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Research Article

Ameliorative Effects of Ethanol Extract of *Ficus sur* on Diethylnitrosamine-induced Hepatotoxicity in Wistar Rats

¹O.E. Yakubu, ²E. Ojogbane, ³V.O. Nwaneri-Chidozie, ⁴P.N. Ibuzo, ¹Ale, E.M. and ¹S.S. Awudu

¹Department of Biochemistry, Faculty of Pure and Applied Sciences, Federal University Wukari, P.M.B. 1020, Wukari, Taraba State, Nigeria

²Department of Medical Laboratory Science, Taraba State University, Jalingo, Taraba State, Nigeria

³Department of Biosciences, Faculty of Pure and Applied Sciences, Salem University, Lokoja, Kogi State, Nigeria

⁴Laboratory Unit, University Health Services, Federal University Wukari, Nigeria

Abstract

Background and Objective: Diethylnitrosamine (DEN) is a hepatotoxic compound found in many foods and drugs capable of causing liver cancer. This study was undertaken in order to investigate the hepatoprotective efficacy of the ethanolic extract of the leaf of *Ficus sur* on liver damage induced by DEN. **Materials and Methods:** Twenty Albino Wistar rats were divided into 4 groups, group A served as the normal control group, received water, group B received 200 mg kg⁻¹ DEN once, group C received 200 mg kg⁻¹ ethanolic extract of *Ficus sur* along with DEN and group D received 200 mg kg⁻¹ silymarin as reference standard along with DEN. The experiment lasted for 2 weeks after which the animals were sacrificed and the blood collected for biochemical analyses. **Results:** Administration of DEN caused a significant ($p < 0.05$) increase in the activities of AST and ALT as well as the concentration of total and direct bilirubin, while the levels of albumin and total protein were markedly ($p < 0.05$) reduced. However, treatment with *Ficus sur* extract ameliorated the levels of these biomarkers in the experimental group. The effect elicited by the extract was not statistically different ($p < 0.05$) when compared to silymarin, a standard drug. **Conclusion:** The ethanolic extract of *Ficus sur* protects against DEN-induced hepatotoxicity on Wistar rats and can play a crucial role in the development of modern hepatoprotective drug.

Key words: Antioxidants, *Ficus sur*, diethylnitrosamine, hepatoprotective, silymarin, ethanol extract

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Corresponding Author: O.E. Yakubu, Department of Biochemistry, Faculty of Pure and Applied Sciences, Federal University Wukari, P.M.B. 1020, Wukari, Taraba State, Nigeria Tel: +2348069078726

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Liver is an important organ that carries out the metabolism, secretion, storage and detoxifying functions in the body and certain alterations in these functions often lead to hepatotoxicity. Drugs and some other chemicals are often the cause of liver damage accounting for about one-half of the cases of acute liver failure and diseases¹. Most of the hepatotoxic chemicals damage liver cells mainly by inducing lipid per oxidation and other oxidative damages. The current treatment for hepatotoxicity includes drugs which influence the P-450 enzyme mechanism either by inhibiting or inducing the metabolic activity of enzymes². Noteworthy is the fact that a number of biochemical parameters have been associated with health indices and are of diagnostic significance in routine clinical evaluation of the state of health³.

Diethylnitrosamine (DEN) is an N-nitroso alkyl compound and a potent hepatotoxin and hepatocarcinogen in experimental animals, producing reproducible tumors⁴. It is also used as a hepatotoxic agent to induce hepatocellular necrosis in experimental animals⁵. DEN is rampant in a variety of foods like cheese, soybean, smoked, salted and dried fish, cured meat, alcoholic beverages and ground water having high level of nitrates⁶ and it has also been reported to be a product of metabolism of certain therapeutic drugs⁷. DEN generates reactive oxygen species (ROS) which produce by-products that often have direct effect on cell development, growth and survival. Considering the above facts, the development of an effective hepatoprotective agent is therefore needful as humans are at potential risk of exposure to DEN and exposure to DEN has become inevitable. The development in the use of plants for therapy has led to the formulation of several herbal medicines for liver disorders⁸. Some plants and plant-derived products have been found to be effective against DEN-induced hepatocarcinogenesis and hepatotoxicity⁹. These plants constitute bioactive substances such as alkaloids, glycosides and flavonoids which account for their pharmacological activities and this forms the bases of modern drug development. Therefore, the potential use of these plants for hepatoprotection makes them an attractive target for future studies and for the identification of their active constituents. *Ficus sur* is among the plants under study. It is commonly called wild Figure. It is a medium sized tree of 6-9 m high with large alternate and spirally arranged leaves with regularly serrated margins. The leaves have found relevance in traditional medicine in the treatment of diarrhoea, anaemia, wounds, stomach problems, infertility, peptic ulcer and gonorrhea. The major phytochemicals revealed by the screening are known to possess a wide range

