and methanol extract of *Vitex doniana* and Liv.52.

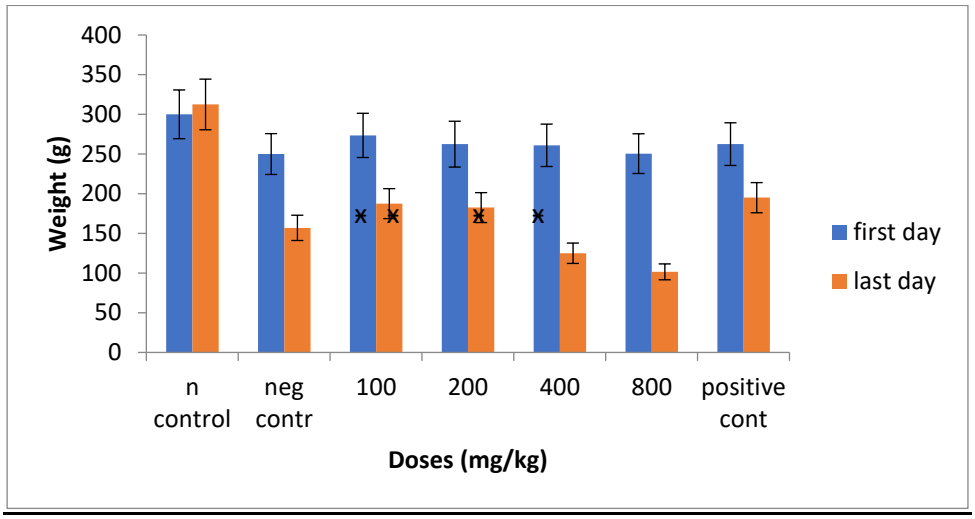



**Figure1: Weights in $CCL_4$ induced Liver damage in Albino Rats treated with methanol extracts of *Vitex doniana* leaf and Orthodox drug Liv.52 (p-control).**

Data are shown as mean ± S.D (n=6). Mean values in bars with (*) have significant differences ($p<0.05$) when compared with the control.

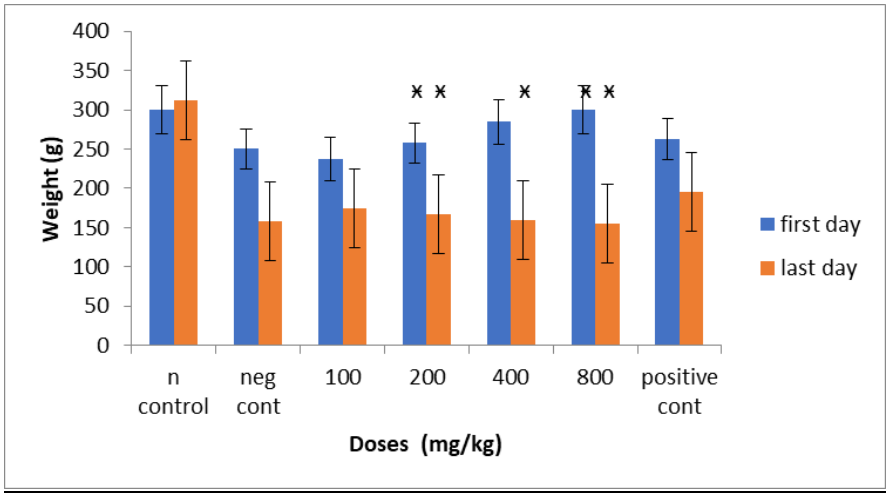
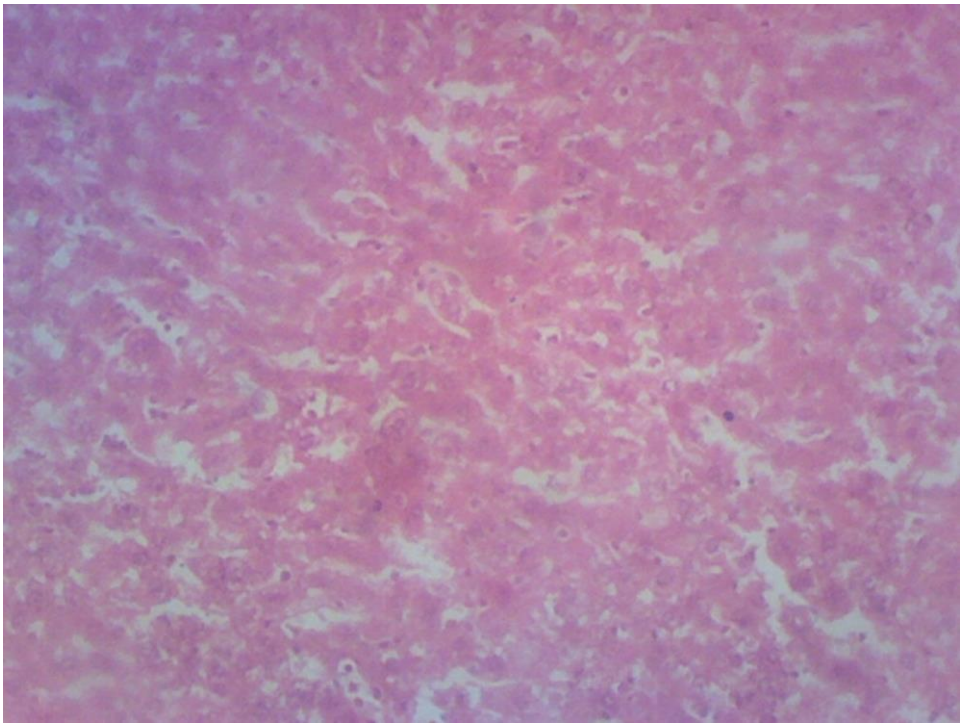


