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Nicotine Induced Liver Toxicity in Wistar Albino Rats: Protective effects of Aqueous Extract of Moringa Olifera (Lam)

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Keywords: moringaolifera, nicotine, hepatotoxicity, antioxidant property, lipid profile.

I. Introduction

The liver is the key organ regulating homeostasis in the body. It involved with almost all the biochemical pathway related to growth, fight against disease, nutrient supply, energy provision and reproduction. The liver is expected not to only perform physiological functions but also to protect against hazards of harmful drugs and chemicals. In spite of tremendous scientific advancement in the field of chemoology in recent years, liver problems are on rise. Cancer is a major disorder that account high death rate.

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Presently a few chemoprotective drugs and that too from natural sources, are available for the treatment of liver disorder. Liver toxicity is an inflammation of your liver in reaction to certain substances to which you're exposed. Liver toxicity can be caused by alcohol, chemicals, drugs or nutritional supplements. In some cases, liver toxicity develops within hours or days of exposure to a toxin.

Nicotine causes vasoconstriction and increased blood pressure[1]. A drop in blood-flow velocity in humans, endothelial damage in rats, and inhibition of platelet aggregation in rabbit's blood, have all been shown experimentally[1]. Nicotine has been reported to up regulate the expression of various proteins such as basic fibroblast growth factor, tumor necrosis factor -α and plasminogen activator inhibitor-1[2]. In addition, nicotine induces mononuclear leukocyte adhesion and expression of adhesion molecules such as vascular cell adhesion molecule-1 and intracellular adhesion molecule in endothelial cells[3]. Most clinical and experimental investigations of the pathophysiology of cigarette smoking have studied the effects of smoke as a whole, while a few studies focused on specific components of cigarette smoke, e.g. nicotine[4]. Nicotine exposure via cigarette smoking has been implicated in pathogenesis of cardiovascular disorders like atherosclerosis and hypertension[1].

Among myriad of plants, Moringaoleifera is one of the best known and most distributed species of Moringaceae family. Moringa is an important tropical crop that is used as human food, medicine and in oil production[5]. Leaves of this plant are traditionally known for or reported to have various biological activities, including hypocholesterolemic agent[6], regulation of thyroid hormone status[7], antidiabetic agent[8], gastric ulcers[9], antitumor agent[10], antihyperglycemic[5] and hypotensive agent[11]. The leaves as well as the flowers, roots, gums, fruits and seeds are extensively used for treating inflammation[12], cardiovascular action, liver disease[13] and hematological, hepatic and renal function[14]. It is generally known in the developing world as a vegetable, a medicinal plant and a source of vegetable oil[15]. Epidemiological studies suggest that specific pharmacologically active agents present in the diet might reduce the relative risk of cancer development[16]. A remarkable surge of interest in

chemoprevention research has led to the identification of many phytochemicals of dietary origin as effective potential chemo preventive agents[10]. Many complications occur as a result of nicotine exposure and its consequence in major and important organs in the body like liver; hence this study was designed to investigate the effect of aqueous extract of Moringaoleiferaleafon nicotine induced hypertension in the liver using wistar albino rats.

II. Materials and Methods

a) Collection and Extraction

The fresh leaves of Moringaolifera was obtained fromIworoko-Ekiti community, Ekiti State and was authenticated at Department of Plant Science, Ekiti State University, Ado-Ekiti.

The leaves were air dried and pulverized. 20% aqueous extract was prepared using distilled water.

b) Animals

Male albino rats of (180-240 g) were used throughout the experiments. Four rats per group(The animals were procured from the Animal House of College of Medicine, Ekiti State University, Ado-Ekiti). The rats were acclimatized for a period of 10 days under standard environmental conditions such as temperature (26 - 30oC), relative humidity (45-55%) and 12 hours dark/light cycle. All the animals were fed with rodent pellet diet and water was allowed ad-libitum under strict hygienic conditions.

c) Experimental Design

The rats were divided into five groups, each consisting of six rats:

Group 1: Normal albino rats treated with normal saline.
Group 2: Nicotine induced rats(1mg/kg body weight).
Group 3: Nicotine induced rats treated with Standard drug (Lisinopril).
Group 4: Treated with 0.5ml of 20% aqueous extract of Moringaoleifera.
Group 5: Nicotine induced rats treated with 0.5ml of 20% aqueous extract of Moringaoleifera.

