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HEPATOPROTECTIVE ACTIVITY OF STEM BARK EXTRACT OF *MANGIFERA INDICA* L. ON CARBON TETRACHLORIDE-INDUCED HEPATIC INJURY IN WISTAR ALBINO RATS

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ABSTRACT: The effects of aqueous stem bark extract of *Mangifera indica* in Carbon tetrachloride induced liver injury were investigated. In vivo animal models using Wistar albino rats was used in this experimental study. Carbon tetrachloride was successfully induced by significantly (P<0.001) increasing serum parameters AST, ALT, and ALP and decreasing serum total proteins and albumin levels (P<0.05). Standard antioxidant drug vitamin C at a dose of 50mg/kg body weight significantly (P<0.05) decreases the elevation of serum enzymes and increases the serum total proteins and albumin levels near to the normal control. It was found that the stem bark extract of *M. indica* exhibit hepatoprotective properties, as apparently observed from changes in the biochemical parameters by decreasing the elevated serum enzymes AST, ALP, and ALP and increasing the serum total proteins and albumin levels. The results of our present findings suggest that stem bark extract of *M. indica* provide effective protection against CCl_4-induced hepatotoxicity. It is therefore evident that *M. indica* can serve as a good source of effective antioxidant against liver injury.

INTRODUCTION: *Mangifera indica* L. (Mango) is one of the largest fruit crops in the world, which grows in tropical and subtropical regions [1] and different parts of the Mango tree has been widely used for the treatment of variety of diseases. *M. indica* is a member of the flowering plant family Anacardiaceae, native to tropical Asia and grown widely in different parts of Africa including Nigeria [2, 3].

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Research has shown that polyphenols from aqueous decoction of Mango leaves possesses antiulcerogenic [4]. A standardized leaf extract has shown to protect NIH/3T3 cells from oxidant-induced cell death [5], antioxidant and neuroprotective [6]. The fruit extracts has shown possess hepatoprotective activity [1] and seeds extracts with antibacterial activity [7]. However, a standardized aqueous stem back extract of *M. indica* is used in Cuba as antioxidant under the brand name VIMANG® showed a greater ability to reduce the formation of reactive oxygen species (ROS) in mice, prevented iron overload in serum as well as liver oxidative stress in rats. It has also shown to inhibit necrosis factor alpha (TNFα) and nitric oxide (NO) in endotoxic shock and microglia [8, 9, 10].

A report has shown that VIMANG® tablets prevent age-associated oxidative stress in elderly humans thereby improving the quality of life for elderly persons [11]. The stem bark extract (VIMANG®) provided significant better protection against TPA-induced oxidative damage when compared with other exogenous antioxidant Vitamin C, Vitamin E, mangiferin as well as β-carotene [12].

Phytochemical investigations of different parts of *M. indica* have been reported to contain flavonoids, phytosterols, tannins and steroids [6, 7]. Among the polyphenols constituents isolated from the leaves extract include mangiferin (C-glucopyranoside of 1, 3, 6, 7-tetrahydroxyxanthone), C-glucosyl-benzophenone (3-C-β-D-glucopyranosyl-4', 2, 4, 6 tetrahydroxybenzophenone) [4]. The stem bark is reported to contains gallic acid, 3,4-dihydroxy benzoic acid, methyl gallate, propyl gallate, mangiferin, (+)-catechin, (-)-epicatechin, and benzoic acid and propyl benzoate [13].

The aqueous decoction of the stem back has been traditionally used for the treatment of menorrhagia, scabies, diarrhea, syphilis, diabetes, cutaneous infections, anemia, malaria, fever, and dysentery [13, 3]. In the Northern parts of Nigeria, the decoction of the stem bark extract is traditionally used in the treatment of wound healing and anemia. Therefore, due to the excellent antioxidant properties of the stem bark extracts of *M. indica* L. previously reported attracted our attention to investigate the hepatoprotective activity of the stem bark extract *M. indica* L. in CCl_4-induced hepatotoxic rats.

MATERIAL AND METHODS:

Reagents: Paraffin oil was obtained from Rauda Pharmacy. Standard assay kits ware obtained from Randox Laboratory Ltd, UK. All other chemicals and reagents were of analytical grade and where obtained from Sigma-Aldrich Company Ltd.

