IOSR Journal of Applied Chemistry (IOSR-JAC)
e-ISSN: 2278-5736.Volume 8, Issue 8 Ver. II (Aug. 2015), PP 114-117
www.iosrjournals.org

Evaluation of the antioxidant and hepatoprotecive activities of Manilkara obovata seed extract in murine models

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Abstract: *Several medicinal plants are currently used to detoxify the liver and to protect the hepatocytes from effects of poison including drugs and chemicals. Certain drugs and chemicals have contributed to the degeneration and inflammatory diseases of the liver. Manikara obvata seeds are among the vast of natural remedies for liver degeneration. Therefore, the present study evaluated the antioxidant and hepatoprotective activities of methanolic and petrolueum ether extracts of Manikara obovata seed against carbon tetrachloride (CCl_4)-induced hepatic damage in rats. Thesubstantially elevated serum enzymatic levels of serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), serum alkaline phosphatase (ALP) and acid phosphatase (ACP) were restored towards normalization by the extracts when compared with the CCl_4-intoxicated control rats. The extracts also protected against CCl_4 induced lipid peroxidation. The acute toxicity, $LD_{50,}$ was also determined to be greater than 5000mg/kg. The results showed that the extracts from the seed of Manikara obovata posses significant antioxidant and hepatoprotective activities.*

Keywords*: Manilkara obovata seed, antioxidant, hepatoprotective, lipid peroxidation, acute toxicity*

I. Introduction

Plants produce a variety of chemical substances and some have therapeutic values. Most of the useful medicinal plants are yet to be authenticated or known. Research in traditionally used medicinal plants has lunched many saved therapeutic plants product but more are needed due to variable disease factors.

Oxidative stress is a threat to the integrity of essential biomolecules such as DNA, RNA, enzymes, proteins andphospholipids responsible for membrane integrity due to the presence of highly reactive species like superoxide, peroxide, hydroxyl and free radicals. The oxidative stress may be due to depletion of endogenous antioxidants or increased formation of the free radicals and other reactive species (1-3). Equally, factors like malnutrition, genetic mutation; direct exposure to toxins like alcohols,cigarette smoke, and halogenated hydrocarbons; tissue injury like burns, ischemia and surgery; bacterial and viral infections cause oxidative stress(4-6). Oxidative stress is linked to many diseases including cardiovascular disease, atherosclerosis(7), malaria (8), inflammation, cataractogenesis(9), aging(10), cancer(11), rheumatoid arthritis (12), sickle cell anaemia(13), neurodegenerative disease(14) and diabetes(15).

Manilkara obovata (family Sapotaceae) is widely distributed in tropical Africa. It is found in lowland, riverine and ground water forest. It is a tree that grows up to 14 m or more and has dark brown bark. The leaf is characterized by the presence of reddish-brown hairs underside. The fruits are edible. The fruit and stem bark are used as spice for cooking. The plant is used as in traditional medicine for treatment of cardiovascular disorders (16, 17). There are many species of this genus but no known antioxidant and hepatoprotective effects of Manilkara obovata has been reported. This work studied the antioxidant and hepatoprotective effects of different fractions of Manilkara obovata seed.

II. Materials and methods

Animals: Both sexes of mice (20-30g) and male wistar albino rats (100-250g) were used for the experiment. The animals were obtained from animal house of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. They were housed in white metallic cages and fed with standard feed and water.

Plant: The seeds of manilkara obovata were purchased from Ogbete main market, Enugu. They were authenticated by Mr. A. Ozioko of the International Centre for Ethno-medicine and Drug Development (InterCEDD), Aku Road, Nsukka, Enugu State, Nigeria. The seeds were dried, pulverized and stored in a dry air-tight container. The voucher specimen of the seed is deposited at the Herbarium of Department of Pharmacognosy, University of Nigeria, Nsukka.

Extraction: A 300g of the powdered seed was exhaustively and successively extracted with petroleum ether and methanol using Soxhlet extractor. The solvents were distilled off and the methanol extract was fractionated

using column packed with silica gel G 60 and eluted successively and exhaustively with chloroform and acetone saturated with HCl to obtain two fractions (F_1 and F_2) respectively.

Acute toxicity: Thirteen mice wereused while the method of Lorke(18) was employed. Nine mice were divided into three groups of three mice each and were given 10, 100 and 1000 mg/kg of the methanolic extract intraperitoneally respectively. One mouse received the vehicle (10% tween-80). They were observed for 24 hours and the number of deaths recorded. In the second phase doses of 1600, 2000, 2900 and 5000 mg/kg of the extract were administered to four mice respectively. The mice were observed for 24 hours and the number of deaths recorded. The LD_{50} was estimated.

Assay for antioxidant and hepatoprotective effects.

The rats were divided into 11 groups of three mice each. Suspensions of the extracts were homogenized by shaking before use. Doses of 200 and 500mg/kg of each of the petroleum ether and methanol extracts, fractions F_1 and F_2 and 1000 IU/kg of vitamin E were given to 9 groups respectively. The administration of the extract was once a day orally for 4 days. Two groups received water. On the third day, 0.75ml/kg of carbon tetrachloride was administered to each of the animals except one group that was given only water. On the fifth day, all the animals were sacrificed and blood samples collected via ocular puncture (19) with sterile capillary tubes into EDTA bottles. The blood samples were centrifuged at 3000 rpm for 10 minutes. The resultant sera were collected and analyzed for total serum, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and acid phosphatase (ACP). The antioxidant effect was determined by estimating thiobarbituric acid reacting substances (TBARS) (20-22).

