



Hepatoprotective Effect of Methanol Stem Bark Extract of *Diospyros mespiliformis* against Carbon Tetrachloride Induced Liver Damage

J. Nwaogu^a, I. M. Fakai^a and I. Yahaya^{a*}

^a Department of Biochemistry, Faculty of Life sciences, Kebbi State University of Science and Technology, Aliero, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This study evaluated the hepatoprotective effects of methanol stem bark extract of *Diospyros mespiliformis* against carbon tetrachloride-induced liver damage in albino rats.

Methodology: Acute toxicity study was determined using limit fixed dose test of 5000mg/kg for 14 days. Six (6) adult albino rats of both sexes were used and randomly divided into six groups of one rat each. Hepatoprotective study; a total of forty two (42) adult albino rats were divided in to seven groups, of six rats each. Methanol stem bark extract of *Diospyros mespiliformis* administered to different groups of rats at the doses of 50 mg/kg, 100 mg/kg, 150 mg/kg and 200 mg/kg orally daily for fourteen. This was followed by CCl₄-administration after every 72 hours for the same period. Silymarin a standard reference drug was administered at a dose of 100mg/kg body weight orally, once daily for fourteen (14) days. Hepatoprotective effect was studied by assaying the activities of liver function indices (aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, and gamma glutamyl transferase, total protein, albumin, direct and total bilirubin) in serum.

Results: The LD₅₀ of methanol stem bark extract of *Diospyros mespiliformis* was greater than 5000 mg/kg. The activities of all the marker enzymes revealed a significant (P<0.05) elevated levels and decreased in the levels of total protein and albumin in CCl₄ treated rats. Group administered with the extract prior to CCl₄ induction show significantly low level of serum liver marker enzymes and increased levels of total protein and albumin compared to CCl₄-induced untreated group and this

*Corresponding author: E-mail: yahayaibr2008@gmail.com;

observations is supported by the histology of liver sections, the result indicate that methanol stem bark extract of *Diospyros mespiliformis* possess significant hepatoprotective property by inhibiting lipid peroxidation.

Conclusion: This property may be attributed to the phytochemical compounds present in the extract.

Keywords: Hepatoprotective; *diospyros mespiliformis*; silymarin.

1. INTRODUCTION

The liver is an essential organ in the body and helps to keep it function properly. It involved in almost all biochemical process: excretion, production, storage and detoxification [1]. While performing several of these metabolic functions, liver undergoes stress, involves oxidative stress leading to liver diseases (condition of liver inflammation or tissue damage that affects liver metabolic functions) ending in liver damage and serious health problems and death [2]. Therefore, the wellbeing of the liver is essential for a adequate metabolic processes of an individual. The most common liver cell injuries are caused by chemotherapeutic agents such as carbon tetrachloride, thioacetamide and microbes [3]. Despite recent strides in medicine, it is still hard to get drugs that protect the liver from damage or regeneration of hepatic cell [2]. Many herbal extracts for liver problems are available in Nigerian but there is no herbal drug that is efficacious in the management of liver diseases [4].

Plants have proven over ages to be important source of all the world pharmaceuticals and form the basis of traditional medicine [5]. Worldwide, particularly in Africa and Asia, plants are an important source of both orthodox and modern medicine. Around 80% of the world population depends on medicine which is predominantly based on plant materials [6]. In Nigeria and other developing countries, people have relied on traditional herbal preparations for treatment of various diseases. In the plant kingdom, there still exist many medicinal plants that are yet to be explored for the scientific importance [7]. *Moringa oleifera*, *Senna alata*, *Cochlospermum tinctorium*, *Uvaria afzelii*, *Vernoni ambigua*, *Acimum americanum* and *Ficus exasperate* are among the plant that have been used in traditional medicine in Nigeria for the treatment of various liver diseases [8]. Another plant that could be used for that purpose is *Diospyros mespiliformis*.