Drugs: The stem bark extract of *Mangifera indica* L. was prepared by decoction in a polar solvent for 1h, concentrated by evaporation at reduced temperature and spray dried to obtain a fine powder. The solid extract was dissolved in distilled water for pharmacological studies [9]. The standard antioxidant drug vitamin C was supplied from Emzor Pharmaceuticals Ltd.

Animals and treatment: Thirty Wister rats of either sex (120-140g) were obtained from Ahmadu Bello University, Zaria and were kept at the animal house of Faculty of Pharmaceutical sciences, Usmanu Danfodiyo University under controlled environment at 22±2^{o}C. A 12 h light and 12 h dark cycle was ensured during which they were allowed to acclimatize under optimum feeds and water access for a period of 2 weeks before the commencement of the experiment. The animals were randomly divided in to six groups of five animals each ($n=5$) and all received the drug by oral feeding cannula. Two control groups received distilled water (1m/kg b.wt, p.o.) once daily for 8 days and the other received distilled water (1m/kg b.wt, p.o.) for 7 days and CCl_4 (0.6ml/kg b.wt in paraffin, p.o.) on the 8th day. One group received standard antioxidant drug Vitamin C (50mg/kg b.wt, p.o.) once daily for 8 days. The other groups received CCl_4 (0.6ml/kg b.wt in paraffin, p.o.) on the 1st day and test extract (25, 50 and 100mg/kg b.wt, p.o.) for 7 days. All the animals were sacrificed under diethyl ether anesthesia, blood sample were collected from each rat withdrawn from carotid artery at the neck in to labeled centrifuging tubes and allowed to clot for 30 min at room temperature [14]. The animal livers were quickly removed, weighed and preserved in 10% formalin solution for histopathological studies. All procedure followed were in accordance with the ethical standard of the European Union Guidelines for Animals Experimentation and approved by the Institutional Animal Care Committee.

Biochemical assessment: The serum was separated by centrifugation at 3000 rpm for 5 min for assessments of biochemical parameters. Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT) [15], Alkaline Phosphatase (ALP) [16] as well as total protein and albumin were estimated.

Histopathological assessment: A small portion of the liver samples of the animals from groups were fixed in 10% buffered formalin solution and then embedded in paraffin. Five micrometer were cut and stained with hematoxylin and eosin.

Statistical analysis: The results were expressed as means±SEM using one-way ANOVA followed by Dunnett's test for multiple comparisons.

RESULTS:

Effects of carbon tetrachloride on Serum biochemical parameters: Carbon tetrachloride significantly (P<0.001) increases serum parameters AST, ALT, and ALP (Fig. 1A) and decreases serum total proteins and albumin levels (P<0.05) in CCl_4 intoxicated rats as compared to the normal control (Fig. 1B).

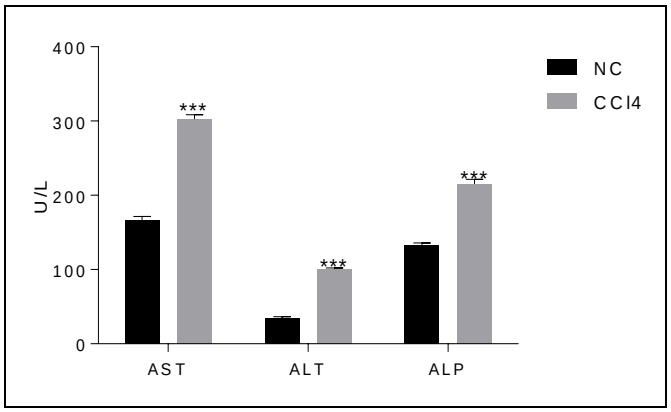


FIG. 1A: EFFECTS OF CCL_4 INDUCED HEPATOTOXICITY ON SERUM ENZYMES ASPARTATE AMINOTRANSFERASE (AST), ALANINE AMINOTRANSFERASE (ALT), AND ALKALINE PHOSPHATASE (ALP). Data are expressed as Mean±SEM, n =5, ***p<0.001, NC: Normal control.