of activities, which may help in protection against chronic diseases. For instance, saponins, flavonoids, tannins and alkaloids are a major component of this plant and possess hypoglycemic and anti-inflammatory activities¹⁰. This plant has been reported to exhibit several pharmacological actions but its hepatoprotective activities still remain vague as there are little or no experimental guided data to support it. Hence, the need for this study to shed light on the ameliorative effect of *Ficus sur* on DEN induced hepatotoxicity in wistar rat.

MATERIALS AND METHODS

Study area: This study was carried between the months of January-August, 2019 at The Federal University Wukari, Taraba State, Nigeria.

Collection and identification of plant materials: The leaves of *Ficus sur* plant were collected within the Biological garden of Federal University Wukari, Taraba State. The plant was identified and authenticated by experts in the Department of Biological Science.

Preparation of ethanolic extract: The ethanolic extract was prepared using the method of Yakubu *et al.*¹¹. The leaves were washes, air dried away from direct sunlight at room temperature for 3 weeks and pulverized in electric grinder. Hundred gram of pulverized leaves was soaked in 400 mL of ethanol for 24 h at room temperature and was continually stirred after every 5 h. After 24 h, the extract was filtered out using clean white sieving mesh and then with Whatman No 1. Filter paper. The ethanol used for extraction was recovered from the extract using rotary evaporator. This helped to concentrate the extract which was dried and stored in refrigerator for further use.

Experimental animals: Twenty (20) Wistar rats of 100-150 g were obtained from the animal house of the Department of Biochemistry, Federal University Wukari, Nigeria and Kept in ambient condition and light cycle (12 h light and 12 h dark) in compliance with ethical guide for care and use of laboratory animals of the Faculty of Pure and Applied Sciences, Federal University Wukari, Nigeria with the approval number, FUW/FPAS/19/030.

Experimental design: This was carried out as described by Yakubu *et al.*¹¹. Liver damage was induced in rats by administering diethylnitrosamine (DEN) intraperitoneally at the dose of 200 mg kg⁻¹ body weight. Group A served as the normal control group and received only normal feed and

water daily. Group B served as the positive control group consisting of the hepatotoxic rats received 200 mg kg⁻¹ DEN once. Group C were the extract treated rats and received 200 mg kg⁻¹ ethanol extract an hour after administration of 200 mg kg⁻¹ DEN. Group D served as the standard group and received 200 mg kg⁻¹ silymarin an hour after administration of 200 mg kg⁻¹ DEN. The experiment lasted for 2 weeks after which the animals were fasted overnight, subjected to mild chloroform anesthesia and the animals were sacrificed and blood collected by cardiac puncture. The blood was centrifuged at 3,000 rpm for 15 min and the clear supernatant used for biochemical assays.

Biochemical analysis: Biochemical parameters which include aspartate aminotransferase (AST), alanine aminotransaminase (ALT), albumin, direct bilirubin, total bilirubin and total protein were analyzed using commercial kits by Randox Laboratories Ltd, UK. AST and ALT were assayed for according to the method described by Reitman and Frankel¹², serum albumin was estimated by the method of Doumas *et al.*¹³, Serum bilirubin was determined colorimetrically according to the method described by Jendrassik and Grof¹⁴ and serum total protein estimated as described by Lubran¹⁵.

Statistical analysis: The results were analyzed by one-way ANOVA, using SPSS statistical package version 26. Values were expressed as mean $\pm$ standard deviation (SD) of 5 animals in each group. The $p < 0.05$ were considered significant.

