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Hepatoprotective property of ethanolic leaf extract of *Moringa oleifera* on carbon tetrachloride (CCl_4) induced hepatotoxicity

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ABSTRACT

The liver serves as a processing laboratory, redistribution centre and a receiving depot of the body. In the light of these roles, the effect of ethanol extract of Maringa oleifera leaves on carbon tetrachloride-induced liver damage in wistar rats was assessed. Carbon tetrachloride CCl_4 (0.5ml/kg,p.i) was used to induce hepatotoxicity. Twenty (28) albino rats of wistar strain (100-175g) were used for the studies and were divided into 7 groups of six (6) rats per each. Group A served as control and was treated with distil water of treatment equivalence, group B, C,D,E, F and G were treated with treated as follows; group B(olive oil treated), C (CCl_4 treated), and group D-G (extract and CCl_4 treated groups). The administered was done via oral gavages with ethanolic leaves extract of Moringa oleifera at 2.5ml/kg/day body weight at scalar doses(10mg/kg,200mg/kg,300mg/kg,1g/kg) daily, the administration of the extract lasted for twenty eight (28) days period after which the animals sacrificed, blood and tissue were obtained for biochemical and histological analysis. Serum enzyme assay results reveals ethanolic extract of Moringa oleifera leaves recorded a marked reduction in the elevated activities of the hepatic enzymes viz; Alkaline phosphatase (ALP) A (1.60±0.07), B (1.63±0.10), C (1.68±0.10) D (1.68±0.09), E (1.20±0.14), F (1.13±0. 10), G (1.48±0.13) levels of rats. Similar trend was observed for Aspartate aminotransferase (AST), but no changes was observed for Alanine aminotransferase (ALT) levels when compared with the control at ($p<0.05$). However, these enzymes still remain elevated CCl_4 treated which did not receive the extract. More so, the micrograph of CCl_4-group compared with control group showed evidence of liver necrosis as indicated by distended hepatocytes, compression of sinusoids, fatty change and vacuolation of cytoplasm. The treated groups at varying concentration of the extract (10%, 20%, and 30%) generally showed mild defects when compared with the untreated CCl_4 group. It is however interesting to know that the 100% M.oleifera treated group showed a healthy liver cells as in the case with the normal olive oil groups. Hence, from the above findings, it is obvious that ethanolic leaves extract of M.oleifera has hepatoprotective and hepatocurative potentials in hepatocellular disorders.

Keywords: Hepatoprotective, Ethanolic Leaf-Extract, *Moringa olifera* leaf, carbon Tetrachloride, hepato-toxicity.

INTRODUCTION

Moringa is promising as a food in the tropics because the tree is in full leaf at the end of the dry season when most foods are typically scarce. A large number of reports on the nutritional qualities of *Moringa* now exist in both the scientific and the popular literature. Moringa leaves have been reported to contain more vitamin A than carrots, more calcium than milk, more iron than spinach, more vitamin C than oranges, and more potassium than bananas, and that the protein quality of Moringa leaves rivals that of milk and eggs. The nutritional properties of Moringa are now so well known that there seems to be little doubt of the substantial wealth benefit to be realized by consumption of Moringa leaf powder in situation where starvation is imminent (Lowell, 1989). The benefits for the treatment, prevention of disease or infection that may accrue from either dietary or tropical administration of Moringa preparation (e.g. extracts, decoction, poultices, creams, oils, emollients, salves, powders and porridges) are not quite so well known (Palada, 1996). It is useful to review the claims that have been made and to assess the quality of evidence available for the more well- documented claims. Wide spread claims of medicinal effectiveness of various Moringa tree preparation have encouraged authors at the John Hopkins university to further investigate some of these possibilities. A plethora of traditional medicine references attest to its curative power and scientific validation of these popular uses is developing countries and to support at least to some of the claims. 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 (Olsen,1987). Recently, 4-(4'-0-accetyl-a-L-rhamnopyranosyloxy)benzylisothiocyanate and the related compound, Niazimicin present in moringa were shown to be potent inhibitors of phorbol ester (TPA) - Induced Epstein- Barr virus early antigen activation in lymphoblastoid (Burkitt's lymphoma) cells. In one of these studies, Niazimicin also inhibited tumour promotion in mouse two-stage DMBA-TPA tumour model (Murakami, 1998). Thus, traditional practice has long suggested that cancer prevention and therapy may be achievable with native plants. The proof required by modern medicine has not been realized because neither the prevention of cancer nor the modification of relevant biomarkers of the protected state has been adequately demonstrated in human subjects.