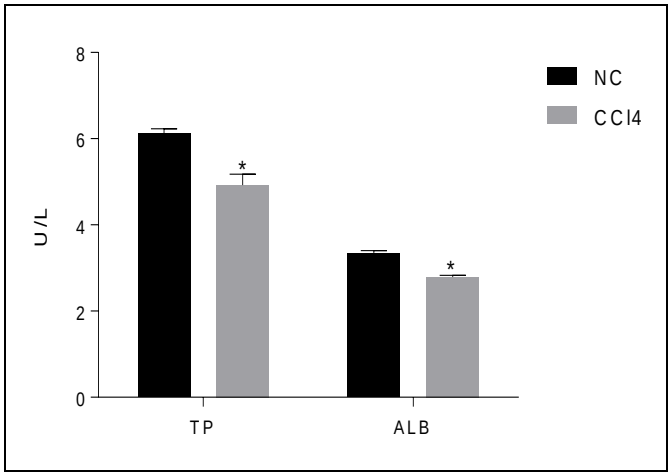


FIG. 1B: EFFECTS OF CCL_4 INDUCED HEPATOTOXICITY ON SERUM TOTAL PROTEINS (TP) AND ALBUMIN (ALB). Data are expressed as Mean±SEM, n =5, *p<0.05, NC: Normal control.

Effects of vitamin C on Serum biochemical parameters: Administration of standard antioxidant drug Vitamin C at a dose of 50mg/kg body weight significantly (P<0.05) decreases the elevation of serum enzymes and increases the serum total proteins and albumin levels which were significantly near to the normal control (**Fig. 2A and 2B**).

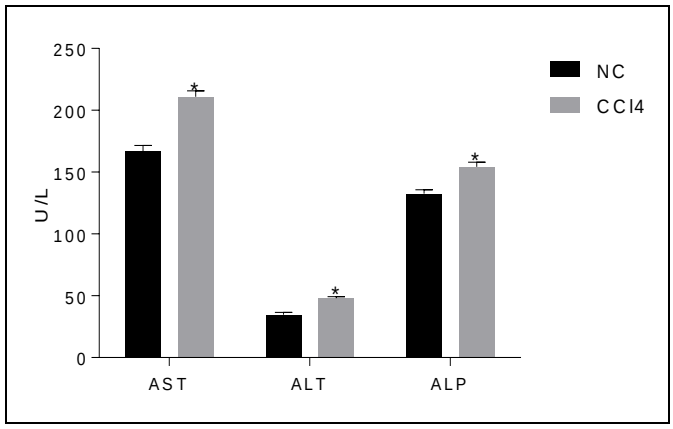


FIG. 2A: EFFECTS OF VITAMIN C ON SERUM ENZYMES ASPARTATE AMINOTRANSFERASE (AST), ALANINE AMINOTRANSFERASE (ALT), AND ALKALINE PHOSPHATASE (ALP) ON CCl_4 TREATED RATS. Data are expressed as Mean±SEM, n =5, *p<0.05, NC: Normal control.

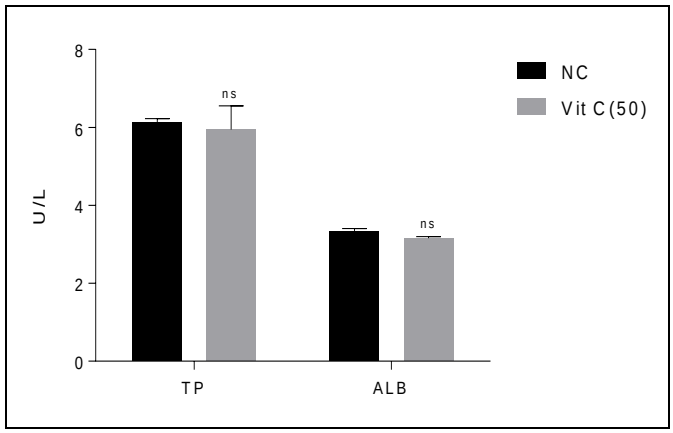


FIG. 2B: EFFECTS OF VITAMIN C ON SERUM TOTAL PROTEINS (TP) AND ALBUMIN (ALB) ON CCl_4 TREATED RATS. Data are expressed as Mean±SEM, n =5, ns: non significance compared to the normal control, NC: Normal control.

Effect of *M. indica* on Serum biochemical parameters: *M. indica* at lower dose (25mg/kg b.wt) did not show any significant changes in the elevation of serum parameters compared to the normal control.

Administration of *M. indica* to CCl_4 treated rats shows significant changes in the serum parameters at dose dependent by decreasing the elevation of serum enzymes levels and increasing the total proteins and albumin levels which were significantly near to the normal control (**Fig. 3A and 3B**).

