The International Journal Of Engineering And Science (IJES)
||Volume||2 ||Issue|| 7 ||Pages|| 54-59||2013||
ISSN(e): 2319 – 1813 ISSN(p): 2319 – 1805

# The Effects of Moringa Extract On Liver Enzymes Of Carbon Tetrachloride Induced Hepatotoxicity In Adult Wister Rats.

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----------***ABSTRACT***----------
*This Work Focuses Primarily On The The Effects Of Moringa Extract On Liver Enzymes Of Carbon Tetrachloride Induced Hepatotoxicity In Adult Wister Rats. Twenty Wister Rats Weighing Between 140 - 220g Were Used. The Rats Were Divided Into Four Groups Of A, B, C And D Of Five Animals Each. Group A Served As Control And Received 0.5ml Of Distilled Water. Group B, C And D Received Different Doses Of Drugs As Follows: Group B Received 0.8ml Of Extract; Group C Received 0.4ml Of Carbon Tetrachloride While Group D Received 0.4ml Of Tetrachloride And 0.8ml Of Extract. The Administration Lasted For Twenty Eight Days Between The Hours Of 12 – 3:30pm Using Intubation Method. Twenty Four Hours After The Last Administration, Liver Tissues Were Removed And Weighed. Blood For Serum Preparation Were Collected Through Cardiac Puncture. Serum Samples Were Separated From Clot By Centrifugation Using Bench Top Centrifuge. Activities Of Serum Aspartate Aminotransferase Alanine Phosphatase Level And Alkaline Phosphatase Level Were Determined Using Randox Kit Method. The Relative Liver Weight For Carbon Tetrachloride Group Were Significantly Higher (P < 0.001) Compared With The Control. The Serum Aspartate Aminotransferase, Alanine Aminotransferase , Alkaline Phosphate Were Significantly Higher ( P < 0.001) Then The Control. The Values For Group B And D Increased Significantly Relative To Control. The Result Showed That No Adverse Biochemical Changes Are Associated With The Use Of Moringa Extract. This May Be Attributed To Its Anti-Oxidant Properties.*

***KEYWORDS:*** *Moringa, Liver Enzymes, Liver Weight, Hepatotoxicity, Wister Rat.*

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Date of Submission: 1July. 2013 Date of Publication: 22.July 2013

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## I. INTRODUCTION

The liver is the largest of the abdominal viscera, occupying a substantial portion of the upper abdominal cavity. It performs a wide range of metabolic activities necessary for homeostasis, nutrition and immune defence. It is composed largely of epithelial cells (hepatocytes), which are bathed in blood derived from the hepatic portal veins and hepatic arteries. There is continuous chemical exchange between the cells and the blood. Hepatocytes are also associated with an extensive system of minute canals, which form the biliary system into which products are secreted. The liver is important in the removal and breakdown of toxic, or potentially toxic, materials from the blood [26].In adults the liver weighs 2% of body mass [15,26].The liver is a highly sensitive organ which plays a major role in maintenance and performance of the homeostasis in our body. It is the chief organ where important processes like metabolism and detoxification take place.Thus the liver is proned to injury due to the chronic exposure to drugs, environmental toxicants and other xenobiotics[23] .The liver disorders are one of the serious health problems, throughout the world. More than 350 million people were affected with chronic hepatic infections and in India above 20,000 deaths were reported every year due to liver disorders. Hepatocellular carcinoma is one of the most common tumors in the world with over 250,000 new cases each year[24]. One of the major functions of the liver is the manufacture and secretion of bile, which is stored in the gall bladder and released in the small intestine. It is the main organ for the synthesis and storage of carbohydrate energy in the form of glycogen. It can store up to 6 to 7% of its weight as glycogen, and readily breaks this down to glucose on demand by the tissues. Inter-conversion of food stuff is accomplished by the liver. Normally, the liver itself stores little if any gross fat, but excess fat may accumulate in it in starvation, excessive carbohydrate feeding, and deficiency of chlorine or as a result of the action of hepatotoxins such as