The liver of an adult comprises one-fiftieth of the body weight (Rappaport, 1982) and it is completely protected by the rib cage and extends from the right fifty intercostals space in the mild clavicular line down to the right coastal margin. From the central vein, cords or plates of the liver cells or hepatocytes extend radially to the periphery of the lobule, separated from each other by blood sinusoids which also run radially. The individual hepatocyte is a polygonal epithelial cell approximately- 25µm in diameter with a well defined plasma membrane that is differentiated into baslaterial (75%) and bile canalicular (15%) domains with different molecular, chemical and antigenic composition. The nucleus of the liver is centrally located round and contains one or more nucleoli. Although binucleate forms are not uncommon, mitotic activity is rare. Nuclei vary in size in the adult, and the great majority are diploid (Ranek *et al*, 1975). 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'. The liver is also capable of synthesizing certain amino acids (the so-called non-essential amino acids) from other amino acids in a process called transamination. It stores important vitamins and minerals, including vitamins A, D, K and B 12 and also has a valuable role in the detoxification of many substances intentionally or accidentally ingested (Patrick and McGee, 1988). The liver is often the target organ for a number of reasons. Most toxicants enter the body via the gastrointestinal tract, and after absorption they are carried by the hepatic vein to the liver. The liver has a high concentration of xenobiotic-metabolizing enzymes (mainly cytochrome P-450) and can detoxify, eliminate many ingested toxicants, mostly by conjugation. However, some toxicants can be concentrated to toxic levels, while others are bio-activated and become more toxic in the liver. In the latter two cases; they may lead to liver damage and other disorder in the body. The toxicology of the liver is complicated by the variety of liver injuries and by the different mechanism through which the injuries are included. The lesion observed depends not only on the toxic agent involved but also on the duration of exposure, hepatocellular lipid accumulation (steatosis) hepatocellular necrosis, or hepatobilliary dysfunction is usually found. Cirrhotic or neoplastic changes are usually considered as a result of chronic exposures (Guo, 1995). Moreover, hepatotoxicity has been shown with mitochondrial respiratory chain

inhibitor such as rotenone, CO, H2S and antimycin A. (Kulkarni and Byczkowski, 1994). peroxidation was first proposed as mechanism of carbon tetrachloride induced liver injury (Recknagel and Ghoshal, 1966). These observations are consistent with the hypothesis that toxicants-induced hepatocellular injury results in the influx of Ca^{2+} (calcium ion) into the cell, initiating a series of cytotoxic events common to various hepatotoxicants and resulting in cell death. Several drugs and chemical inhibit hepatocyte antioxidant defines against lipids peroxidation, thus increasing the severity and duration of peroxide damage (Kulkarni and Byczkowski, 1994). Cirrhosis is a chronic morphologic alteration of the liver and characterized by the presence of septae of collagen distributed throughout most of the liver (Schinella and Becker, 1975). Separated by these fibrous sheaths, clusters of hepatocytes appear as nodules and invariably, the pattern of hepatic blood flow is altered. The pathogenesis is not fully understood, but in a majority of cases, cirrhosis seems to originate from single cell necrosis associated with a deficiency in the repair mechanism. This condition then leads to fibroblastic activity and scar formation. Cirrhosis can be induced in animals by chronic administration of carbon tetrachloride or aflatoxin or several chemical carcinogens (Guo, 1995). In humans however, the single most important cause of cirrhosis is chronic ingestion of alcoholic beverages (Rankin *et al*, 1975. 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 (Wilkinson, 1976). The amino transferases (transaminases) are one group of enzymes that are sensitive indicators of liver cell injury (Mayers and Jones, 1990). Their serum levels are especially altered in hepatocellular disease particularly in acute diseases and they are often referred to as hepatocellular enzymes (Wilkinson, 1976). 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 (Kaplowitz, 1992). Liver diseases were among the first disorders to which serums test were applied and have proved to be useful in diagnostic purposes. At present over fifty enzymes are known to exist in the serum or plasma and all these have been known to have abnormal values in patients with hepatic diseases (Ukoha, 1998).