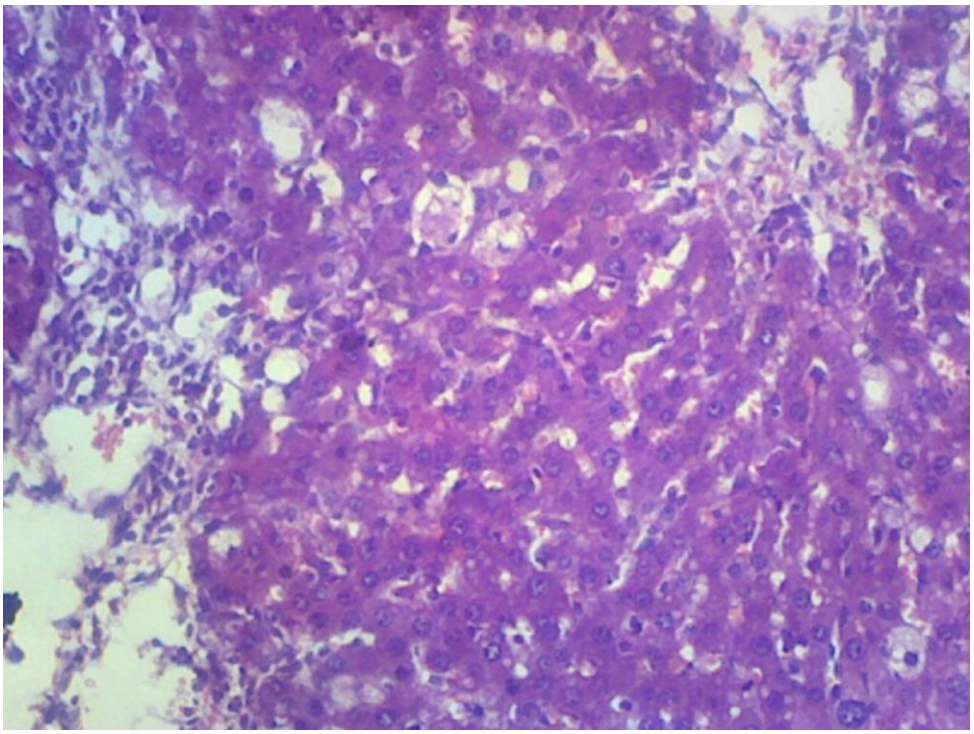

**Figure 2: Weights in $CCL_4$ induced Liver damage in Albino Rats treated with aqueous extracts of *Vitex doniana* leaf and Orthodox drug Liv.52 (p-control).**

Data are shown as mean ± S.D (n=6). Mean values are in bars with (*) have significant difference ($p<0.05$) when compared with the control.



