ASSESSMENT OF THE HEPATOPROTECTIVE POTENTIAL OF METHANOL SEED EXTRACT OF (*CARICA PAPAYA)* PAWPAW IN CCL4 INDUCED HEPATIC DAMAGE IN WISTAR RATS

Abstract

The liver is one of the most vital organs that functions as a centre for metabolism of nutrients and excretion of waste metabolites among numerous important metabolic tasks. A total loss of liver function could lead to death within minutes, demonstrating the liver's great importance. Thus, the aim of this study was to assess the hepatoprotective potential of methanol seed extract of $_{\text{Carica papaya}}$ on CCl_4 induced hepatic damage. *Carica papaya*seed obtained from ripe pawpaw fruit were processed into fine powder and subsequently extract. Thirty adults male Wistar rats were divided into five groups of five rats each. Groups II-VI were administered carbon tetrachloride (2 mL/kg body weight) and olive were mixed 1:1 to induce acute liver injury. Group I was the normal control and was administered 1 mL/kg of distilled water. Group II was not administered CP extract. Group III was administered 100 mg/kg bw of CP extract. Group IV was administered 200 mg/kg bw of CP extract. Group V was administered 300 mg/kg bw of CP extract. Group VI was administered 100 mg/kg bw of standard drug (Silymarin). Animal body weight was determined twice i.e., atthe commencement the study and at the completion of the study. Administration of carbon tetrachloride significantly raised the activities of the liver enzymes i.e., Aspartate aminotransferase (AST), Alanine transaminase (ALT) and Alkaline phosphatase (ALP). However, oral administration of CP seed extract significantly ($P<0.05$) lowered the activities of the said enzymes though to levels which were significantly ($P<0.05$) higher than that reported for the normal control. A contrary observation was however made on the activity of AST. The body weight of rat at the completion of experiment was significantly ($P<0.05$) higher than that reported at the commencement of the study. Meanwhile, a contrary observation was made on group II. In conclusion, it can be deduced from this study that that methanol seed extract of *Carica papaya* may have the ability to ameliorate chemically induced hepatic damage.

Keywords: Liver, Enzymes, *Carica papaya*, Hepatoprotective, Silymarin

Introduction

The liver is the most vital and largest solid organ of the body saddled with the task of nutrient metabolism as well as evacuation of metabolic waste products[1]. Primarily, it is concerned with the control of the flow as well as the safety of substances absorbed from the gastrointestinal tract (GIT) prior to distribution of substances to systemic circulation [2]. Thus, a complete loss of

liver function could translate to death within a short time, thereby demonstrating the significance of liver to life[3].

The harmful effects of ambient particulate matter or carbon black on the liver are well documented. Several animal models have clearly shown that exposure to PM or CB can cause direct hepatotoxicity [4]. Incidence of hepatic damage resulting from exposure to particulate matter is more prevalent in developing countries owing to the ever increasing human population and consequent demand to meet human need through industrialization in a system with a wobbling environmental laws and attendant weak adherence which culminate to an alarming levels of air pollution and its attendant consequences notably liver damage among others [5].

Plant-based therapeutic options remain the pivot upon which trado-medical practices rest since prehistoric times. In fact, an estimated 80% of the global population rely on plant-based therapeutic substances to meet their basic health care need [6]. This could be attributed to the fact that they are relatively safe for consumption in addition to being affordable among numerous other advantages[7].

Carica papaya popularly known as papaya is has been used in the treatment of diverse human ailments. It is a member of the *Caricaceae* family. Its cultivation predominates in the tropics, sub-tropical and temperate zones such as Australia,Brazil, China, Hawaii, Nigeria etc[8];[9]. It owes it therapeutic potential to its rich phytochemical contents. Major phytochemicals present in the plant include tannins, steroids, terpenoids, saponins, phenols, flavonoids etc. [10]. Evidently, *Carica papaya* fruit pulp extract has been relied upon to protect against CCl_4 induced hepatic toxicity in rats [11]. Similarly, *C. papaya* root extract had also been used to protect against sodium arsenate induced hepatic damage in mice[12].Unfortunately, these plant parts are not

sustainable as the fruit serves mainly as food while the root plays key role to the survival of the plant itself. Thus, the need to evaluatethe *Carica papaya* fruit seed which could be considered irrelevant to human as food or are not required by the plant to survive becomes imperative.