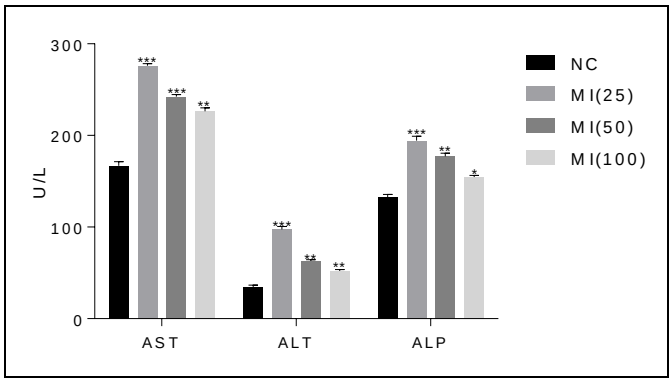


FIG. 3A: EFFECTS OF DIFFERENT DOSES OF *M. INDICA* ON SERUM ENZYMES ASPARTATE AMINOTRANSFERASE (AST), ALANINE AMINO-TRANSFERASE (ALT), AND ALKALINE PHOSPHATASE (ALP) ON CCl_4 TREATED RATS. Data are expressed as Mean±SEM, n =5, ***p<0.001, **p<0.01, *p<0.05, NC: Normal control.

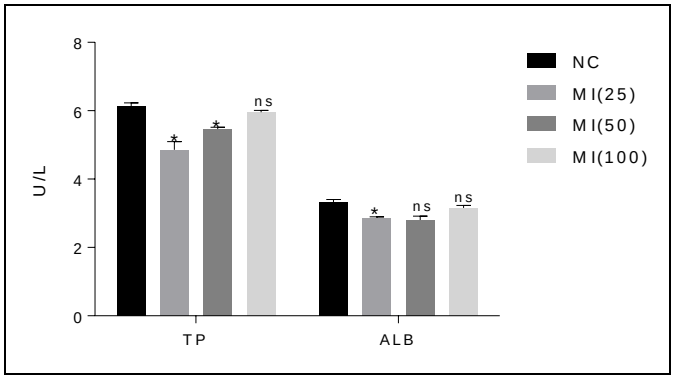


FIG. 3B: EFFECTS OF DIFFERENT DOSES OF *M. INDICA* ON SERUM TOTAL PROTEINS (TP) AND ALBUMIN (ALB) ON CCl_4 TREATED RATS. Data are expressed as Mean±SEM, n =5,*p<0.05, ns: non significance compared to the normal control, NC: Normal control.

Effect of carbon tetrachloride, vitamin C, and *M. indica* on liver histology: The hepatocytes of the normal control group showed preserved hepatic lobular architecture arranged in one to two cell thick cords separated by sinusoids. The portal and central areas showed vascular congestion and mild limphocitic infiltration within the lobules (**Fig. 4A**).

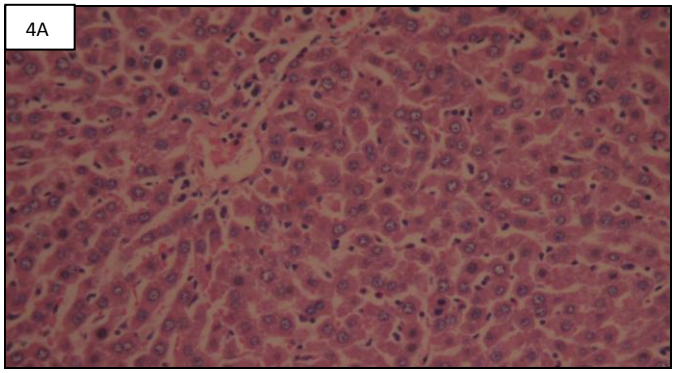


FIG. 4A: PHOTOMICROGRAPHS OF LIVER SECTIONS OF A NORMAL CONTROL RAT AT X200 MAGNIFICATION

A section of the liver tissue in CCl_4 controlled group showed preserved hepatic lobular architecture with marked congestion within the portal and central vessels. The hepatocytes showed severed or extensive balloon degeneration and micro-vesicles showing evidence of liver damage (**Fig. 4B**).