The consumption of variety of local herbs and vegetables by man is believed to contribute significantly to the improvement of human health in terms of prevention and cure of disease because plants have long served as a useful and rational source of therapeutic agents (Ukoha, 1998). The liver is an organ of paramount importance, which plays an essential role in the metabolism of foreign compounds entering the body (Athar *et al*, 1997). Conventional drugs used in the treatment of liver diseases are often inadequate and thus, it is therefore necessary to search for alternative drugs for the treatment of liver disease to replace the currently used drugs of doubtful efficacy and safety. 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.

MATERIALS AND METHODS

Collection and Preparation of Plant Materials

Fresh horseradish leaves were purchased at Sabon Gari market in Kano State. The leaves were dried in an airy room for about 3 days of drying, away from direct sunlight to avoid possible damage to their phyto-constituents. 40g of the dried leaves was soaked in 250ml of ethanol, shaken for 10minutes and then allowed to stay in refrigerator for 48 hours at 4^oC. The mixtures were first filtered with cheese cloth, then with WhatMan No 1 filter paper (24cm). The filtrates were separately concentrated *in vacuo* using Rotary Evaporator (Model RE52A, China) to 10% of its original volume at 37o C - 40o C. These were concentrated to complete dryness in water bath in order to obtain the crude extract (Won, *et al*, 2005)

Laboratory Animals

Twenty (28) albino rats of wistar strain (100-175g) were obtained from the animal holding unit, Department of biochemistry department, university of Port Harcourt, Nigeria and were allowed acclimatization period of fourteen (14) days in well ventilated room with a temperature and relative humidity of 29±2^{o}c and 70% respectively. They were maintained with commercial rat chow (Vital Feeds LMT) and water *ad libitum*. The animals were housed in a cage and were exposed to 12 hour light-dark cycle and handled according to standard protocol. At the end of the

acclimatization period, they were divided into 7 groups of six (6) rats per group. Group A served as control and was treated with distil water of treatment equivalence, group B, C,D,E, F and G were treated with treated with 0.5ml olive oil/kg, 0.5ml CCl_4 dissolved in olive oil (1:1) and given intraperitoneally, dose of 0.5ml CCl_4/kg body weight in 0.5ml of olive oil and with 100mg/kg of the crude extract of *Moringa oleifera*, 0.5ml CCl_4/kg body weight in 0.5ml of olive oil with 200mg/kg of the crude extract of *Moringa oleifera*, 0.5ml CCl_4/kg body weight in 0.5ml of olive oil with 300mg/kg of the crude extract of *Moringa oleifera*, 0.5ml CCl_4/kg body weight in 0.5ml of olive oil with1g/kg of the crude extract of *Moringa oleifera.* The administration of the extract lasted for twenty eight (28) days period after which the animals sacrificed after 24hrs after the last administration in chloroform saturated chamber in accordance with the guidelines of the European Convention for the Protection of Vertebrate animals and other scientific purposes –ETS-123 (European Treaty Series, 2005).

Serum enzyme assay and Histomorphological Assessment

Alanine transaminase (ALT) and Aspartate transaminase (AST)activities was assayed using the method of Reitman and Frankel, (1957) while Alkaline Phosphatase (ALP) serum level estimated by the principle of Tietz, (1995). The method described by Baker and Silverton (1985) was adopted in the preparation of slices or fixed tissues (liver) for histological examinations. The livers were removed immediately after blood collection and a part of the right lobe was sliced and fixed in 10% buffered formal saline .Following decalcification, dehydration, impregnation, embedding and section cutting, the tissues were stained using the Mayer's acid alum haematoxylin and eosin staining techniques and mounted in neutral balsum. The slides were then examined microscopically for histological changes and micrograph of each section taken.

Statistical Analysis

The results of the proximate analysis and anti-nutrient screening were analysed for statistical significance by one way ANOVA (F- ratio) (Welkowitz, 1976) and student 't' test were applicable values at ($p<0.05$) were regarded as significant in comparison with appropriate control. All data were expressed as means of ± SEM.