Material and Methods

Collection of plant material

Ripe fruits of Carica papaya (pawpaw) were procured from Eke Market, Afikpo, South Eastern Nigeria. They were identified and authenticated at the herbarium unit of the Department of Forestry, Micheal Okpara University of Agriculture, Umudike South Eastern University.

Extraction

Pawpaw fruits were dissected and the seeds collected were washed using clean get rid of debris. The clean seeds subsequently sun-dried for 48 hr and then oven-dried at 40^{o}C for 1 week to ensure complete dryness. With the aid of an electric blender, dried seeds were thoroughly ground into fine power. Exactly 500 g of the papaw seed powdered sample was wrapped in a thimble and placed in a 500 cm 3 Soxhlet extractor (M&G Scientific Co., England). The sample was Soxhlet extracted following standard analytical laboratory method at 60^{o}C in methanol for 72 h. The extract was evaporated to a paste form at 40^{o}C for 8 hr to produce 36% yield of raw sample of *C. papaya* seed extract. The powder plant-drug was stored for use in the experiment in a refrigerator

Animals

Adult male Wistar rats weighing between 150 and 180 g were purchased from the Animal House of the Department of Science Laboratory Technology,AkanuIbiam Federal Polytechnic Unwana,

Afikpo. The animals were housed in transparent plastic cages and fed rat pellets. They were allowed access to water *ad-libtum* with a 12-h dark-light cycle. The animals were allowed an acclimatization period of 14 days prior to the commencement of extract administration.

Acute Toxicity Test

Acute toxicity test was determined in line with the description of Lorke [13]. In this test, two phases were involved. At the initial phase, nine (9) adult rats were divided into three (3) groups of three rats each and were subsequently administered 10, 100 and 1000 mg/kg b.w.of extract respectively. This was followed by a close monitoring of the animals for manifestations of signs of toxicity for a period of 24 hrs in the absence of which the second phase was initiated with three adults male Wistar rats which were divided into three groups of one rat per group and were administered 1600, 2900 and 5000 mg/kg b.w of extract orally respectively. the animals were observed for 24 hours for clinical signs of toxicity. The LD_{50} was calculated as the geometric mean of the non-lethal dose and lowest lethal dose.

Induction of Hepatic Damage

Carbon tetrachloride (2 mL/kg body weight) and olive were mixed 1:1 to induce acute liver injury in rats [14]. Olive oil was used as the vehicle and treatment lasted for 28 days.

Animal grouping

Group I (Normal Control): Animals were orallyadministered 1 ml of distilled water daily and olive oil (2 ml/kg body weight) thrice weekly

Group II: Animals were induced hepatic damage without treatment and olive oil (2 ml/kg body weight) thrice weekly

Group III: Animals with hepatic damage treated with 100 mg/kg of CP extract and olive oil (2 ml/kg body weight) thrice weekly

Group IV: Animals with hepatic damage treated with 200 mg/kg of CP extract and olive oil (2 ml/kg body weight) thrice weekly and olive oil (2 ml/kg body weight) thrice weekly

Group V: Animals with hepatic damage treated with 300 mg/kg of CP extract

Group VI: Animals with hepatic damage treated with 100 mg/kg bodyweight of silymarin

Determination of Body Weight

With the aid of an electronic weighing scale, the weights of the animals were determined at the beginning of the experiment and at its termination after which the animals were humanely sacrificed and samples collected for analysis.

Biochemical analysis

Sample preparation: In order to performliver function tests, exactly 2 mL of blood introduced intoan EDTA tube and subsequently centrifuged at 4,000 rpm for 15 min and the plasma obtained was used for biochemical analysis. Kits were used to determine the activity of aspartate aminotransferase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP).

Histopathological studies

Liver tissue harvested waspreserved in 4% formaldehyde solution, embedded in paraffin wax, and subsequently sliced. Sliced sections were fixed on slides and stained with H&E staining. The tissue sections were observed under a microscope (×400) to identify changes in the hepatocyte[15].

Data 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.