Diospyros mespiliformis (Hochst) (Ebenaceae) is a medicinal plant commonly called Jackal-berry

or African ebony, locally called Kaiwa or kanya among Hausa's [9]. *Diospyros mespiliformis* is of the genera *Ebenaceae* and has been used for the treatment of various ailments which included liver disease. Research on medicinal plants with acclaimed effects in reducing hepatic disorders therefore becomes imperative in order to improve the quality of life of the people. In view of the fact that there are claimed by the traditional healers regarding the use of this traditional medicinal plant stem bark (*Diospyros mespiliformis*) in the management of liver diseases. Moreover, there are little to no available scientific report regarding the use of the plant stem bark as hepatoprotective agent. Therefore, this study evaluated the hepatoprotective effect of the methanol stem bark extract of *Diospyros mespiliformis* in CCl₄-induced rat liver damaged.

2. METHODOLOGY

2.1 Study Area

2.1.1 Plant sample collection and identification

The plant sample of *Diospyros mespiliformis* stem bark was collected from Ngaski Local Government area of Kebbi State, Northern Nigeria. It was taken to the Herbarium section of the Department of Plant Science and Biotechnology, Kebbi State University of Science and Technology Aliero; where it was identified and authenticated by a plant taxonomist Prof. Dramendra Singh. The voucher number assigned to the plant was: V.N: 182 for future reference.

2.1.2 Drug used

Silymarin was purchased from Sigma Chemical Company, USA.

2.1.3 Chemicals and reagents

All chemicals and reagents used were of analytical grades. Carbon tetrachloride was purchased from Benzer Multitech, India.

Methanol, ferric chloride, hydrochloric acid (HCl), sulphuric acid (H₂SO₄), benzene, sodium hydroxide (NaOH) and acetic anhydride were purchased from British Drug House (BDH), England. Chloroform (GPR) was purchased from Sigma-Aldrich chemical limited, UK, Normal saline was purchased from Kernel and distilled water (AR) was purchased from SD chemical limited. Similarly some reagents used for experiments were commercial kits, which are products of Randox, (UK), Chemelex Lab kit S.A and Cayman Chemicals, USA.

2.1.4 Experimental animals

Sixty (60) albino rats of both sexes weighing 150-200g were procured from the Animal House, Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto. The animals were allowed to acclimatize to laboratory condition for two (2) weeks with free access to food and water. Dark and light cycles were also maintained at 12 hours each.

2.2 Methods

2.2.1 Preparation of plant sample and extraction

The stem bark of *Diospyros mespiliformis* was washed with distilled water and air dried under shade away from sun, dust and intense heat in the laboratory for two (2) weeks. The stem bark was pounded into powder using a wooden mortar and pestle. It's weight was measured and then stored in a specimen bottle until required for used.

The plant material (200g) was extracted with two liters of 98% methanol and was left in an air tight aspirator bottle for 72 hours with occasional shaken. The mixture was then filtered with sterile Muslim cloth; the filtrate was evaporate using hot air ovum at 45oc and subsequently dried in a drying cabinet at 45oC. Extract was subsequently weighed and recorded for calculation of percentage yield, labeled and stored in a closed container until required for reconstitution in distilled water.

2.2.2 Experimental design for hepatoprotective study

Hepatotoxicity was induced according to the method of Rao et al. [10] with slight modification.

Forty two (42) adult albino rats weighting 150-200g were divided in to seven groups, of six rats each.

Group I: Received distilled water (1 ml/kg body weight P.O) for fourteen days, in addition to normal diet.

Group II: Received 30% carbon tetrachloride (1ml/kg body weight, I.P) in liquid paraffin for every 72 hrs for fourteen (14) days, in addition to normal diet.

Group III, IV, V and VI: Received the methanol stem bark extract of *Diospyros mespiliformis* at dose of 50, 100, 150 and 200mg/kg respectively; once a day for fourteen days following by CCl₄ induction for every seventy two (72) hours.

Group VII: Received silymarin at a dose of 100mg/kg body weight per oral, once daily for fourteen (14) days followed by CCl₄ induction for every seventy two (72) hours.