The nicotine induced groups rats were induced with nicotine intraperitoneally at every other day.

Interval with nicotine (1mg/kg body weight) in normal saline for 21 days to induce hypertension.

d) Preparation of Organs Homogenate

At the completion of the experiment, the rats were quickly dissected; the liver was removed.

10% of the organ homogenate was then prepared in 0.25M sucrose solution using the Teflon homogenizer. The homogenate was centrifuged at 10,000rpm for 10 minutes at 40C to obtain a clear supernatant which was stored at 80C and used for measurement of biochemical contents.

Plasma sample was prepared from the whole blood collected from the heart into EDTA bottles and spinned at 3000 rpm.

e) Biochemical Analyses

Biochemical parameters like aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), creatine kinase (CK), total protein, malondialdehyde (MDA),superoxide dismutase (SOD), catalase (CAT), glutathione (GSH); some lipid profiles like triglyceride, HDL-Cholesterol, LDL-Cholesterol and Total Cholesterol were analysed according to the standard methods.

f) Statistical Analysis

The values were expressed as mean± SD. Statistical analysis was performed by one way analysis of variance (ANOVA) followed by Duncan multiple comparison tests. p value <0.05 were considered as significant.

III. Results and Discussion

Table 1: Effect of Aqueous Extract of Moringaoleifera on Some Biomarker Enzymes (U/L) in Nicotine-Induced Diabetic Rats.

	AST		CK		ALT		ALP	
	Plasma	Liver	Plasma	Liver	Plasma	Liver	Plasma	Liver
1	6.785 ± 2.71b	7.24 ± 0.68ab	4.464 ± 1.98b	3.264 ± 4.46b	4.943 ± 0.78b	8.145 ± 1.29a	21.342 ± 4.22a	17.455 ± 2.45c
2	8.382 ± 1.36c	6.66 ± 0.72a	7.338 ± 0.06a	1.264 ± 3.33b	12.11 ± 1.01b	6.404 ± 1.01c	36.347 ± 3.58d	8.352 ± 1.34a
3	6.272 ± 0.67b	8.297 ± 0.49b	3.654 ± 0.52a	3.887 ± 3.65b	5.082 ± 0.8bc	9.676 ± 1.53a	25.345 ± 3.94c	15.541 ± 1.39c
4	4.795 ± 0.19a	12.19 ± 0.59c	2.944 ± 0.54a	3.121 ± 0.22a	2.714 ± 0.43a	14.133 ± 2.23b	16.32 ± 1.39a	26.231 ± 3.55d
5	3.277 ± 1.03a	9.214 ± 7.91b	3.972 ± 0.56b	4.225 ± 3.97c	5.151 ± 0.8bc	8.494 ± 1.34a	25.342 ± 2.49c	16.632 ± 2.34b

Each value is a mean ± SD., n = 3. Values not sharing a common superscript (a-c) differ significantly with each other (P < 0.05) in all the groups

Table 1.0 showed the results of aqueous moringaolifera extracts on some biomarkers (AST, CK, ALT&ALP).In the assessment of the liver damage by Nicotine, determination of AST, CK, ALT and ALP is largely used. This study assessed the effect of nicotine-induced toxicity on the activity of ALP, AST, ALT on the plasma and liver. Generally, necrosis or membrane damage releases the enzyme into circulation and hence it can be the plasma[17]. ALP and CK occur in most tissues of the body as an isoenzyme such as the liver, kidney, bone, placenta and intestine etc. ALP is diagnostic of bone or liver disease or a tumor in these organs;it is found in liver cells and is associated with osteoblastic activity in the bone[18]. As shown in Table 1, from the results obtained in this study, it was observed that there was a significant increase in the activity of ALP in the plasma of animals in group induced with nicotine (group 2) compared to the control and reduction in the enzyme liver level. Healthy and active persons show higher values of serum CK activity. Moreover, CK values are lower in women than men and are usually lower in the morning than in the evening[19]. Significant increase were also observed for ALT and AST in the plasma of nicotine induced group with concomitant reductions in the liver, significant reduction in the concentrations of ALT and AST enzymes were recorded in this study. (This may be attributed to loss of membrane components due to a possible reaction between the drug (nicotine) and the liver tissues. Therefore, enzymes from diseased organs may become manifested in the plasma resulting in increased activity since they must have leaked from the diseased organ. A treatment with the administration of aqueous extract of the plant and the standard drug bring about a significant reduction in the enzymes plasma level, significant restoration towards the control values and significant rise in the biomarker enzyme activities in the liver cells were observed. Nicotine-intoxication caused a significant increase in plasma CK of rats when compared with normal. Furthermore, liver creatinine kinase levels decreased significantly in untreated nicotine induced rats. However, administration of the plant extract significantly reversed the adverse effects of nicotine on both the plasma and liver of the animals. In standard drug treated hepatotoxic rats, CK level in the plasma significantly decreased when compared to normal. Extract alone was able to reverse the nicotine-induced increase in plasma CK levels to value that were statistically similar to normal.