alcohol, carbon tetrachloride and phosphorous. In addition to manufacturing the large variety of enzymes and other proteins required by its own cells and large amount of protein for 'export' [25].The liver contains thousands of enzymes some of which are also present in serum in very low concentration. These enzymes have no known function in serum other than to provide information about hepatic state and disorders. These enzymes are distributed in plasma and in interstitial fluid and have characteristic half-lives, usually measured in days. The elevation of a given enzyme activity in serum reflects its increase rate of entrance into serum from damaged liver cells like AST, ALT, ALP. Specific isoenzymes of AST are present in the liver cell mitochondria and cytoplasm whereas ALT is confined to the cytoplasm[22]. The amino transferases (transaminases) are one group of enzymes that are sensitive indicators of liver cell injury[12]. Their serum levels are especially altered in hepatocellular disease particularly in acute diseases and they are often referred to as hepatocellular enzymes[22]. Elevated serum transaminase levels are typically associated with acute hepatocellular necrosis and reflect the release of enzymes from the cytoplasm of dying cells. Other features such as fatty degeneration, infiltration by inflammatory cells are variable and reflect the severity of injury[11].Moringa oleifera is the most widely cultivated species of a monogeric family, the Moringacea e that is native to the sub-Himalayan tracts of India, Pakistan,Ban gladesh and Afghanistan. Moringa oleifera or the horseradish tree is a pan-tropical species that is known. It is believed to have variety usages which include combating malnutrition, anticancer and is being promoted as a panacea[4,6,7,816]. Thus this study was undertaken to investigate the hepatoprotective nature of Moringa oleifera on induction of $CCl_4$ known to cause liver damage in wistar rats since it has been used non-conventionally in the treatment of certain diseases associated with liver, kidney, cough, diarrhoea etc. Cirrhosis can be induced in animals by chronic administration of carbon tetrachloride or aflatoxin or several chemical carcinogens[10].Moringa preparation have been cited in the scientific literature as having antibiotic, anti inflammatory, hypocholesterolemic, and hypoglycaemic activities, as well as having considerable efficacy in water purification by flocculation, sedimentation, antibiosis and even reduction of schitosome cercariae titer[14].

Some plants with reported hepatoprotective properties are *Garcinia kola* Ker Gaul (Clausiaceae), *Tinospora cordifolia* (A. Rich.), *Ricinus communis* Linn (Euphorbiaceae), *Curcuma longa* Linn (Zingiberaceae), *Enicostemma littorale Blume* (Gentianaceae), *Flaveria trinervia* Linn (Asteriaceae), Moringa and *Boerhaavia diffusa* Linn (Nyctaginaceae) [3,19,20]. Plants are a major source of novel anti-oxidant and hepatoprotective agents since many industrial drugs are derived as a result of knowledge got from folklore medicine[2]. Some of the herbal preparations speed up the natural healing processes of the liver[17]. *Phellinus rimosus* (Berk) Platt (Hymenochaetaceae), a mushroom has been shown to protect the liver from acute and chronic carbon tetrachloride ($CCl_4$)-induced hepatotoxicity in rats by restoring the liver anti-oxidant status, inhibiting the phase I and enhancing the phase II enzyme activities[1].This study focuses on the effects of moringa extract on liver enzymes of carbon tetrachloride induced hepatotoxicity in adult wister rats.

## II. MATERIALS AND METHODS

**PROCUREMENT OF PLANT:** The leaves of Moringa oleifera was procured from Nibo in Awka south (Anambra) and authenticated at the department of Botany Nnamdi Azikiwe University, Awka.

**Preparation of extract**: Fresh leaves of Moringa oleifera were collected, shade-dried and pounded into powder before extraction. The powder was macerated into absolute alcohol at room temperature. The filtrate was concentrated under reduced pressure and later evaporated in a water bath using evaporating dish at 45$^{o}$C. A greenish paste was obtained.

**Experimental animals:** Twenty (20) adult Wistar rats weighing 150 to 210 g were obtained for the study. The animals were fed with standard diet and water and were adapted to the laboratory environment in the Department of Human Anatomy for two weeks in order to acclimatize. The administration lasted for twenty eight days between the hours of 12 – 3:30pm using intubation method.

Wistar rats weighing between140 and 220 g were grouped into four (4) groups of A, B, C and D of five animals each. Group A served as control and received 0.5ml of distilled water. Group B, C and D received different doses of drugs as follows:

- group B received 0.8ml of extract.
- group C received 0.4ml of carbon tetrachloride.
- group D received 0.4ml of tetrachloride and 0.8ml of extract.

Oral route of administration was used and the administration lasted for twenty days.