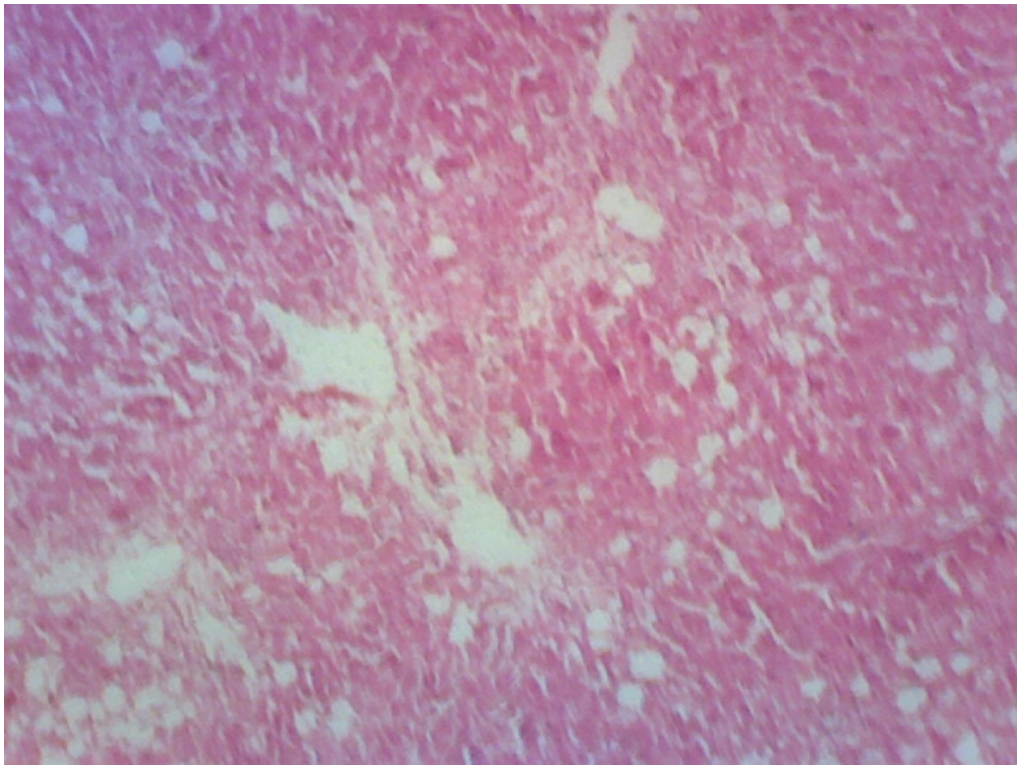
**Magnification : $X_{100}$**

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



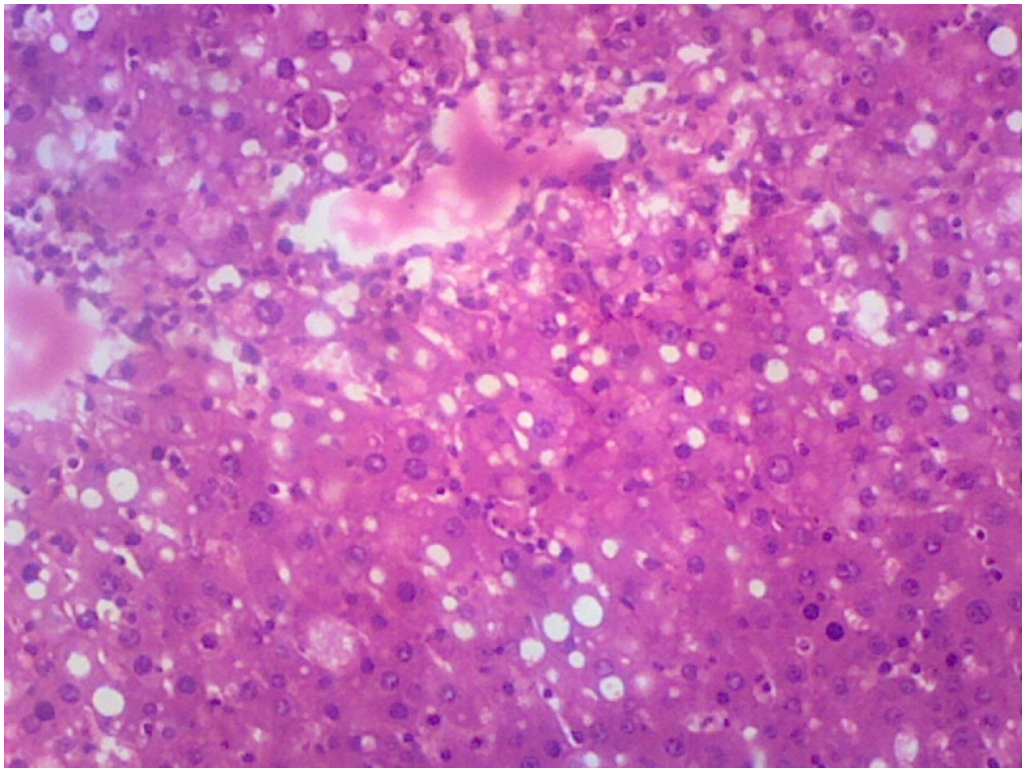
**Magnification : $X_{150}$**

**Plate 2: Photomicrogram of liver in $CCl_4$ induced liver damage in albino rats without treatment stained with H/E showed distortion of hepatic architeture( DHA), extensive fatty change (EFC), infiltration of infilamatory cell (IIC) arround portal traid otherwise portal hepatitis.**



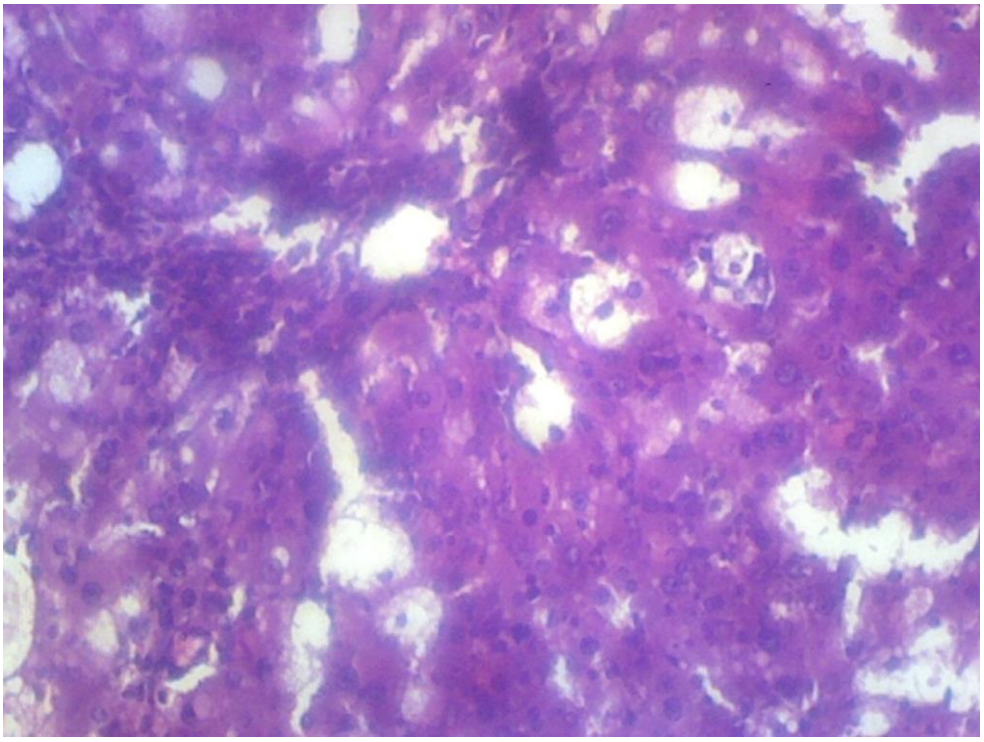
**Magnification : $X_{150}$**

**Plate 3: Photomicrogram of liver in $CCl_4$ induced liver damage in albino rats treated with Orthodox drug liv. 52 stained with H/E showed distortion of hepatic archtecture (DHA), mild fatty change (MFC).**