The extract of the plant and lisinoprilsignificantly reversed these changes toward the control ones and minimized the adverse effects of nicotine (Table 1). These findings are similar to the report of 20 that nicotine causes disruptions to membrane of organs thereby compromising the membrane integrity.

Table 2: Effect of Aqueous Extract of Moringaoleiferaon some Antioxidant Enzymes against Nicotine-Induced Hepatoxicity in Rats

	MDA X 10-7		SOD		CAT		GSH	
GP	Plasma	Liver	Plasma	Liver	Plasma	Liver	Plasma	Liver
1	3.008 ± 1.8b	6.692 ± 3.57c	9.120 ± 1.09c	7.680 ± 83.84c	0.003 ± 0.00c	0.002 ± 0.00c	14.561 ± 2.2bc	9.34 ± 1.76b
2	7.897 ± 1.52c	2.662 ± 1.13b	5.665 ± 0.54b	4.267 ± 5.54b	0.001 ± 0.00a	0.001 ± 0.00b	4.28 ± 1.03a	3.12 ± 0.76a
3	3.627 ± 0.55b	3.256 ± 1.41b	14.312 ± 0.19a	2.133 ± 9.81a	0.001 ± 0.00a	0.0004 ± 0.00a	12.855 ± 2.32b	7.49 ± 2.6ab
4	2.505 ± 0.64a	1.708 ± 0.46a	12.509 ± 0.29a	5.760 ± 5.33b	0.002 ± 0.00b	0.002 ± 0.00c	15.322 ± 3.43c	14.35 ± 4.8c
5	2.559 ± 0.41a	1.281 ± 0.89a	14.193 ± 1.19c	2.290 ± 1.29a	0.002 ± 0.00b	0.001 ± 0.00b	12.545 ± 2.45b	9.43 ± 2.09b

Each value is a mean ± SD., n = 3. Values not sharing a common superscript (a-c) differ significantly with each other (P < 0.05) in all the groups

From table 2.0, induction of nicotine with the animals in group 2 there is a significant increase in the levels of lipid perioxidation; as a result of enhance lipid perioxidation leading to tissue damage and failure of antioxidant defense mechanism to prevent formation of excessive free radicals and decrease in the levels of antioxidant (GSH) and antioxidant enzyme (SOD and CAT) when compared to the control animals. Decrease in enzyme activity of superoxide dismutase (SOD) is a sensitive index in hepatocellular damage and is the most sensitive enzyme index in liver injury. These antioxidant enzymes are significantly decreased in the organ (liver) and plasma due to the inadequacy of the antioxidant defenses in combating ROS mediated damage and when they are treated with aqueous leave extract of the plant, the activities of these enzymes was increased and may help to control the free radicals when compared to the hypertensive rats and the effect produced by aqueous leave extract of the plant was comparable with that of standard drug Lisinopril. Treatment with aqueous extract of Moringaoleifera leaf and lisinopril was found to produce a significant

decrease in TBARS and increase in GSH, catalase (CAT) in the liver homogenate of the hypertensive rats. The effect of aqueous extract of Moringaoleifera leaf on group not induced with nicotine showed significant increase in the levels of superoxidedismutase (SOD), catalase (CAT) and GSH and reduction in lipid perioxidation. MDA level is the most important factor indicating increased peroxidative level. Enzymatic antioxidants are important antioxidant for scavenging free radicals. From the figure, these reports suggest that doxorubicin produces renal, cardiac and hepatic injury. The major role of catalase (CAT) is to scavenge H2O2 that has been generated by free radicals or by superoxide dismutase (SOD) in its removal of superoxide anions, and convert it to water[21]. And significant reduction in concentration of superoxide dismutase (SOD), GSH and catalase (CAT) were recorded in the group treated with nicotine.