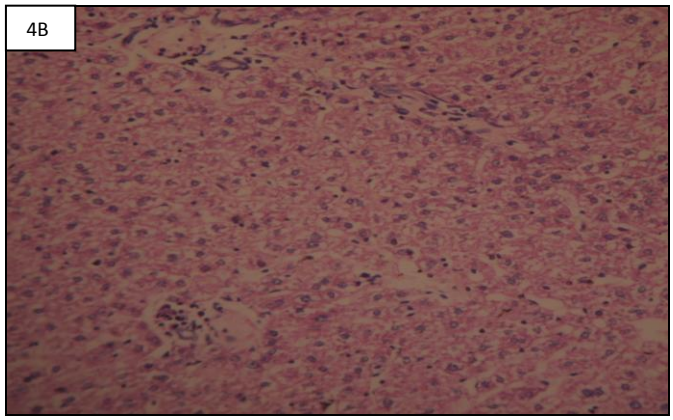


FIG. 4B: PHOTOMICROGRAPHS OF LIVER SECTIONS OF A CCL_4 INTOXICATED RAT AT X200 MAGNIFICATION

Vitamin C treated group showed preserved hepatic lobular architecture with mild congestion within the portal and central vessels. The hepatocytes showed moderate to marked balloon degeneration and microvessicules (Fig. 4C).

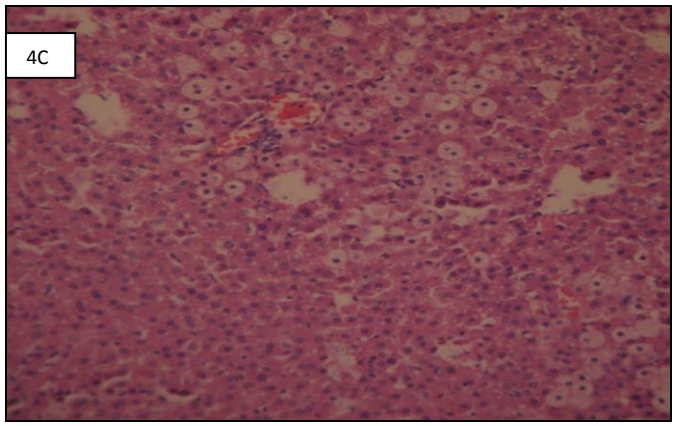
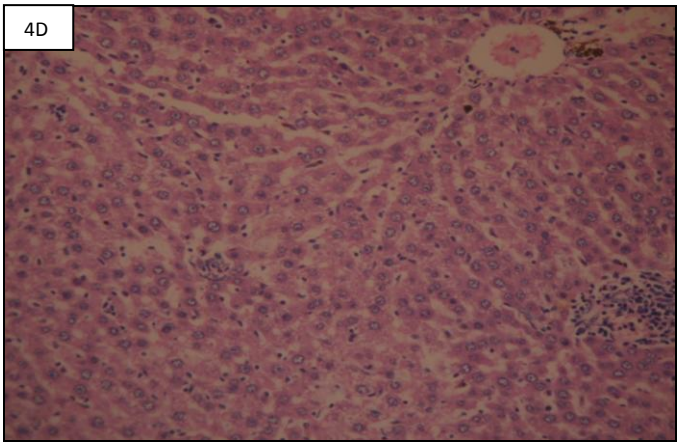
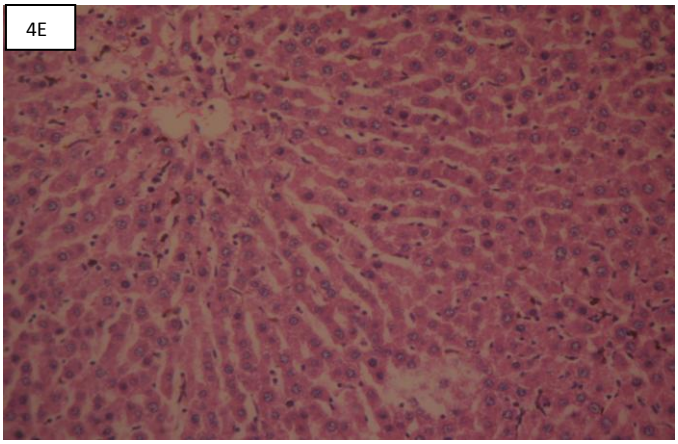


FIG. 4C: PHOTOMICROGRAPHS OF LIVER SECTIONS OF VITAMIN C TREATMENT ON CCL_4 INTOXICATED RAT AT X200 MAGNIFICATION.

The group treated with *M. indica* (25, 50 and 100mg/kg b.wt) showed preserved hepatic lobular architecture arranged in one to two cell thick. The portal and central areas showed vascular congestion some of which exhibit moderate cytoplasmic vacuolation. The hepatocytes showed mild to moderate microvesicular changes indicating possible reversible injury as shown in Fig. 4D, 4E and 4F.