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 plates;

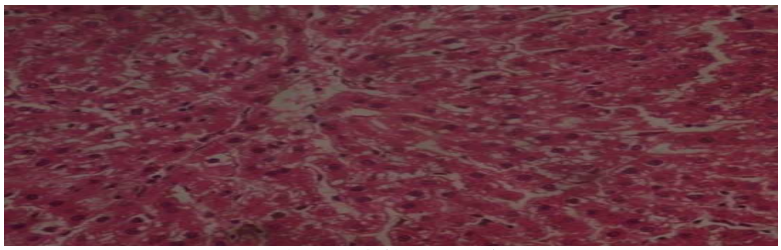
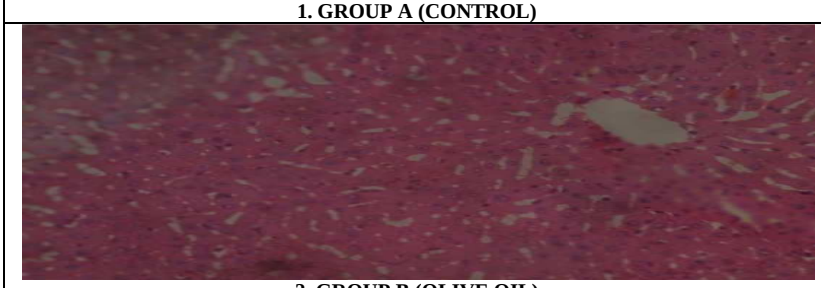
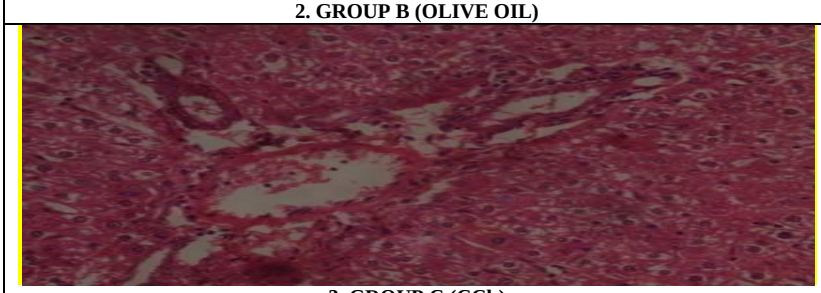
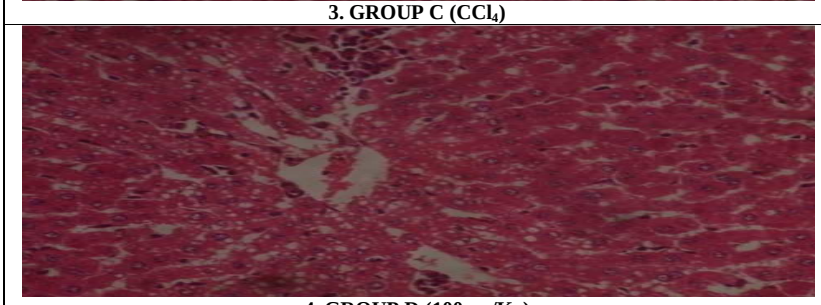
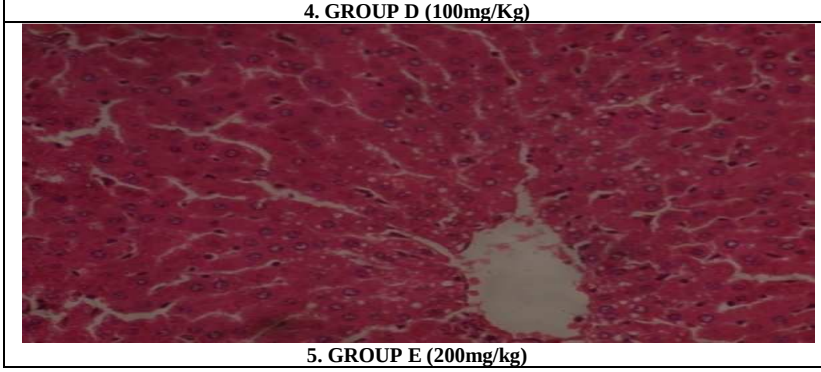
Statistical evaluation reveals that for AST levels, the extract recorded no significant ($p>0.05$) changes in group B (87.50±1.91) when compared with the control (90.55± 0.40). However, significant ($p<0.05$) increase was recorded for group C (135.86±3.00), D (117.05 ±1.20), E (117.23±3.86), F (114.64±7.04) and G (111.59 ± 7.97) all when compared with the control (90.55± 0.40). Beside, significant ($p<0.05$) decreased was observed in moringa treated group when compared with those that only CCl_4. 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.