Table 3: Effect of Aqueous Extract of Moringaoleifera on Some Lipid Profile (mg/dl) in Nicotine-Induced Hepatotoxicity Rats

	Triglycride		HDL-Cholesterol		Total Cholesterol		LDL-Cholesterol	
GP	Plasma	Liver	Plasma	Liver	Plasma	Liver	Plasma	Liver
1	291.59 ± 20.4a	285.86 ± 26.11a	32.98 ± 5.04b	19.70 ± 4.58a	15.804 ± 1.69a	14.68 ± 6.01a	26.37 ± 3.41b	14.49 ± 2.44a
2	440.28 ± 20.4d	335.33 ± 25.55c	24.99 ± 5.83a	18.72 ± 5.83a	29.067 ± 3.04c	30.41 ± 8.44c	67.37 ± 6.43d	21.34 ± 2.58c
3	395.95 ± 35.0b	319.63 ± 39.12b	49.50 ± 11.5c	30.44 ± 0.44c	18.986 ± 3.3ab	22.02 ± 5.32b	34.74 ± 3.55c	17.44 ± 3.38b
4	414.66 ± 11.2c	348.92 ± 36.94c	22.48 ± 2.46a	20.66 ± 4.82a	22.044 ± 0.78b	34.61 ± 16.0d	18.43 ± 2.53a	11.644 ± 1.48a
5	419.33 ± 7.12c	461.79 ± 26.60d	21.14 ± 4.9a	20.83 ± 2.0ab	25.030 ± 3.34c	32.51 ± 7.7cd	17.245 ± 2.44a	37.25 ± 4.56d

Each value is a mean ± S.E.M., n = 3. Values not sharing a common superscript (a-c) differ significantly with each other (P < 0.05) in all the groups

Table 3 shows the levels of total cholesterol, triglycerides, HDL-cholesterol and LDL-cholesterol in plasma and hepatic tissues of experimental animals. The levels of total cholesterol, and LDL- cholesterol in plasma and the liver of experimental animals increased significantly in the nicotine treated group. Administration of nicotine caused a significant increase in the levels of triglycerides in the plasma and in the liver, compared with positive control rats. This shows the hyperlipideamic effect of nicotine. However, after the rats were treated with the extract, significant improvement in the levels of triglycerides was observed in the plasma. Hypertriglyceridemic patients are at a risk for cardiovascular disease often develops a lipoprotein profile characterized by elevated triglyceride, dense LDL, and low HDL cholesterol which causes myocardial membrane damage. Significant reduction, similar protection and biochemical restoration of levels of Total cholesterol, LDL-cholesterol and Triglyceride were seen in the plant extract and standard drug (Lisinopril) and nicotine treated animals. The group, given only the extract showed the protective potential and effectiveness of the plant in both plasma and the liver. The study suggests that the intake of the extract decreases the absorption of triglycerides and cholesterol, and these findings are in accordance with the report of22that reported Green tea intake also decreases the absorption of triglycerides and cholesterol.

IV. Conclusion

The present study indicates that a decrease in the antioxidant status is one of the main factors contributing to nicotine toxicity to the Liver. The observed significant increase in the LPO and oxidative stress markers and lipid profile in the liver of nicotine-induced animals, suggests that the tissues are subjected to increased oxidative stress. Reversible oxidative/antioxidant, biomarker enzymes and the lipid profile modifications were observed when treated with the aqueous extract of the plant. Treatment found to remove the continuously generated free radicals, to prevent the endogenous antioxidant enzymes decrease and act to prevent oxidative cell damage induced by nicotine.

Conflict of Interests

The authors declare that no conflict of interest exist.

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