RESULTS

Result of serum AST and ALT activities: The results presented in Fig. 1a, b revealed an elevated level of AST and ALT respectively in the positive control (group B) that received only DEN and the increase was significant ($p < 0.05$) when compared to the normal control (group A) that received water only but the administration of the extract in group C caused a significant ($p < 0.05$) reduction in the activities of these enzymes when compared with the positive control. In addition, silymarin treated animals (group D) produced similar result as group C and there was no significant ($p < 0.05$) difference in the levels of these enzymes in both groups.

Result of serum albumin and total protein: The administration of DEN resulted in a marked reduction in the level of albumin (Fig. 1c) and total protein (Fig. 1d) and this reduction was significantly ($p < 0.05$) different from the normal control. However, their concentrations were raised when the

extract was administered and this increase was significant ($p < 0.05$) compared to the positive control. Also, treatment with silymarin in group D produced similar trend of result as the extract and no significant ($p < 0.05$) difference was observed in the levels of these biomarkers in group C and D.

Results of total bilirubin and direct bilirubin: The results shown in Fig. 1d, e revealed that DEN caused a significant ($p < 0.05$) increase in the serum level of total bilirubin (Fig. 1d) and direct bilirubin (Fig. 1e) when compared with the normal control but their concentrations were declined upon administration of the extract and this reductions were significant ($p < 0.05$) when compared with the positive control. Moreso, Silymarin treated animals (group D) produced similar result as the extract and there was no significant ($p < 0.05$) difference in the levels of these biomarkers in group C and D.

DISCUSSION

The results of this study revealed an elevated level of AST (Fig. 1a) and ALT (Fig. 1b) in the positive control (group B) that received only DEN and the increase was significant ($p < 0.05$) when compared to the normal control (group A) but the administration of the extract in group C caused a significant ($p < 0.05$) reduction in the activities of these enzymes when compared with the positive control. Conversely, the administration of DEN resulted in significant reduction ($p < 0.05$) in the level of albumin (Fig. 1c) and total protein (Fig. 1d). However, the concentrations were raised when the extract was administered and this increase was significant ($p < 0.05$) compared to the positive control. In like manner to that of the enzymes activities, treatment with DEN led to a significant ($p < 0.05$) increase in the serum level of total bilirubin (Fig. 1d) and direct bilirubin (Fig. 1e) when compared with the normal control but the administration of the extract rescued the situation by reducing their concentrations and these reductions were significant ($p < 0.05$) when compared with the positive control. In addition, silymarin produced similar result in group D as the extract in all the tested parameters and there was no significant ($p < 0.05$) difference in the levels of these biomarkers in group C and D.

DEN has been reported to be a carcinogenic drug inhuman system which produces oxidative stress through the generation of ROS and therefore damaging the antioxidant defense system in tissues¹⁶. DEN induces oxidative stress possibly due to the generation of reactive oxygen species (ROS), which are capable of initiating per oxidative damage to the cell¹⁷. DEN is biotrans formed by mixed-function

