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

Abstract

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 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 options. Hence, the aim of this study was to evaluate the restorative potential of the methanol seed extract of *Spondias mombin* on Carbon tetrachloride induced hepatic damage. Freshly harvested leaves of *Spodias mombin* were dried at room temperature prior to being ground into a fine powder. The powdered plant sample was steeped in 1 L of 50% methanol for a period of 72 hours, during which the mixture 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 samples collected and analyzed using standard procedures. The solvent was filtered over a layer of gauge and then the filtrate evaporated to dryness at 55°C. Qualitative phytochemical screening of the resulting extract revealed the presence of flavonoids, tannins, alkaloids, phenols, and cardiac glycoside. Oral administration of extract significantly ($P \leq 0.05$) reduced the activities of Aspartate amino transferase (AST), Alanine transaminase (ALT) and Alkaline phosphatase (ALP). The body weight of rats significantly ($P \leq 0.05$) increased in all groups except **Group II,** which was not administered extract. In conclusion, methanol leaf extract of *S. mombin* wields the potential to restore damaged hepatocytes.

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

Introduction

Liver diseases have been identified as one of the major public health concerns in recent times and have contributed maximally to the global burden of diseases, accounting for most deaths worldwide [1]. Conventional treatment approaches are either expensive or unsustainable, factors that have rendered them unreliable and consequently out of context, a development that has paved the 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].

Botanical therapies have been part of 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 support from plant based medicinal preparations [3].

Spondias mombin belongs to the *Anacardiaceae* family and is commonly referred to as yellow mombin, hog plum, or ubos. *Spondias mombin* can grow to a height of 15-20 metres 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 world, with high genetic variability among populations [4]. Numerous reports on the medicinal properties of *S. mombin* leaf have revealed that it can decrease anxiety, halts convulsions, and relieve pain [5]. Further research efforts have shown that it is rich in vitamins A and C [6] and has 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 a paucity of data on the potential of the seed of the said plant to protect the liver against external insults, an observation that underscores the imperativeness of this study.

Materials and Methods

Spondias mombin seeds were harvested from a farm in Afikpo North Local Area, 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

Spondias mombin L. seeds were dried at room temperature. The dried seeds were ground with a mortar and pestle blender. The cold extraction method was employed to extract 500 g of powdered seed samples with methanol as solvent. The powdered plant sample was soaked in one litre of 50% methanol for a period of 72 hours, during which the mixture was shaken twice daily. The solvent was filtered over a layer of gauge, and then the filtrate evaporated to dryness in vacuo at 55°C. The resulting methanol seed extract of *Spondias mombin* (MSESP) was appropriately preserved for use.

Animals

Mature male Wistar rats that weighed between 130 and160 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 the commencement of the experiment which followed the Guide to the Care and Use of Animals in Research and Teaching [9].

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 hours 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, each of which was separately administered 1600, 2900, and 5000 mg/kg of extract, and afterwards, animals were observed for 48 hours for signs of toxicity, according Lorke [10].

Phytochemical screening

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

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 MSESP.

Group IV: Rats were administered with 200 mg/kg MSESP.

Group V: Rats were administered with 400 mg/kg MSESP.

Methanol seed 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 freshly prepared CCl_4 in liquid paraffin (2ml/kg bw, 1:1 intraperitoneally) one hour after administration of the last dosing. The body weights of all rats were recorded at the commencement of the experiment and after the last dosing. After 48 hours, rats were anesthetized using diethyl ether prior to sacrifice. Blood was obtained by cardiac puncture.

Measurement of biochemical parameters

Blood was spun at 3000 rpm for 10 minutes at 4ºC to separate serum into vacutainer vials and stored at 4ºC until used for analyses. "The serum collected was used to determine Alanine

Aminotransferase (ALT), Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP) using Randox diagnostic kits". [11]

Statistical Analysis

The data generated were expressed as mean ± standard deviation using SPSS (Ver. 23). Data were analysed using one way analysis of variance (ANOVA). Differences in the mean were compared using the Turkey Test. *p-values* less than 0.05 were considered statistically significant.

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]
MSESP 100 mg/kg	111.23± 2.80[d]	261.13 ± 1.92[d]	278.32 ± 2.63[d]
MSESP 200 mg/kg	102.21± 2.10[c]	229.51± 2.81[c]	260.36± 2.62[c]
MSESP 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 ($P \leq 0.05$).

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

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

+ [abundant], ++ [more abundant]

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 MSESP	139.00±8.59[a]	142.01±4.21[b]
200 mg/kg MSESP	151.70±4.21[a]	168.20±9.21[b]
400 mg/kg MSESP	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 ($P<0.05$).

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 the activities of the serum hepatomarkers such as ALT, AST, and ALP has provided a powerful tool for the assessment of liver function" [12]. Table 1 shows the activities of serum hepatomarkers (ALT, AST, and ALP) following oral administration of the methanol seed 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 ($P \leq 0.05$), reduced the activities of the enzymes in a dose dependent manner which however was significantly ($P \leq 0.05$) 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] who showed that "methanol stem bark and leaf extracts of *Spondias mombin* significantly reduced the activity of serum hepatomarkers. Table 2 shows the outcome of qualitative phytochemical screening on a methanol seed 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 glycosides were more abundant that other phytochemicals reportedly present. Changes in body weight have been used as indicator of adverse effects of drugs and chemicals" [13]. Table 3 shows the body weight of rats administered methanol leaf extract of *Spondias mombin*, indicating that a significant decrease was observed in the negative control, contrary to the observation made in other treatment groups. The body weight increase observed in the treated groups could be a result of the extract effect on the appetite centre of the hypothalamus. The findings established through this research effort are 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 a stem bark extract of *Spondias mombin*.

Conclusion

It is concluded that 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.

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