

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

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Abstract

Aqueous extract of Moringaolifera (Lam) was evaluated for protective and antioxidant activities in rats. The plant extract showed a remarkable chemo-protective activity on nicotine induced liver toxicity as judged by serum maker enzyme and some antioxidant levels in the liver of male albino rats weighing between 180 and 200g. The animals were grouped into five of six rats each which were intraperitoneally induced with nicotine at 1mg/kg body weight except the control group. Some biochemical parameters (Aspartate aminotransferase (AST), Alanineaminotransferase (ALT), alkaline phosphatase (ALP), creatinine kinase malondialdehyde (MDA)); some antioxidant indices and lipid profiles were monitored. Induction of nicotine produced a significant increase in the level of malondialdehyde and decrease in the levels of superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH) as compared to the control animals. Treatment with aqueous extract of Moringaoleifera leaf was found to produce a significant decrease in TBARS and increase in GSH, SOD and CAT in the plasma and liver homogenate of the nicotine induced rats. The levels of total cholesterol, triglycerides, HDL-cholesterol and LDL-cholesterol in plasma and hepatic tissues of the experimental animals were also monitored. Significant protection was seen in the extract and standard drug (Lisinopril) treated nicotine induced animals. Administration of aqueous extract of the plant brings about a significant restoration towards the control values. The results however showed the protective and antioxidant effects of the extract against nicotine-induced liver toxicity.

Index terms— moringaolifera, nicotine, hepatotoxicity, antioxidant property, lipid profile.

1 Nicotine Induced Liver Toxicity in Wistar Albino

Rats: Protective Effects of Aqueous Extract of Moringa Olifera (Lam)

Oseni Olatunde Abass[?], Akindolire Olajumoke[?] & Musbau Aderonke[?] Abstract-Aqueous extract of Moringaolifera (Lam) was evaluated for protective and antioxidant activities in rats. The plant extract showed a remarkable chemo-protective activity on nicotine induced liver toxicity as judged by serum maker enzyme and some antioxidant levels in the liver of male albino rats weighing between 180 and 200g. The animals were grouped into five of six rats each which were intraperitoneally induced with nicotine at 1mg/kg body weight except the control group. Some biochemical parameters (Aspartate aminotransferase (AST), Alanineaminotransferase (ALT), alkaline phosphatase (ALP), creatinine kinase malondialdehyde (MDA)); some antioxidant indices and lipid profiles were monitored. Induction of nicotine produced a significant increase in the level of malondialdehyde and decrease in the levels of superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH) as compared to the control animals. Treatment with aqueous extract of Moringaoleifera leaf was found to produce a significant decrease in TBARS and increase in GSH, SOD and CAT in the plasma and liver homogenate of the nicotine

induced rats. The levels of total cholesterol, triglycerides, HDL-cholesterol and LDL-cholesterol in plasma and hepatic tissues of the experimental animals were also monitored. Significant protection was seen in the extract and standard drug (Lisinopril) treated nicotine induced animals. Administration of aqueous extract of the plant brings about a significant restoration towards the control values. The results however showed the protective and antioxidant effects of the extract against nicotine-induced liver toxicity.

2 I. Introduction

he 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 chemooology in recent years, liver problems are on rise. Cancer is a major disorder that account high death rate.

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 - γ 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 *Moringaoleifera* leaf on nicotine induced hypertension in the liver using wistar albino rats.

3 II. Materials and Methods

4 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.

5 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.

6 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.

7 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 spun at 3000 rpm.

8 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.

9 f) Statistical Analysis

The values were expressed as mean $\pm$ 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. Each value is a mean $\pm$ 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 moringaoleifera 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.

10 III. Results and Discussion

The extract of the plant and lisinopril significantly 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.

11 Table 2: Effect of Aqueous Extract of Moringaoleifera on some Antioxidant Enzymes against

Nicotine-Induced Hepatotoxicity in Rats From table 2.0, induction of nicotine with the animals in group 2 there is a significant increase in the levels of lipid peroxidation; as a result of enhance lipid peroxidation 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 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

153 superoxide anions, and convert it to water 21 . And significant reduction in concentration of superoxide dismutase
154 (SOD), GSH and catalase (CAT) were recorded in the group treated with nicotine.

155 Table ?? : Effect of Aqueous Extract of Moringaoleifera on Some Lipid Profile (mg/dl) in Nicotine-Induced
156 Hepatotoxicity Rats Table ?? shows the levels of total cholesterol, triglycerides, HDL-cholesterol and LDL-
157 cholesterol in plasma and hepatic tissues of experimental animals. The levels of total cholesterol, and LDL-
158 cholesterol in plasma and the liver of experimental animals increased significantly in the nicotine treated group.
159 Administration of nicotine caused a significant increase in the levels of triglycerides in the plasma and in the
160 liver, compared with positive control rats. This shows the hyperlipideamic effect of nicotine. However, after
161 the rats were treated with the extract, significant improvement in the levels of triglycerides was observed in the
162 plasma. Hypertriglyceridemic patients are at a risk for cardiovascular disease often develops a lipoprotein profile
163 characterized by elevated triglyceride, dense LDL, and low HDL cholesterol which causes myocardial membrane
164 damage. Significant reduction, similar protection and biochemical restoration of levels of Total cholesterol, LDL-
165 cholesterol and Triglyceride were seen in the plant extract and standard drug (Lisinopril) and nicotine treated
166 animals. The group, given only the extract showed the protective potential and effectiveness of the plant in both
167 plasma and the liver. The study suggests that the intake of the extract decreases the absorption of triglycerides
168 and cholesterol, and these findings are in accordance with the report of22that reported Green tea intake also
169 decreases the absorption of triglycerides and cholesterol.

170 12 IV. Conclusion

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

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Figure 1: Table 1 :

.1 Conflict of Interests

The authors declare that no conflict of interest exist.

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