Evaluation of the Restorative Potential of Methanol Seed Extract of *Spondias mombin* on CCl_4 induced Hepatic Damage

Abstract

The liver is indisputably one of the most important organs of the body. It is saddled with the responsibility of detoxifying xenobiotics. The use of the conventional synthetic drugs in restoring the hepatic life and functionality is characterized by a number of pitfalls and thus, necessitates the need for plant based option. Hence, the aim of this study was to evaluate the restorative potential of methanol seed extract of *Spondias mombin* on CCl_4 induced hepatic damage. Freshly harvested leaves of *Spodias mombin* were dried at room temperature prior to being ground into fine powder. The powdered plant sample was steeped in1 L of 50% methanol for a period of 72 hours during which themixture was shaken twice daily. Twenty five adult male Wistar rats were divided into five groups of five rats each. **Group I** was the normal control. **Group II** was induced without treatment, while **Groups III-V** were separately administered 100, 200 and 400 mg/kg of extract respectively for 28 days after which animals were sacrificed and blood sample collected and analyzed using standard procedure. The solvent wasfiltered over a layer of gauge and then the filtrate evaporated to drynessat 55°C. Qualitative phytochemical screening on the resulting extract revealed the presence of flavonoids, tannins, alkaloids, phenols and cardiac glycoside. Oral administration of extract significantly (P<0.05) reduced the activities of Aspartate amino transferase (AST), Alanine transaminase (ALT) and Alkaline phosphatase (ALP). Body weight of rats significantly (P<0.05) increased in all groups except Group II which was not administered extract. In conclusion, methanol leaf extract wields the potential to restore damaged hepatocytes.

Keywords:*Spondias mombin*, Hepatomarkers, Phytochemicals, Body, Weight

Introduction

Liver diseases has been identified a one the major public health concerns in recent times and has contributed maximally to the global burden of diseases accounting for most deaths worldwide [1]. Conventional treatment approaches are either expensive or unsustainable, factors which have rendered them unreliable and consequently out of context, a development which has paved way for a renewed interest in natural products notably plants as viable candidates for the development of ideal drugs for the treatment and management of liver diseases [2].

Comment [1]: Replace with: Carbon tetrachloride-induced

Comment [2]: Replace with: The liver is indisputably one of the most important organs in the body. It is saddled with the responsibility of detoxifying xenobiotics. The use of conventional synthetic drugs in restoring hepatic life

Comment [3]: Replace with: thus necessitates the need for plant-based options.

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Comment [9]: Replace with: 200, and 400 mg/kg of extract, respectively, for 28 days, after which animals were sacrificed and blood samples collected and analyzed using standard procedures.

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Comment [11]: Replace with: screening of the resulting extract revealed the presence of flavonoids, tannins, alkaloids, phenols, and cardiac glycosides. Oral administration of extract significantly (*P*= .05) reduced the activities of aspartate amino transferase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP). The body weight of rats significantly (*P*= .05) increased in all groups except Group II, which was not administered extract. In conclusion, methanol leaf extract has the

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Botanical therapies have been part of the human culture since prehistoric times and have remained a dependable source of treatment for diverse human and animal illnesses evident by the fact that an estimated 80% of the global population sources medical supports from plant based medicinal preparations [3].

Spondias mombin belongs to the*Anacardiaceae* family and commonly referred to as yellow mombin, hog plum, or ubos. *Spondias mombin* can grow to a height of 15-20 metre tall with the trunk measuring 60-75 cm wide. The tree is commonly found in Nigeria among many other tropical and sub-tropical forests of the worldwith high geneticvariability among populations [4]. Numerous reports on the medicinal properties of *S. mombin* leaf have revealed that it can decrease anxiety, haltconvulsions and relieve pain [5]. Further research efforts have shown that it is rich in vitamins A and C[6] with enormous enzyme inhibition potential [7].Different parts of a particular plant could be uniquely endowed with bioactive ingredients of immense health significance. The leaf and stem bark of *Spondias mombin* have demonstrated varying degrees of therapeutic strength in protecting the liver against external insults [8]. However, there is paucity of data on the potential of the seed of the said plant to protect the liver against external insults, an observation which underscores the imperativeness of this study.

Materials and Methods

Spondias mombin seeds were harvested from a farm in Afikpo North Local Area of Ebonyi State Nigeria. The leaves were conveyed in a black polythene bag to the herbarium of the Department of Forestry, Micheal Okpara University of Agriculture, Umudike Abia State South-Eastern Nigeria for identification.

Processing and extraction of plant material

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Spondias mombin L. seed were dried were dried at room temperature. The dried seed were ground with mortar and pestle blender. Cold extraction method was employed to extract 500 g of powdered seed sample with methanol as solvent. The powdered plant sample was soaked inone litre of 50% methanol for a period of 72 hours during which themixture was shaken twice daily. The solvent wasfiltered over a layer of gauge and then the filtrate evaporated to drynessin vacuo at 55°C.

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Animals

Mature male Wistar rats which weighed between 130-160 g were obtained from a commercial Animal House within Okigwe metropolis, Imo State. The animals were housed in well ventilated aluminium cages under standard laboratory conditions and were allowed unrestricted access to food and water. Animals were acclimatized for two weeks prior to commencement of experiment which follow the Guide to the care and use ofanimals in research and teaching [9].

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Median Lethal Dose 50% (LD50%)

Nine (9) adult Wistar rats were involved in the first phase of the experiment. The rats were divided into three groups of three rats each. The groups labeled A, B, and C administered 10, 100 and 1000 mg/kg of extract orally respectively. The rats were observed for 24 h to possibly identify signs of toxicity. Being that mortality was not recorded in the first phase, the second phase was initiated and involved another three groups of one rat each was each which was separately administered 1600, 2900 and 5000 mg/kg of extract and afterwards, animals were observed for 48 h for signs of toxicity according to Lorke [10].