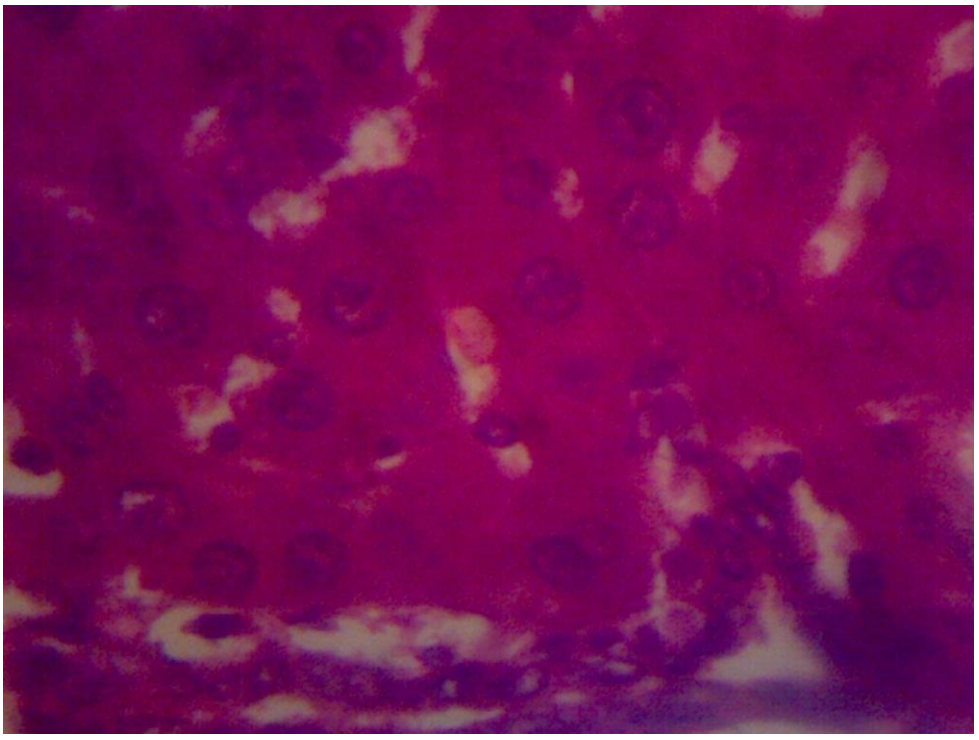
**Magnification : $X_{150}$**

**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 infilamatory cells (MIIC).**



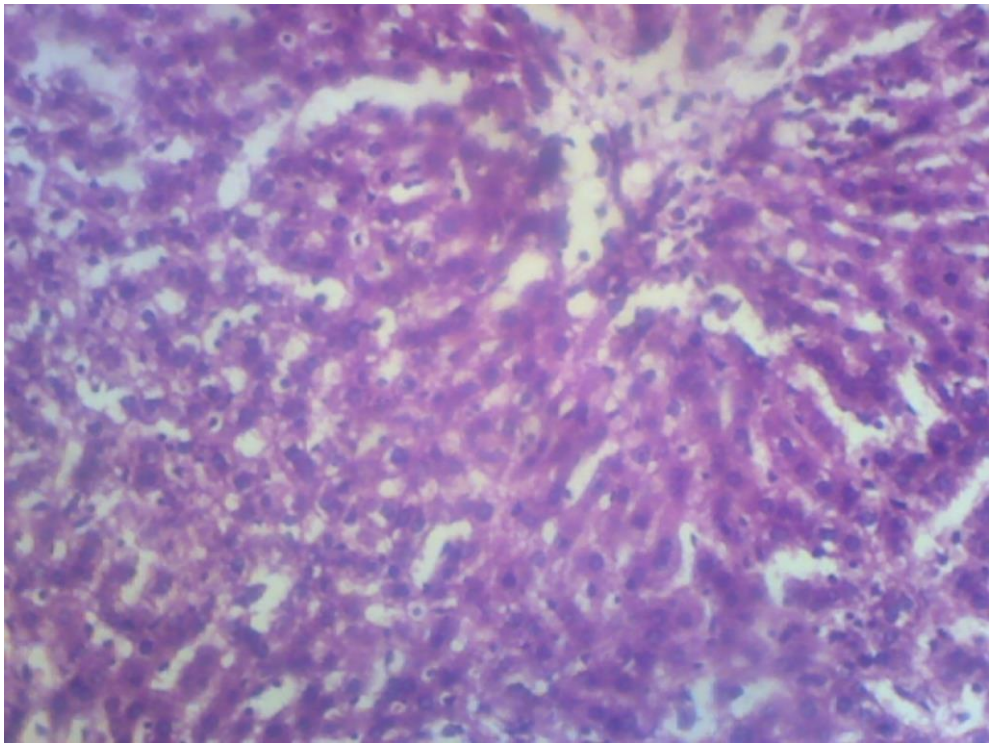
**Magnification : $X_{150}$**

**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 infilamatory