III. Results and Discussion

The petroleum ether and methanol extractions gave percentage yields of 7.6 and 35% respectively. The petroleum ether extract was light brown liquid and the methanol extract was viscous brown solid. Each possessed sweet smell. The result showed an LD_{50}greater than 5000mg/kg. The effects of ingestion of different doses of different fractions of the seed on the serum enzymes and malondialdehyde (MDA) concentrations were shown inTable 1.

Table 1: The effects of different doses of the extracts/fraction and vitamins E on various liver function markers in rats injected with carbon tetrachloride.

Agents	Dose (mg/kg)	AST(UI)	ALT(UI)	ACP(UI)	ALP(UI)
Methanol extract	200	16.0±7.0	16.25±3.75	25.25±5.0	62.10±6.9
	500	13.0±3.00	12.50±5.0*	17.7±3.53*	60.45±10.7
	1000	11.0±4.0*	11.25±3.75*	15.15±5.05*	57.70±2.50*
Petroleum extract	200	17.03±0.55	27.0±3.89	25.48±3.34	68.0±3.34
	500	18.75±1.5	13.8±0.9*	12.40±0*	44.67±0*
F_1	200	25±0.55	27.6±13.8	29±1.35	74.7±3.3
	500	20.7±1.50	17.4±6.8	19.8±0.2	73.8±0
F_2	200	18.6±0.5	18.37±5.5	37.3±3.4	81±5.50
	500	16.44±1.02	17.4±4.5	35±1.88	105±4.5
Vitamin E	1000IU	13±0.00*	16.62±3.13*	20.2±0.00*	56.6±1.40*
Normal (water)	1 ml	10.00±3.00	10.62±3.13	20.20±0.00	56.60±1.40
CCl_4	0.75ml/kg	18.50±8.50	20.00±5.00	39.40±1.30	96.60±2.80

All values are mean ±SEM, n = 3 rats in each group. $p<0.05$ as compared with normal control
F1 = Chloroform fraction of methanol extract; F2 = Acetone-HCl fraction of methanol extract
AST = aspartate aminotransferase, ALT = alanine aminotransferase; ALP = alkaline phosphatase; ACP = acid phosphatase

Table 2: Malondialdehyde (MDA) levels in rats treated with extracts of Manikara obovata seed

Agents	Dose (mg/kg)	MDA(µg/ml)
Methanol extract	200	18.47±5.43
	500	13.52±2.09*
Petroleum extract	200	31.09±9.31
	500	15.37±0.8*
F_1	200	32.7±13.6
	500	20.35±0.75
F_2	200	46.67±2.88
	500	27.75±13.2
Vitamin E	1000IU	7.48±0.88*
Normal (water)	1 ml	8.33±2.23
CCl_4	0.75ml/kg	41.55±20.40

All values are mean ±SEM, n = 3 rats in each group. $p<0.05$ as compared with normal control
Seeds of M. obovata exhibited antioxidant and hepatoprotective effects and was found to be relatively safe with an LD_{50} greater than 5000mg/kg.

There was a significant reduction ($p<0.05$) of the melanodialdehyde(MDA) in the serum of the rats treated with 1000 mg/kg and 500mg/kg of the methanol and 500mg/kg petroleum ether extracts when compared with the group that received the toxicant (CCl_4) only. This reduction in MDA caused by the extracts was dose dependent, the higher the dose, the more the reduction. F_2 did not show any effect while F_1 had moderate effect on the MDA reduction when compared with the methanol extract. These indicate significant role the extract can play in reduction of lipid peroxidation caused by free radicals.

A 0.75mg/kg of CCl_4 was found to cause drastic effects on the liver of the rats as the enzyme biomarkers for hepatitis were relatively high when compared to those of the rats given only water. Increase in the levels of the liver function serum enzymes above the normal depictsliver damage and the ability of the administered extracts to reduce the levels in the presence of a toxicant (CCl_4) shows its hepatoprotective ability. The acute hepatotoxicity of CCl_4 lies in its biotransformation to trichloromethyl free radical (CCl_3) or trichloroperoxyl radical ($CCl_3O_2^-$) produced by the mixed-function cytochrome P450 oxygenase system of the endoplasmic reticulum, which causes oxidative stress and membrane damage[22]. There were dose dependent drastic reduction ($P<0.05$) in the levels of the liver function enzymes (ACP, ALP, AST and ALT) of the rats fed with methanol and petroleum extracts when compared with those of the rats given CCl_4only. The results were comparable with enzyme markers in rats given vitamins E. F_2 had no effect. Oral administration of methanol extract of Manilkara obovate seed to mice which have received a LD_{50} dosage of CCl_4 resulted in a significantly reduced mortality rate. Since the pathological effect of CCl_4 in the animals has been proved to be mainly restricted to the liver and lethality of high-dose CCl_4 is mostly related with organ failure following acute liver failure instead of direct injury to other organs[23], it is reasonable to hypothesize that administration of methanol and petroleum extracts of Manilkara obovate seed can reduce animal mortality mainly through attenuating acute liver damage by CCl_4, and facilitating the preservation and restoration of liver functions.

Islam et al (2012) has reported the antioxidant activity of the ethanol extract of Manilkarazapota Leaf[23]. Mital Kaneria and Sumitra Chanda (2012) also collaborated their findings[24]. However, no known antioxidant and hepatoprotective activity of Manilkara obovate seed has been reported.

IV. Conclusion

We found out that the seed of Manilkara obovate possesses strong beneficial effects in a mouse model against acute liver injury caused by CCl_4. The holistic formulation of the seed as a food supplement can be very beneficial in protecting the liver against oxidants. Also the protective effect of Manilkara obovate seed represents a clinical potential in the development of novel therapeutic agents for acute liver injury.

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