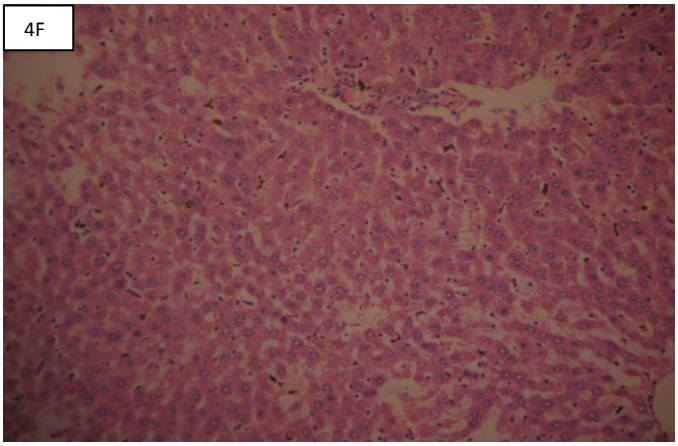


FIG. 4D, 4E, AND 4F: PHOTOMICROGRAPHS OF LIVER SECTIONS OF AQUEOUS STEM BACK EXTRACT OF *M. INDICA* (25, 50, AND 100MG/KG B.WT) TREATMENT ON CCL_4 INTOXICATED RAT AT X200 MAGNIFICATION

DISCUSSION: Liver injury due to chemicals or infectious agents may lead to progressive liver fibrosis and ultimately cirrhosis and liver failure [17]. Carbon tetrachloride (CCl_4) induced hepatotoxicity model is considered to be a valid animal model to test for hepatoprotective effects of drugs. The hepatotoxic effects of Carbon tetrachloride are largely due to its active metabolite, the trichloromethyl radicals.

These activated radicals bind covalently to the macromolecules and induced lipid peroxidative degeneration of biomembranes, leading to liver damage [18]. The rise in serum enzyme levels of AST, ALT and ALP as well as decreased in serum levels of total proteins and albumin has been attributed to the damaged structural integrity of the liver [14]. Although, serum enzyme levels are not a direct measure of hepatic injury, they show the status of the liver. However, the lowering of enzymes levels is definite indication of hepatoprotective action of the drug [19].

Our present findings also suggest that CCl_4 (0.6mg/kg b.wt) significantly increases serum AST, ALT, and ALP and decreases serum total proteins and albumin levels in CCl_4 intoxicated rats. Research has shown that standard antioxidant vitamins such as vitamin C decreases the activities of AST, ALT, and ALP while increasing the total protein in rats anesthetized with halothane [20].

However, hepatoprotective effects of vitamin C on sodium nitrite-induced lipid peroxidation in albino rats and on thioacetamide-induced liver cirrhosis in wister male rats has also been reported [21, 22]. It is therefore, evident that vitamin C, in our present findings provide antihepatotoxic effects on CCl_4-induced hepatotoxicity.

The fruit extract of *Mangifera indica* has been reported to protect liver injury associated with oxidative stress. However, our present study showed evident of reversible injury on CCl_4 intoxicated rats. The antihepatotoxic effects of stem bark extract of *M. indica* may be due to the presence of its main phytochemical constituent polyphenols which may react with trichloromethyl radicals thereby preventing activated radicals against lipid peroxidation.

Polyphenolic compound in plant extract has been known to act as free radicals scavengers thereby exerting antioxidant activities. A standardized extract of *M. indica* polyphenols has been shown to provide effective protection on in vitro human T cells AICD by diminishing the increase intracellular ROS and free Ca^{2+} induced by T cell receptor (TCR) triggering [23]. The stem bark extract used in Cuba as antioxidant under the brand named VIMANG® has been shown to provide significant better protection against TPA-induced oxidative

damage when compared with the standard antioxidant vitamin C [12]. This is a clear evident to our present histopathological finding which showed stem bark extract of *M. indica* at 50 and 100mg/kg b.wt provide better protection against liver injury when compared with the standard antioxidant vitamin C.

CONCLUSION: The results of our present study suggest that stem bark extract of *M. indica* provide effective protection against CCl_4-induced hepatotoxicity. However, we can suggest that the anti-hepatotoxic effect may be due to the free radical scavenging activities of the polyphenolic constituents present in the plant extract. It is therefore evident that *M. indica* can serve as a good source of effective antioxidant against liver injury.

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