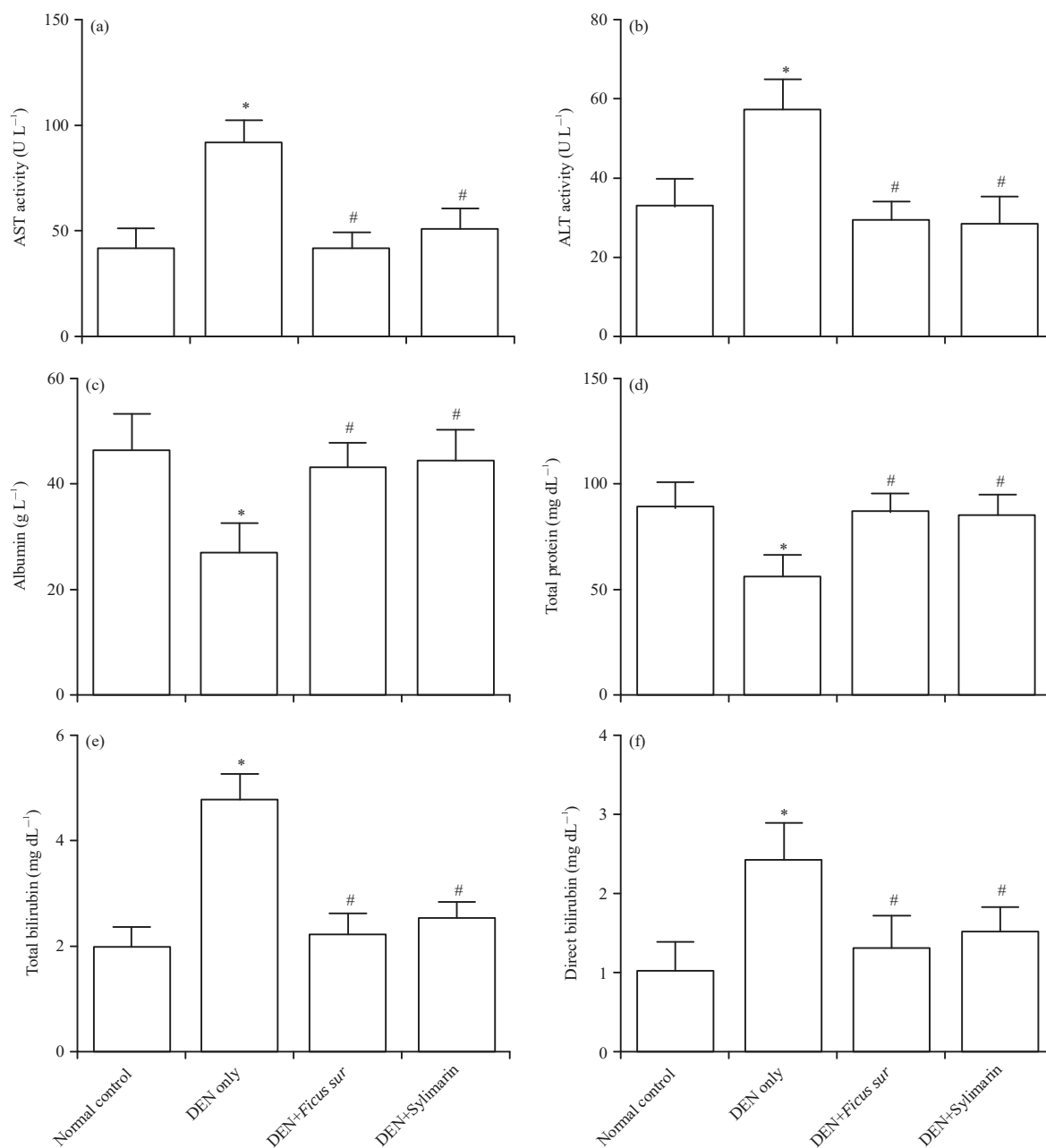


Fig. 1(a-f): Effect of *Ficus sur* extract on serum (a) AST, (b) ALT, (c) Albumin, (d) Total protein, (e) Total bilirubin and (f) Direct bilirubin of rat

Data are expressed as mean $\pm$ SEM of 8 animals in each group, * $p < 0.05$ as compared to normal control, (group A): # $p < 0.05$ as compared to DEN only (group B)

cytochrome P450 dependent mono oxidase systems and its metabolic activation is reported to be responsible for the onset of the toxic effects¹⁸. Intermediate reactive compounds originating from the bioactivation of DEN are known to form covalent bonds with important cell constituents, thus inducing the onset of mutations, cancer and necrosis¹⁹. Microsomal activation of DEN involves cytochrome P450 and

hence compounds that selectively activate cytochrome P450 systems are being widely used by several investigators to induce hepatocellular carcinoma in experimental animals. Toxicity in the liver is often reflected from abnormal increase in the level of liver biomarkers in the serum. This is usually consequent to the destruction of the integrity of the membrane phospholipid bilayers of the hepatic cells causing

these biomarkers to leak into the blood. Estimation of the activity of liver markers such as AST, ALT, albumin, total protein, direct bilirubin and total bilirubin is therefore a way of diagnosing liver problem²⁰.