cells at the portal traid (IIC),distortion of hepatic archtecture (DHA).**



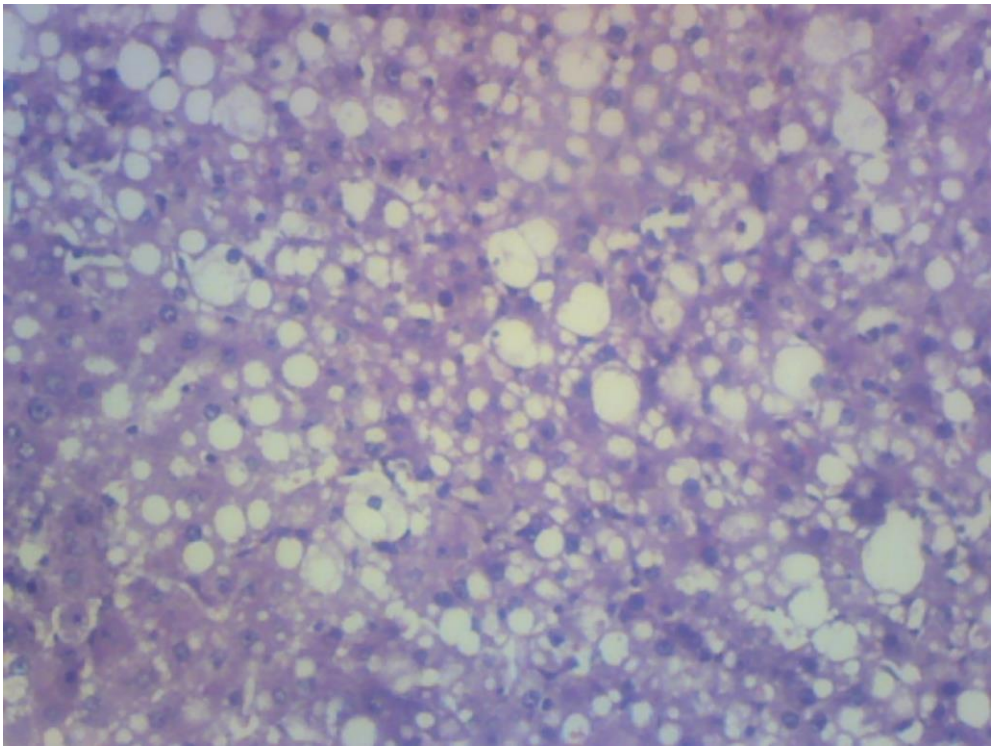
**Magnification : $X_{600}$**

**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 conjestion of hepatic cell (MCHC), mild inflamatory cell (MIC), mild fatty change (MFC).**