**Tissue processing and staining:** Twenty four hours after the the last administration, liver tissues were removed and weighed. Blood for serum preparation were collected through cardiac puncture. Serum samples were separated from clot by centrifugation using bench top centrifuge. Activities of serum aspartate aminotransferase alanine phosphatase level and alkaline phosphatase level were determined using randox kit method. They were then dissected and the liver tissues were removed, and immediately fixed in 10% formalin. The tissues were transferred into an automatic processor where they went through a process of dehydration in ascending grades of alcohol (ethanol) 70, 80, 95% and absolute alcohol for 2 changes each. The tissues were then cleared in Xylene and embedded in paraffin wax. Serial sections of 5 micron thick were obtained using a rotary microtome. The tissue sections were deparaffinised hydrated and stained using the routine haematoxylin and eosin staining method (H&E). The stained sections were examined under the light microscope.

## III. RESULTS

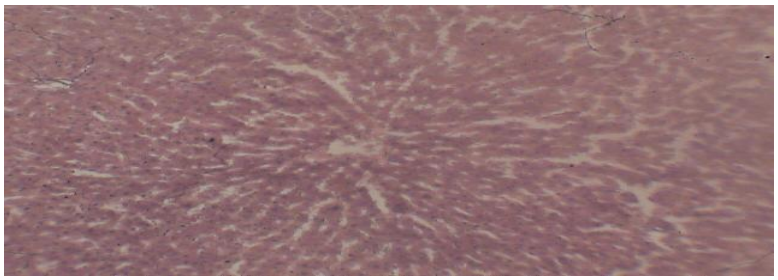
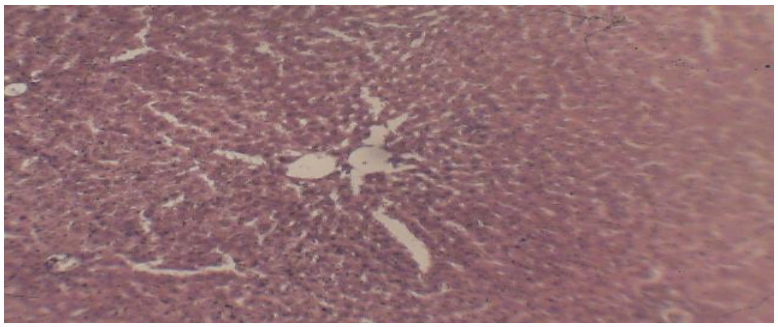
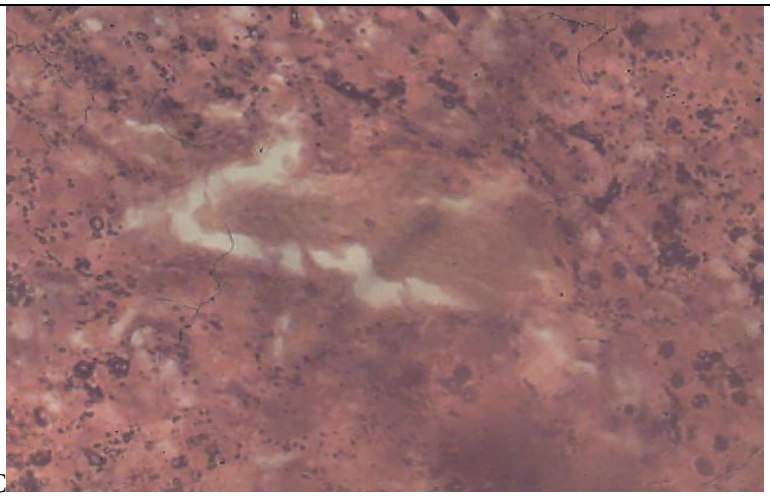
In the study, ALT, AST, ALP activities were used to measure the protective effect of M.oleifera on $CCl_4$ induced hepatotoxicity in wistar rats was examined and interpreted and the result is presented in table 1 and figure below; However, significant ($p<0.05$) increase was recorded for group C (135.86±2.20), when compared with the control (90.55± 0.40). Beside, significant ($p<0.05$) decreased was observed in moringa treated group B (87.40±2.0), when compared with those that only $CCl_4$. Significant ($p<0.05$) decrease was observed in Group D compared to group C. More so, no significant ($p>0.05$) changes was observed for serum ALT levels all compared with the control. Furthermore, similar trend observed for serum AST levels was also recorded for ALP levels.