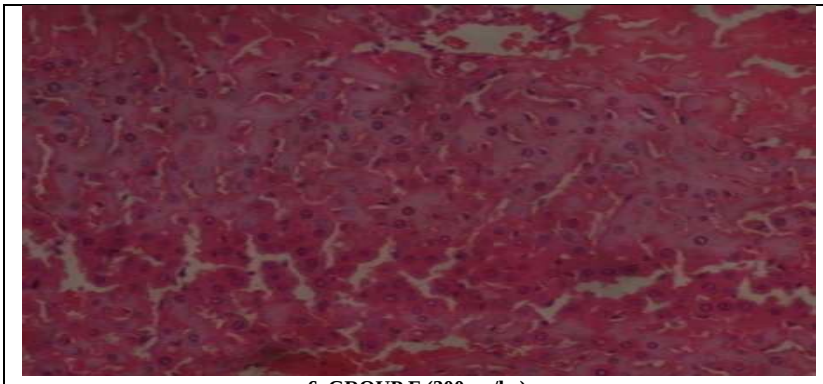
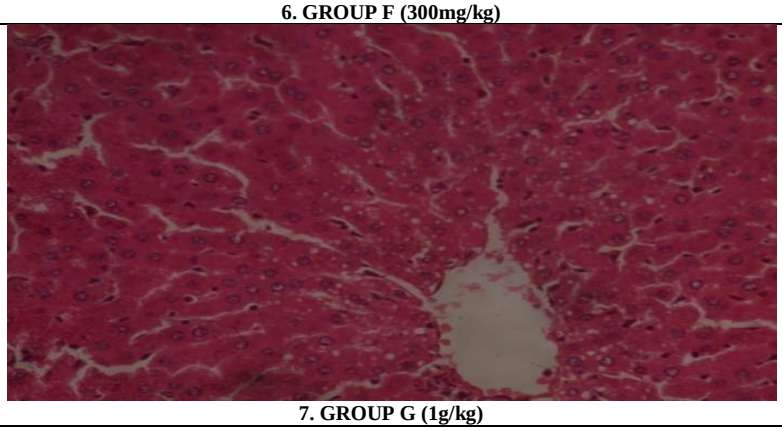
TABLE 1 Effect of crude extract of M.oleifera on serum enzyme activities of ccl_4 induced hepatotoxicity induce rats

Treatment	Dose $CCl_{4/M.olifera}$	ENZYME ACTIVITY		
		AST (U/l)	ALT (U/l)	ALP(U/l)
A (Control)	-------	90.55± 0.40	23.45±2.39	1.60±0.07
B	0.5ml/kg	87.50±1.91[a]	23.45±2.39[a]	1.63±0.10[a]
C	0.5ml/kg	135.86±3.00[b]	23.45±2.39[a]	1.68±0.10[b]
D	0.5ml/100mg/kg	117.05 ±1.20[b]	23.45±2.39[a]	1.68±0.09[b]
E	0.5ml/200mg/kg	117.23±3.86[b]	23.45±2.39[a]	1.20±0.14[b]
F	0.5ml/300mg/kg	114.64±7.04[b]	23.45±2.39[a]	1.13±0.10[b]
G	0.5ml/1g/kg	111.59 ± 7.97[b]	23.45±2.39[a]	1.48±0.13[b]

Values are means ±standard deviation (n=6 for each group)
a= values are not significantly different from control at P≤ 0.05
b= values are significantly different from control at P≤ 0.05

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

PLATES/ GROUPS	DESCRIPTION
 **1. GROUP A (CONTROL)**	Cords of hepatocytes with well preserved cytoplasm, not vacuolated, sinusoidal well demarcated, no area of necrosis, no fatty changes, no fatty degeneration.
 **2. GROUP B (OLIVE OIL)**	Cords of hepatocytes are distinct essentially normal, no fatty change, cytoplasm not vacuolated.
 **3. GROUP C (CCl_4)**	Hepatocytes are vacuolated, enlarged cytoplasm, nuclear darkly stained, and area shows extensive fatty change (steatosis), presence of necrosis.
 **4. GROUP D (100mg/Kg)**	Presence of steatosis mid-way between the portal tract and the central. Few inflammatory cells observed.
 **5. GROUP E (200mg/kg)**	Patchy areas of necrosis, prominent nucleoli, evidence of activity and regeneration. There is evidence of recovery although the liver has not reverted to normal.