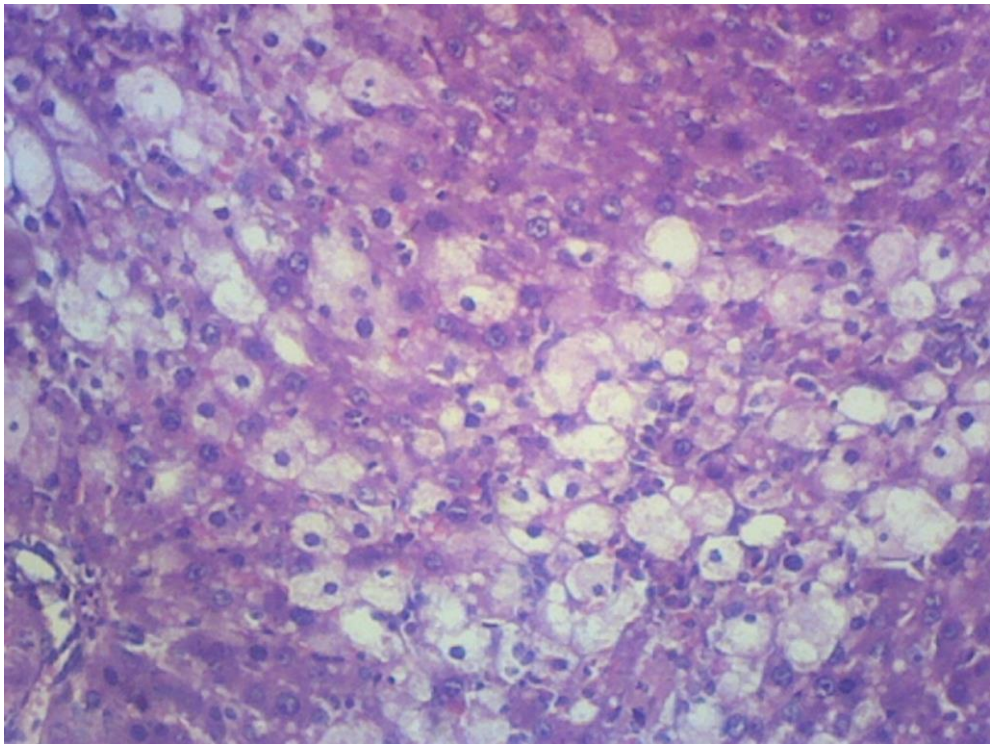
**Magnification : $X_{150}$**

**Plate 7: Photomicrogram of liver in $CCl_4$ 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 inflamatory cells(MIC).**