In this study, we were able to document the hepatoprotective activity of the ethanolic leaf extract of *Ficus sur* against liver injury induced by DEN in rats. The administration of DEN resulted in an increase in the level of AST (Fig. 1a) and ALT (Fig. 1b) and these increase were significantly ($p < 0.05$) different from the normal control (group A). The liver releases AST and ALT and an elevation in plasma concentration of these enzymes is an indicator of liver damage^{21,22}. This may be consequent to the leakage of these enzymes from the liver tissue into the system circulation of the animals as a result of the hepatocellular damage caused by the administered DEN. Upon administration of the extract, a significant ($p < 0.05$) decrease in both ALT and AST activities were observed in the animals as compared to animal treated with DEN. However, there was no significant difference when compared with animals treated with silymarin. This suggests a protective effect of the extract against DEN induced hepatotoxicity.

Furthermore, the levels of albumin (Fig. 1c) and total protein (Fig. 1d) were markedly reduced when DEN was administered and these were significantly ($p < 0.05$) different from the control. This suggests a reduction in the protein synthetic function of the liver, which could be a consequence of possible damage to the hepatocytes induced by DEN. Most protein found in the plasma are synthesized by the hepatocytes and secreted into circulation. This result reveals that the extract may modulate the reduced protein synthetic function of the liver caused by DEN.

Moreover, the result presented in Fig. 1d, e showed that the total bilirubin and conjugated bilirubin levels were increased in the DEN treated rats and the increase were significant ($p < 0.05$) when compared to the normal control. Interestingly, administration of the extracts led to a significant reduction ($p < 0.05$) in their levels. Elevation of total bilirubin which results from decreased uptake and conjugation of bilirubin by the liver is caused by liver cell dysfunction, while increased levels of direct or conjugated bilirubin is due to decreased secretion from the liver or obstruction of the bile ducts. The marked reduction in total and conjugated bilirubin by *Ficus sur* extract further show its protective effect against DEN induced liver toxicity. The extract perhaps protects the liver cell from damage, thereby enhancing bilirubin uptake and conjugation by the liver and subsequent secretion into the bile ducts.

Intriguing and enigmatic studies have shown that some species of *Ficus* possess antioxidant activity and that their antioxidant activity is related to their phenolic content^{23,24}. Bruneton²⁵ also reported that the antiradical activity of *Ficus sur* could be due to the high content of tannins and flavonoids present in the leaves. Flavonoids have important antioxidant and antiradical activities. Their protective effects in biological systems are linked to their ability to transfer electrons to free radicals, chelate metals, activate antioxidant enzymes, reducing radicals of alpha-tocopherol or to inhibit oxidases. It is therefore worth while to attribute the hepatoprotective action of *Ficus sur* to these essential bioactive constituents. It can be suggested that these antioxidative phytochemicals may be interfering with the biotransformation processes of DEN which often lead to the damage of hepatic cells or that these bioactive substances may be donating electrons to free radicals or chelate metals that are generated from metabolism of DEN. It could as well be that they are activating the antioxidant enzymes in counteracting the oxidative stress that results from biotransformation of DEN.

Technically speaking, *Ficus sur* in present study, maintained the structural integrity of the hepatocellular membrane and offered protection against liver damage induced by the administration of DEN and this was evident from its ability to ameliorate the levels of the liver markers in the serum and this justifies the use of the plant as local herbal medicines. This study is limited to laboratory animals and therefore has to be conducted on human subject in order to vehemently ascertain the hepatoprotective efficacy of the plant. Also, further studies need to be carried out to isolate the bioactive compound in the plant and to elucidate the mechanisms of action.

CONCLUSION

The results of this study have shown that ethanol leaf extract of *Ficus sur* can be used to remedy the abnormal increase in the liver biomarkers observed in rats with liver damage and justify the use of this plant in the management as liver problems.

SIGNIFICANT STATEMENT

This study shed light on the hepatoprotective action of *Ficus sur* and serves as template for further research in the design of modern drug and the elucidation of mechanisms of action as well as the pharmacokinetics of the drug.

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