2.2.3 Collection and preparation of blood sample

After the 14th day, the animals in various groups were sacrificed just after 48 hours of 30% CCl₄ induction under chloroform anesthesia, blood and liver samples were collected. A blood sample collected into clean non-heparinised bottles was allowed to clot and the serum was separated by centrifuging at 3000rpm for 5 minutes. The serum was collected according to groups using pasteur pipette into the sample bottle. The serum was used for the analysis of biochemical parameters like GGT, AST, ALT, ALP, Total protein, albumin and bilirubin.

2.2.4 Biochemical analysis

"Serum Alanine Aminotransferase and Serum Aspartate Aminotransferase (AST) activities were ascertained using the method of Reitman and Frankel" [11]. "Total protein in was determined using Biuret method as desc Young" [12]. "Total and conjugated bilirubin was determined using the method of Jendrassik and Grof" [13], "Alkaline phosphatase was estimated using Colorimetric method of Young (2001), Albumin was determined by the dye binding technique utilizing Bromocresol green (BCG) as modified by" Doumas et al. [14] was employed. "Gamma-Glutamyl Transferase Activity Assay (GGT) by the method of Szasz and Bergmeyer" [15].

2.3 Statistical Analysis

Results were presented as mean \pm standard error of mean (SEM). For data comparison

between the groups, one-way analysis of variance (ANOVA) was applied using statistical package for the Social Sciences (SPSS) version 20.0. Duncan post Hoc comparison test was used to check differences between the individual group and values were considered statistically significant at $P < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Methanol Extract of *Diospyros mespiliformis* Stem Bark

The extract obtained was soluble in water, dark-brown in colour, with a characteristic of unpleasant smell. The percentage yield of extract obtained was = 13.50%.

3.2 Acute Toxicity Studies (LD_{50})

3.2.1 Effect of acute administration of 5000 mg/kg body weight of methanol stem bark extract of *Diospyros mespiliformis* (MSEDM)

Acute administration of 5000mg/kg body weight of methanol stem bark extract of *Diospyros mespiliformis* (Table 1) produced no mortality after 48 hrs of observation. The median lethal dosage (LD_{50}) of the MSEDM was therefore estimated to be greater than 5000 mg/kg body weight. The extract did not produced any grossly negative behavioral changes such as etching, depression, tremor, weakness, food and water refusal, salivation, discharged from eyes and ears, skin changes and hair removal.

3.3 Assessment of Serum Liver Function Indices

3.3.1 Effect of administration of MSEDM on serum liver function indices

Administration of MSEDM at different doses on treated groups showed a remarkable protective

effect across the various liver function indices. The CCl_4 -induced control group showed significant ($p < 0.05$) increased in alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (TB), direct bilirubin (DB), alkaline phosphatase (ALP), and gamma glutamyl transferase (GGT) levels, while albumin and total protein levels decreased significantly ($p < 0.05$) compared to normal control (Table 2).

3.4 Discussion

The acute oral toxicity test of MSEDM did not produced any negative behavioral changes such as etching, depression, weakness, food and water refusal and salivation. There was also no mortality recorded after a single oral administration of 5000mg/kg of MSEDM after 14 days of the acute toxicity. Loomis and Hayes [16] "a substance administered orally having an LD_{50} ranging between 5000-15000 mg/kg is marked as non-toxic. The main objective of toxicity testing is basically to classify substances according to their toxicity profile or potency, this is aim to protect public health by regulating exposure to potentially dangerous substances" [17]. Therefore, estimated LD_{50} of methanol stem bark extract of *Diospyros mespiliformis* is above 5000mg/kg.