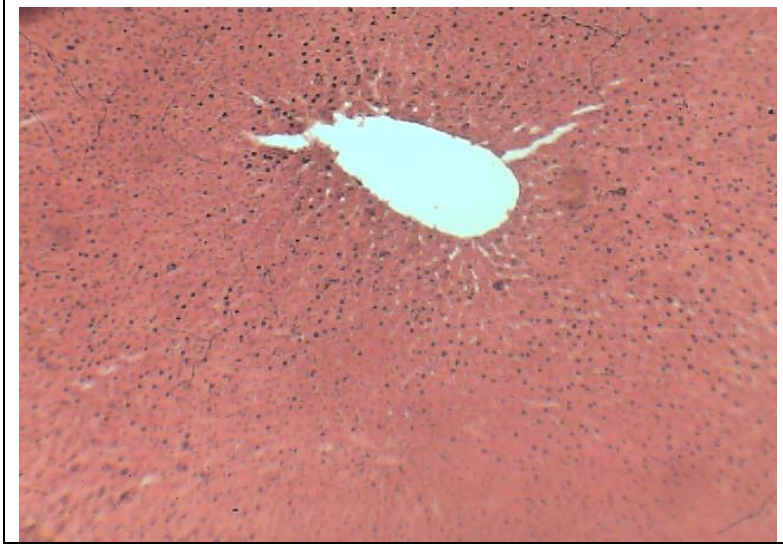
TABLE 1 Effect of crude extract of M.oleifera on serum enzyme activities of ccl4 induced hepatotoxicity induce rats.

| Groups | Treatment | Dose | Enzyme activity | | |
|---|---|---|---|---|---|
| | | | AST | ALT | ALP |
| A | Control | …… | 90.56±0.40 | 23.50±2.25 | 1.60±0.06 |
| B | Moringa extract | 0.8ml of moringa extract. | 87.40±2.0 | 23.50±2.25 | 1.67±0.12 |
| C | $CCl_4$ | 0.4ml of CCl4 | 135.05±2.20 | 23.50±2.25 | 1.68±0.09 |
| D | Moringa extract and carbon tetrachloride | 0.8ml of moringa extract and 0.4ml of $CCl_4$ | 114.64±7.04 | 23.50±2.25 | 1.13±0.10 |

Values are means ± standard deviation (n=6 for each group).

FIGURE 1 Histopathological examination of the hepatoprotective activity of M.oleifera on $CCl_4$ hepatotoxicity induced rats.

| FIGURES | DISCUSSION |
|---|---|
| Fig. A  | Cords of hepatocytes with well preserved cytoplasm, not vacuolated, sinusoidal well demarcated, no area of necrosis, no fatty changes, no fatty degeneration. |
| Fig.B  | Cords of hepatocytes are distinct essentially normal, no fatty change, cytoplasm not vacuolated. |
|  Fig.C | Hepatocytes are vacuolated, enlarged cytoplasm, nuclear darkly stained, and area shows extensive fatty change (steatosis), presence of necrosis. |
| Fig. D | Very little fatty change. Most areas appear to have recovered, hepatocytes well preserved and not vacuolated. Liver is essentially normal. There is further improvement when compared with Fig.D |



## IV. DISCUSSION

Liver is an organ involved in many metabolic functions and is prone to xenobiotic injury because of its central role in xenobiotic metabolism[18]. Hepatotoxic drugs cause damage to the liver[18,19]. Carbon tetrachloride was used in this study to induce the liver damage (Fig. C) and it was reported to be hepatotoxic[13,21]. Based on the results obtained, we therefore inferred that Moringa oleifera leave extract has some protective effect on the liver as shown by the reduced damage in group D (Fig. D). The reduced necrosis of cells in the group III study might be due in part to the presence of chemical constituents which have hepatoprotective properties. Liver protective herbal drugs contain a variety of chemical constituents like phenols, coumarins, lignans, essential oil, monoterpenes, carotinoids, glycosides, flavonoids, organic acids, lip ids, alkaloids and xanthenes[9], this may be present in the Moringa oleifera and so responsible for this effect. From this study, we therefore inferred that ethanolic leave extract of Moringa oleifera has an appreciable ability to prevent damage to the liver.

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