 **6. GROUP F (300mg/kg)**	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 200mg/kg
 **7. GROUP G (1g/kg)**	There is no fatty change; cords of hepatocytes are well preserved. Sinusoids well demarcated and no area of necrosis. It is like the normal liver.

DISCUSSION

Carbon tetrachloride (CCl_4) is one common hepatotoxin used in the experimental study of liver disease (obi et al, 1998; Ulicna et al 2003; Yan-Junluo *et al*, 2004). In agreement with the report of previous workers (Reinke *et al*, 1988; Obi et al., 1998; Ulicna *et al*, 2003; Ragesh and Latha, 2004; Yan-Junluo *et al*, 2004), the results showed that CCl_4 caused an elevation in the serum content of ALT, AST and ALP. This indicates liver injury especially the rise in ALT activity (Lin and Wang, 1986; Reinke et al., 1988; Ngaha *et al*, 1989). Hence serum or plasma enzymes levels have been used as indices for monitoring chemically induced tissue damages. From the results of this study, it was observed that treatment of rats with ethanolic extracts of *Moringa oleifera* leaf in different percentage concentration after CCl_4 administration caused a significant reduction in hepatotoxicity for rats treated with CCl_4. This is evidenced in marked decrease in serum ALT, AST and ALP activities of those treated with *Moringa oleifera* extract ($P<0.05$) relative to the group treated with CCl_4 alone. This could be that since high level of the enzymes are associated with hepatic injuries probably; this cellular protection could be due to the presence of β-carotene in *Moringa oleifera* plants, a precursor of vitamin A (Greervani and Devi, 1981). The marked decrease in the activities of these three marker enzymes ALT, AST and ALP agrees with the studies carried out by other worker on CCl_4 hepatotoxicity of other herbal plants such as hibiscus rosasinenis (Obi *et al*, 1998); Rooibios Tea (Aspalathus Linearis) (Ulicna *et al*, 2003).

Hispathological studies as shown in plate 1-6 demonstrated that CCl_4 induced fatty degeneration, fatty change, distended hepatocytes compression of sinusoids and vacuolation of cytoplasm. These could be as a result of biochemical changes that occurred in liver cells. As observed in this study, increase in the liver enzyme activities (ALT, AST etc) was secondary to the liver dysfunction and is also associated with disruption of cellular structure and sometimes accompanied by high levels unconjugated billirubin as reported by Ekam et al., (2012). This is in agreement with the findings of Murakami, (1998). He also observed that xenobiotic caused an elevation of liver enzyme activity resulting to severe liver damage. Moringa oleifera of 10%, 20% and 30% doses in the treated groups generally showed significant improvement. Furthermore, it is interesting to know that 100% Moringa oleifera treated plate showed healthy liver cells as in the case with the normal control plate. This observation was in agreement with the biochemical test results. It has been commonly reported that Moringa plant is rich in compounds

containing the simple sugar, rhamnose and a fairly unique group of compounds called glucosinolates and Isothiocyanate, niazimicin, pterygosperm, benzyl isothiocyanate and 4-(a-rhamnopyranosyloxy) benzyl glucosinolate and a number of vitamins and minerals as well as other more commonly recognized phytochemicals such as carotenoids (β-carotene or pro-vitamin A) (Lowell, 1989). This could be the possible reason for its hepatoprotective activity. Although the biochemical mechanism of the protective effect of the leaf extract of M. oleifera against CCl_4 induced hepatotoxicity was not examined in this study, it is possible that the extract may have acted directly or indirectly in protecting the liver against damage. Directly, it may be breaking the sequence of events between the reductive dechlorination of CCl_4 and subsequent obstruction of hydrogen from unsaturated fatty acids in the membrane and peroxide formation or indirectly, it may be inhibiting the activities of the cytochrome P-450 isoenzymes (Sipes *et al*, 1977) required for trichloromethyl radical production or impair their elaboration. Conclusively, from the research findings, we can conclude that oral administration of ethanolic leaf extract of *Moringa oleifera* might have a hepatoprotective potential. Hence, *Moringa oleifera* leaf appears to be aid full in the management of hepatic inflammation or injury since it possess hepato protective potentials.

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