

Toxicological Evaluation of Selected Medicinal Plants on Hepatic and Renal Functions

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Abstract

The aim of this study was to evaluate the toxicity of the methanol leaf extract of *Anacardium occidentale* (cashew) *Carica papaya* (pawpaw) and *Psidium guajava* (guava) on hepatic and renal functions of wistar rats. Indices of hepatic and renal functions which were measured using standard procedures revealed a non-significant ($P>0.05$) difference in the activities of Aspartate transaminase (AST) of the treated groups (II-IV) and that of the normal control group. However, for Alanine aminotransferase and Alkaline phosphatase the value (ALT) (41.67 ± 1.20 IU/L), (43.67 ± 1.20 IU/L), (40.33 ± 1.46 IU/L) and (ALP) (65.67 ± 1.76 IU/L), (60.67 ± 6.36 IU/L), (60.33 ± 5.78 IU/L) respectively recorded for the treated groups were significantly ($P<0.05$) higher than those of the normal control groups (36.67 ± 1.20 IU/L) and (57.67 ± 43.33 IU/L) respectively. Measurement of renal function indices revealed that the value of urea for the treated groups (II-IV) (38.67 ± 0.88 mmol/L), (39.00 ± 2.086 mmol/L) and (37.00 ± 2.08 mmol/L) was significantly ($P<0.05$) higher than that of the normal control group (28.67 ± 3.17 mmol/L). However, for total protein and albumin, there was no significant ($P>0.05$) difference in values recorded for the treated groups and the normal control group. In conclusion, findings made in this work indicate that pawpaw, guava and cashew leaves extract may contain compounds with potential to induce serum hepatomakers which however may not be suggestive of any form of liver damage.

Keywords: Hepatic; Renal; Toxicity; Injuries; Protein.

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1. Introduction

Medicinal plants as the name implies are plants whose part or parts have been recognized for its therapeutic abilities. Plants' parts such as the leaf, stem bark, and root used both for prophylactic and therapeutic purposes against diseases affecting mankind have been documented in reputable scholarly articles [1]. About 1500 are known globally for their therapeutic potentials. The use of medicinal plants in the health care system has been in existence since prehistoric time and has been absorbed into the African cultural systems where plant derived medicinal preparations have been relied upon to treat diverse infectious and non-infectious diseases[2].

In accordance with World Health Organization (WHO) report, about 90% of the populations of developing countries depend on the use of plant based preparations to address numerous diseases ravaging them [3]. The interest in the use of medicinal plants in the treatment of diseases has been propelled by the characteristic toxic influence of their synthetic counterparts on biological systems [4].

It is a widely held belief, that therapeutic preparations derived from medicinal plants are not toxic. This impression is misleading as research has established the toxic effects of such preparations on the health of subjects [5] which is dependent on factors such as the strength of secondary metabolite and the quantity of the material consumed [5].

Carica papaya (Pawpaw) which is predominantly cultivated in the tropics is a member of the family *Caricaceae* [6]. Research has shown that parts of *C. papaya* are embodiment of compounds with wide therapeutic potentials.

Notable examples of such compounds are the anti-oxidants and minerals reportedly present in different parts of the plants [7]. Leaf of *C. papaya* is used in the treatment of diseases such as dyspepsia, hyperacidity, dysentery and constipation etc [8].

Psidium guajava (guava) is a well known tropic tree which is mainly grown for its fruit. Belonging to the family *myrtaceae*, it is cultivated in South Africa [9]. The leaf has been extensively explored in the treatment of numerous human diseases [10]. It is consumed not only as food but also as a folk medicine in sub-tropical areas all over the world due to its pharmacologic activities in addition to its anti-inflammatory and anti-diabetic properties [11]. *Anacardium occidentale* which can also be referred to as cashew belongs to the family *Anacardiaceae* [12]. The tree is native to Brazil, although it is found in tropical countries. Different parts of the *Anacardium occidentale* have been used in the treatment of diverse diseases ravaging mankind. These include bacterial and fungal infections and oxidative stress related conditions [12]. Although these plants are inherently endowed with therapeutic potentials, the possibility of the presence toxic components cannot be completely ruled off and thus has informed the basis for this research [13].

2. Materials and methods

Collection of plant material

Fresh leaves of Cashew (*Anacardium occidentale*), pawpaw (*Carica papaya*) and Guava (*Psidium guajava*)

were collected from Zaria metropolis. Identification and authentication of the leaves were performed at the Herbarium unit of the Department of Biological Sciences, Ahmadu Bello University, Zaria.