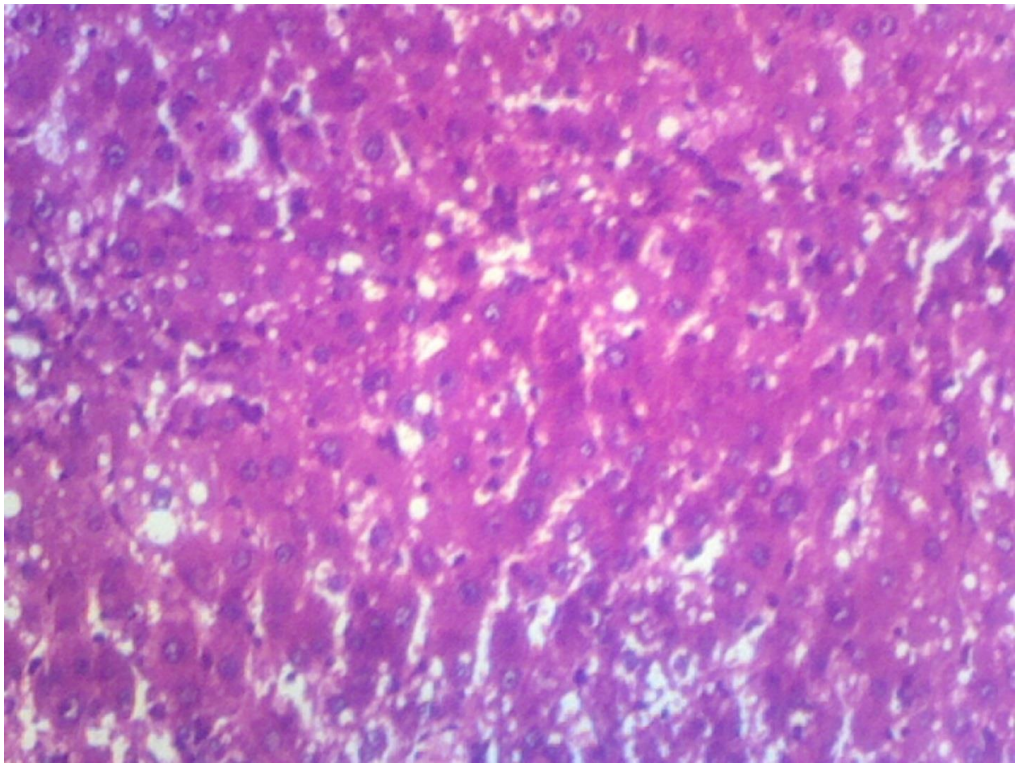
**Magnification : $X_{150}$**

**Plate 8: Photomicrogram of liver in $CCl_4$ induced liver damage in albino rats treated with 100 mg/kg methanol leaf extract of *Vitex doniana* stained with H/E showed extensive fatty change (EFC), mild infiltration of infilamatory cell (MIIC).**



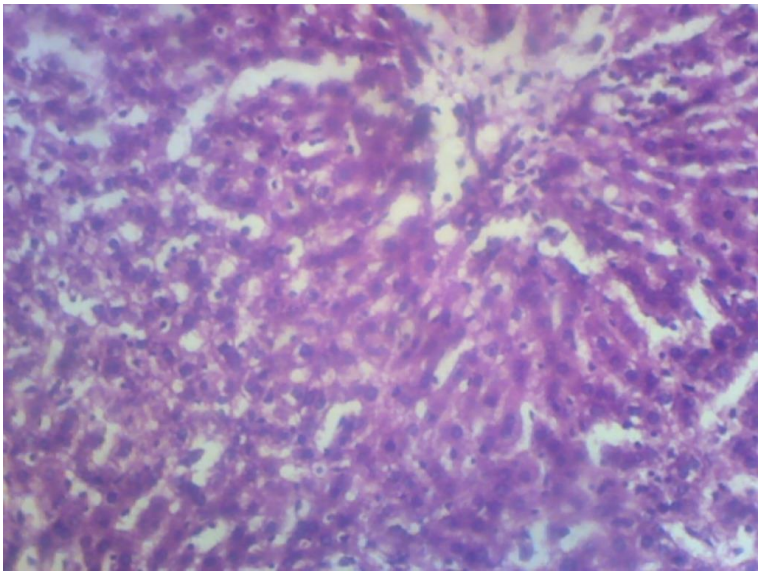
**Magnification : $X_{150}$**

**Plate 9: Photomicrogram of liver in $CCl_4$ induced liver damage in albino rats treated with 200 mg/kg of methanol leaf extract of *Vitex doniana* stained with H/E showed mild infiltration of infilamatory cells (MIIC), extensive fatty change (EFC), well perfused tissue.**



**Magnification : $X_{150}$**

**Plate 10: Photomicrogram of liver in $CCl_4$ induced liver damage in albino rats treated with 400 mg/kg of methanol leaf extract of *Vitex doniana* stained with H/E showed well perfused tissue,mild fatty change (MFC), mild infiltration of infilamatory cell (MIIC).**



**Magnification : $X_{150}$**

**Plate 11: Photomicrogram of liver in $CCl_4$ induced liver damage in albino rats treated with 800 mg/kg of methanol leaf extract of *Vitex doniana* stained with H/E showed moderate fatty change (MFC), mild inflamatory cells (MIC).**

## IV. Conclusion

The result of histological analysis revealed that, the organs of rats that received $CCl_4$ without treatment were highly distorted when compared with those that received the toxicant and treated with the extract at different doses and Liv.52, the healing increased with increase in concentration of the extract administration, indicating that the extract has hepatoprotective effect on organs.

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