Table 1: Effect of *Carca papaya* Seed Extract on Serum Hepatomarkers

Treatment	AST (U/l)	ALT (U/l)	ALP (U/l)
Group I (Normal control)	9.90 ± 2.30^{a}	15.00 ± 5.97^{a}	55.00 ± 3.24^{a}
Group II (Negative control)	42.10 ± 3.10^{d}	29.04 ± 3.20^{d}	115.00 ± 6.20^{c}
Group III (100 mg/kg.)	10.00 ± 3.02^{a}	21.00 ± 1.21^{c}	66.00 ± 2.20^{b}
Group IV (200 mg/kg)	10.20 ± 1.32^{a}	20.01 ± 1.23^{c}	64.00 ± 1.43^{b}
Group V (300 mg/kg)	10.00 ± 4.23^{a}	19.00 ± 2.32^{c}	60.00 ± 1.65^{b}
Group VI Std drug (silymarin)	10.05 ± 2.10^{a}	$15.2.00\pm2.10^{a}$	56.00 ± 9.74^{a}

Results are expressed as mean ± standard of five determination. Values with the same superscript in a column are not significantly ($p<0.05$)

Table 2: Body weight of rats with Hepatic damage treated with *Carica papaya* Extract

Treatment	Initial wt (g)	Final wt (g)
Group I (Normal control)	125 ± 21.87^{a}	157 ± 21.00^{b}
Group II (Negative control)	161 ± 2.52^{b}	135 ± 2.56^{a}
Group III (100 mg/kg.)	153 ± 2.18^{a}	165 ± 2.05^{b}
Group IV (200 mg/kg)	165 ± 2.26^{a}	177 ± 5.36^{b}
Group V (300 mg/kg)	163 ± 2.36^{a}	178 ± 21.65^{b}
Group VI Std drug (silymarin)	130 ± 4.20^{a}	135 ± 4.55^{b}

Results are expressed as mean ± standard of five determination. Values with the same superscript in a column are not significantly ($p<0.05$)

Results and Discussions

The liver is one the critical organs of the body saddled with the task of detoxification of xenobiotics. It is also highly susceptible to damage through exposure to diverse environmental pollutants. Table 1 shows the hepato-protective effect of methanol seed extract of *Carica*

*papaya*in Wistar rat. Exactly 2 ml of $CCl_4$was orally administered to induce hepatic damage. However, following oral administration *Carica papaya* seed extract, there was a significant ($P<0.05$) reduction in the activity of the aspartate amino transaminase compared to the activity reported for the untreated control (group II). It is important to note that groups III and IV administered 100 and 200 mg/kg of extract of *Carica papaya* seed was not significantly ($P>0.05$) different from that reported for the standard control (group VI) administered 100 mg/kg of the standard drug silymarin. On the activity of the ALT, the reported activity for the normal control (group 1) was not significantly ($P>0.05$) different from that reported for the standard control (group VI) but significantly ($P<0.05$) lower than that reported for the negative control (group II). However, the activity of ALT reported for groups III, IV and V administered 100, 200 and 300 mg/kg of *Carica papaya* extract was not significantly ($P<0.05$) different but was significantly ($P<0.05$) lower than the activity of ALT reported for group II and higher than that reported for the normal control (group I). Similar observation was made on the activity of the alkaline phosphatase (ALP). Observed decline in the activity of the liver enzymes following oral administration of extract could be attributed to the presence of phytochemicals inherent in theseed. This finding is consistent with the finding of Shuban et al.[11] who established the prophylactic and restorative effect of *C. papaya* Linn pulp water extract against CCl_4 induced hepatic damage.It is also in tandem with the outcome of a work done by Ojo et al. [12] which showed that 150 mg/kg of aqueous extract of pawpaw root protected against arsenate induced damage.Table 2 shows the body weight of rats with hepatic damage administered with *Carica papaya* seed extract indicating that although hepatic damage could cause a marked reduction in the weight of the experimental animals, treatment with the aforementioned extract resulted in a significant ($P<0.05$) increase in weight of rats.

Conclusions

In order to maximize the benefits of pawpaw consumption, and to shift attention from dependence on certain vital parts of the plant for use as medicine, it is imperative to explore the efficacy of some of its relegated parts such as the seed. Thus, it can deduced from this study that that methanol seed extract of *Carica papaya* may has the ability to ameliorate chemically induced hepatic damage.

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