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Phytochemical screening

The extract was quantitatively assayed for the presenceof phytochemicals such as saponins, tannins, alkaloids, terpenoids,cardiac glycosides and flavonoids as described by Trease and Evans, [11].

Comment [37]: Replace with: glycosides, and flavonoids, as described by Trease and Evans

Experimental design

Animal Grouping

A total of 25 Wistar rats were randomly divided into four (5) groups of five (5) rats each. The groups were treated thus:

Group I (Normal control): Rats were administered 2 mL of distilled water.

Group II: Rats 2ml/kg bw, 1:1 intraperitoneally only.

Group III: Rats were administered with 100 mg/kg MLESP

Group IV: Rats were administered with 200 mg/kg MLESP

Group V: Rats were administered with 400 mg/kg MLESP

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Methanol leaf extract of *Spondias mombin* was administered daily via oral gavage for seven days. On the seventh day, Groups II to V were administered with a mixture of freshlyprepared CCl_4 in liquid paraffin (2ml/kg bw, 1:1 intraperitoneally) onehour after administration of the last dosing. Body weights of all ratswere recorded at the commencement of experiment and after the last dosing. After 48 hours rats were anesthetized using diethyl ether prior tosacrifice. Blood was obtained by cardiac puncture.

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Measurement of biochemical parameters

Blood was spun at 3000 rpm for 10minutes at 4ºC to separate serum into vacutainer vials and stored at4ºC until used for analyses. The serum collected was used to determine Alanine Aminotransferase(ALT), Aspartate Aminotransferase (AST), Alkaline Phosphatase(ALP) using Randox diagnostic kits.

Statistical Analysis

Data generated were expressed as Mean ± Standard Deviation using SPSS (Ver. 23). Data were analysed using one way Analysis of Variance (ANOVA). Differences in mean were compared using Turkey Test. *p-values* less than 0.05 was considered statistically significant.

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Table 1: Liver Enzymes Activities in Rats administered Methanol Leaf Extract of *Spondias mombin*

Groups	ALT (IU/L)	AST (IU/L)	ALP (IU/L)
Normal control	52.62 ± 2.10[a]	193.11 ± 0.83[a]	216.01 ± 2.30[a]
Negative control	143.21± 1.20[e]	318.62 ± 2.81[e]	325.83± 3.90[e]
MLESP $_{100\ mg/kg}$	111.23± 2.80[d]	261.13 ± 1.92[d]	278.32 ± 2.63[d]
MLESP$_{200\ mg/kg}$	102.21± 2.10[c]	229.51± 2.81[c]	260.36± 2.62[c]
MLESP$_{400mg/kg}$	73.86± 2.10[b]	202.38 ± 0.35[b]	236.31± 2.60[b]

Results are expressed as mean ± standard deviation from three determinations. Values with same superscript in column are not significantly different at (P<0.05).

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Note: SMLE= *Spondias mombin* Leaf extract

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Table 2: Result on the Qualitative Phytochemical Screening on Methanol Leaf Extract of *Spondia mombin*

Phytochemicals	Abundance
Flavonoids	+
Taninins	+
Alkaloids	+
Phenols	++
Cardiadiac glycosides	++

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Table 3: Body weight changes in Rats administered Methanol extract of *Spondia mombin*

Treatment	Initial weight	Final weight
Normal Control	116.30±6.32[a]	137.00±5.38[b]
Negative Control	129.21±6.42[b]	110.28±2.39[a]
100 mg/kg MLESP	139.00±8.59[a]	142.01±4.21[b]
200 mg/kg MLESP	151.70±4.21[a]	168.20±9.21[b]
400 mg/kg MLESP	140.70±3.29[a]	145.70±3.29[b]

Results are expressed as mean ± standard deviation from three determinations. Values with same superscript in column are not significantly different at (P<0.05).

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Results and Discussions

When the liver cell membrane is damaged, a variety of enzymes located in the cytosol are released into the blood stream. Measurement of s of the activities of the serum hepatomarkers such the ALT, AST and ALP have provided powerful tool for assessment of liver function [12]. Table 1 shows the activities of serum hepatomarkers (ALT, AST and ALP) following oral administration of methanol leaf extract of *Spondias mombin*. Damage to the liver following intraperitoneal administration of CCl_4 in paraffin triggered increased activity of ALT, AST and ALP. However, oral administration of *Spondias mombin* leaf extract significantly ($P<0.05$) reduced the activities of the enzymes in a dose dependent manner which however was significantly ($P<0.05$) higher than that reported for the control. The decreased serum activities of the serum hepatomarkers following ingestion of *Spondias mombin* extract could be attributed to the presence of phytochemicals. This is consistent with the finding of Nwidu et al. [8] which showed that methanol stem bark and leaf extract of *Spondias mombin* significantly reduced the activity of serum hepatomarkers. Table 2 shows the outcome of qualitative phytochemical screening on methanol leaf extract of *Spondias mombin* indicating the presence of flavonoids, tannins, alkaloids, phenols and cardiac glycosides. It could be observed from the study that phenols and cardiac glycoside were more abundant that other phytochemicals reportedly present. Changes in the body weight have been used as indicator of adverse effects of drugs and chemicals [13]. Table 3 shows the bodyweight of rats administered methanol leaf extract of *Spondias mombin*indicating that a significant decrease was observed on the negative control contrary to the observation made on other treatment groups. The body weight increase observed on the treated groups could be as a result of extract effect on the appetite centre of the hypothalamus. The findings established through this research effort is consistent with the outcome of a work by Nwidu et al [8] which showed a dose dependent increase in the body weight of animals administered stem bark extract of *Spondias mombin*.

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