Many toxicants are responsible for hepatotoxicity. Carbon tetrachloride and paracetamol have been critically examined. In this research, CCl_4 -treated rats indicated significant elevated levels of AST, ALT, ALP, GGT, total and direct bilirubin. There was also a remarkable decrease in total protein and albumin levels after CCl_4 administration as compared with that of normal as well as the extract and silymarin treated groups. Normally, when there is elevation in serum AST and ALT, in most cases, it is caused by changed in the permeability of the hepatocyte membrane leading to a change in the

Table 1. The effect of acute oral administration of MSEDM

Dose	Groups	Number of Animal	Number of Death
DTW (1ml/kgbw)	A	1	0
MSEDM 5000 mg/kgbw	B	1	0
MSEDM 5000 mg/kgbw	C	1	0
MSEDM 5000 mg/kgbw	D	1	0
MSEDM 5000 mg/kgbw	E	1	0
MSEDM 5000 mg/kgbw	F	1	0

Key: DTW-distilled water MSEDM- methanol stem bark extract of *Diospyros mespiliformis*. The A- received distilled water and served as control. The remaining B to F were administered with single oral dose of 5000mg/kg body weight of methanol stem bark extract of *Diospyros mespiliformis*

Table 2. Effects of administration of methanol stem bark extract of *Diospyros mespiliformis* on serum liver biochemical indices in rats with CCl₄-induced hepatotoxicity

Biochemical Parameters	AST(U/L)	ALT (U/L)	ALP (U/L)	GGT (U/L)	ALB (g/dl)	TP (g/dl)	TB (g/dl)	DB (g/dl)
GROUP I	61.17±2.34 ^a	48.50±1.87 ^a	245.49±3.17 ^a	76.61±3.32 ^a	6.90±0.56 ^{cd}	9.53±0.65 ^d	1.57±0.31 ^a	0.97±0.18 ^{ab}
GROUP II	83.17±3.87 ^c	67.50±2.65 ^b	381.19±4.61 ^e	126.99±1.82 ^d	2.89±0.23 ^a	4.90±0.82 ^a	3.93±0.18 ^c	2.35±0.35 ^c
GROUP III	68.33±5.30 ^{ab}	58.50±3.26 ^{ab}	340.48±3.10 ^d	113.23±3.20 ^c	3.32 ±0.30 ^a	5.94±0.26 ^{ab}	4.00±0.30 ^c	2.06±0.22 ^c
GROUP IV	68.33±5.30 ^{ab}	56.83±3.81 ^{ab}	304.46±1.23 ^c	103.37±1.53 ^b	4.70 ±0.19 ^b	7.59±0.33 ^b	3.09±0.38 ^b	1.77±0.24 ^{bc}
GROUP V	65.83±2.59 ^a	51.33±3.81 ^a	265.11±2.33 ^b	93.38±1.42 ^a	4.27±0.15 ^b	8.08±0.62 ^{bc}	2.13±0.33 ^a	1.17±0.28 ^{ab}
GROUP VI	63.67±1.61 ^a	62.17±1.54 ^a	259.29±2.47 ^b	82.21±9.96 ^a	6.10 ±0.42 ^c	8.74±0.33 ^{ab}	1.83±0.38 ^a	1.37±0.41 ^{ab}
GROUP VII	61.00±4.57 ^a	49.16±0.98 ^a	247.68±5.2 ^a	79.27±1.90 ^a	6.86±0.28 ^d	9.47±0.40 ^{cd}	1.64±0.22 ^a	1.02±0.18 ^a

Values were expressed as mean ± standard error of mean (SEM), n = 6 in each group. Figures in parenthesis are percentage of protection of the activity to normal values as compared to CCl₄ control. Values are expressed as mean ± Standard error of mean. Mean values having different superscript letters in a column are significantly different at (p<0.05).

Key: ALB-Albumin, TP-Total protein, ALT-Alanine aminotransferase, AST-Aspartate aminotransferase, TB-Total bilirubin, DB-Direct bilirubin, ALP-Alkaline phosphatase, GGT - Gamma Glutamyl Transferase

Group I: received liquid paraffin (1ml/kg body weight P.O)

Group II: received 1ml/kg body weight I.P of 30% CCl₄ in liquid paraffin for every 72 hrs for 14 days

Group III: received 50 mg/kg body weight of the extract once daily and 1ml/kg, body weight of 30 % CCl₄ in liquid paraffin for every 72 hrs for 14 days

Group IV: received 100 mg/kg body weight of the extract once daily and 1ml/kg body weight of 30 % CCl₄ in liquid paraffin for every 72 hrs for 14 days

Group V: received 150 mg/kg body weight of the extract once daily and 1ml/kg body weight of 30% CCl₄ in liquid paraffin for every 72 hrs for 14 days

Group VI: received 200 mg/kg body weight of the extract once daily and 1ml/kg body weight of 30 % CCl₄ in liquid paraffin for every 72 hrs for 14 days

Group VII: received 100 mg/kg peros of silymarin once daily and 1ml/kg body weight of 30 % CCl₄ in liquid paraffin for every 72 hrs for 14 days.

synthesis or catabolism of amino transferases [18]. This generally implies that the synthetic function of the liver is affected [19,20]. When there is a decrease in the total protein and albumin levels could be due to defective protein biosynthesis which is caused by hepatocellular injury from the effect of CCl₄ [21]. These findings agree with the findings of Hassan et al. [22], Alhassan et al. [23] and Abbas et al. [18]; “who similarly reported that the serum levels of ALT, AST, ALP, GGT, total and direct bilirubin were elevated while they was a decrease in total protein and albumin levels in the CCl₄-treated group when compared with the untreated group. Therefore, the elevated serum level of AST, ALT and ALP in CCl₄ treated animals indicated cellular breakage and loss of functional integrity of cell membranes” [24]. “GGT is a membrane bound enzyme and an indicator of cell and tissue damage. When there is a rise in the level of serum conjugated bilirubin, it is likely due to the regurgitation of bilirubin glucuronides from hepatocytes back into plasma. This could also indicate that erythrocytes are highly degraded” [18].

“CCl₄ is the most extensively used chemical agent for investigation of hepatoprotective activity on various experimental animal model” [24]. The hepatotoxicity induced by CCl₄ is due to its metabolite generated during its metabolism [19]. When there is a hepatic dysfunction or damage, liver enzymes are released into circulation. This causes the serum concentration of these enzymes to rise significantly [25]. “The ability of silymarin to reduce the injurious effects or to preserve the normal hepatic physiological mechanisms, which have been disturbed by a hepatotoxin, is the index of its protective effects” [20].

“Reduction in the levels of serum/liver homogenate AST, ALT and GGT after treatment with the MSEDm is an indication of regeneration process of hepatocytes. Reduction in ALP level with decreases in the raised bilirubin levels suggests the stability of biliary function” [26]. “The concentration of serum total protein and albumin levels reduced in the group treated with CCl₄. On administering MSEDm, it restored serum total protein and albumin levels normalizing these to their control values. The significant increase in protein and albumin levels shows the stabilization of endoplasmic reticulum leading to protein synthesis” [25].

“Administration of MSEDm at different doses support the hepatoprotective role of plant extracts in present study. The ability of the MSEDm to significantly protect the level of serum total and direct bilirubin could suggest the potential of the extract in clearing the level of bilirubin in the serum when it is elevated. The extract also protects decrease in protein and albumin levels are an indication of stabilization of endoplasmic reticulum leading to protein synthesis” [26]. This research showed that the effect of different doses of treatment of MSEDm effectively protected against liver injury. The standard drug has both hepatoprotective and regenerative actions [26]. The hepatoprotective effect of the extract overall at 200mg/kg was comparable with that of silymarin. These findings suggest that *Diospyros mespiliformis* stem bark extract most likely protected the structural integrity of the cell membrane of hepatocytes through a mechanism similar to that of silymarin.

4. CONCLUSION

From the results obtained, the altered biochemical profiles due to the deleterious effects of CCl₄ on the liver, this was reversed towards normalization by the extract. The contents of the extract not only increased the regenerative and reparative capacity of the liver but, at the same time prevented from oxidative damage. Beneficial effects of methanol stem bark extract of *Diospyros mespiliformis* illustrated in this study is likely due to some phytochemicals that are present in the plant.

COMPETING INTERESTS

Authors have declared that they have no known competing financial interests or non-financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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