Preparation of plant extracts

Leaves sourced from the three different plants were separately dried at room temperature, after which they were individually ground to powder using an electric blender. The resulting powder was sieved to obtain fine powder. 500 g of the powdered plant sample was separately suspended in 2 Liters of 70% methanol for 72 hours and stirred intermittently. The extracts were filtered and the filtrates concentrated at 40°C [14].

Animals

Adult male wister rats (150-200g) were procured from the animal house of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria. The animals were housed in well ventilated cages and fed with rat chow and water *ad libitum*.

Acute toxicity study

The acute toxicity test was performed in accordance with the method of Lorke [15]. The initial phase was characterised by the division of the rats into 3 groups of 3 rats per group and were orally administered with 10mg, 100mg and 1000mg of the extracts per kg body weight.

They were observed for 24 hours for signs of toxicity. In the absence of which the second phase was initiated and made up of 4 rats which were divided into 4 groups of 1 rat per group. The LD₅₀ was determined from the results of the final phase as the square root of the product of the lowest lethal dose and the highest non-lethal dose.

Experimental design

Twenty adult male wistar rats were divided into four groups of five rats each.

Group I: Normal control was fed with only rat chow and water *ad libitum*.

Group II: Rats were administered with 200 mg/kg b. w of methanol extract of *Carica papaya* (pawpaw) leaf orally.

Group IV: Rats were administered with 200 mg/kg b.w of methanol extract of *Anacardium occidentale* leaf orally.

Group V: Rats were administered with 200 mg/kg b.w of methanol extract of *Psidium guajava* (guajava) leaf orally.

Collection of blood sample and preparation of serum

The experiment was terminated on the 28th day of starting of the experiment, after which rats were sacrificed and blood samples collected in red topped tubes and allowed to stand at room temperature for 15-30 minutes to clot. Blood samples were centrifuged at 1500 x g for 10 minutes. The serum was transferred into a collecting tube with the aid of a pipette.

Renal Function Test

Serum urea concentration:

Exactly 2 ml of water was introduced into test tubes; this was followed by the inclusion of 10 µL of test sample into. 10 µL of standard reagent was added into a separate test tube. 2 ml of uric acid colour reagent was introduced into all test tubes and incubated for 20 minutes. The blank was used to zero the machine. The absorbance of the test samples and the standard were read at 500 nm and the concentration of the test sample was determined [16].

Determination of total proteins

Exactly 1 ml of biuret reagent was added to sample test tubes containing 20 µL of test sample. Precisely 20 µL of the standard reagent introduced into a separate test tubes was allowed to incubate for 10 minutes under room temperature. The blank was used to zero the machine and absorbance read at 540nm [17]

Determination of albumin concentration

Precisely 1 ml of bromocresol green reagent was introduced into sample test tubes containing 20 µL of test sample. This was followed by the addition of 20 µL of the standard reagent was added into another test tube. The test tubes were incubated for 10 minutes under room temperature. The blank was used to zero the machine and the absorbance was read at 630nm [18]

Evaluation of Markers of Liver Function

Determination of aspartate aminotransferase

Exactly 1 ml of reagent was introduced into sample test tubes containing 500 µL of test sample and 50 µL of the standard reagent was added into separate test tube. The test tubes were incubated at room temperature for 20 minutes, mixed immediately and first absorbance of test was read exactly at 1 minute and thereafter at interval of 30, 60, 90 and 120 seconds at 340 nm. The mean change in absorbance was determined [19].

Determination of alanine aminotransferase activity

Precisely 1 ml of reagent was introduced into sample test tubes containing 500 µL of test sample. Exactly 50 µL of the standard reagent was added into a separate test tube. Samples were incubated at room temperature for 20 minutes, it was mixed immediately and first absorbance of test was read at exactly 1 minute and thereafter, 30, 60, 90 and 120 seconds at 340nm. The mean change in absorbance per minute was determined [19].

Determination of alkaline phosphatase activities

Precisely 3 ml of substrate solution was incubated at 37°C for 15 minutes after which 0.5 ml of test samples was introduced. The mixture was shaken vigorously. 0.05 ml of the mixture was removed and mixed with 9.5 ml of 0.085 N NaOH. This corresponded to zero time assay (blank). The remaining solution (substrate+enzyme) was incubated for 15 minutes at 37°C and then 0.5 ml was drawn and mixed with 9.5ml of 0.085N NaOH. Absorbance was measured at 405 nm against the reference blank [20].

Statistical Analysis

Results were expressed as mean $\pm$ standard error of mean (SEM). The data was analyzed with the aid of the analysis of variance (ANOVA). The differences in mean among groups were compared using the Duncan Multiple Range Test. P value less than 0.05 was considered significant.

3. Result

Table 1.0: Liver Enzymes activity of Rats treated with selected medicinal plants

Groups	Treatment	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
Group I	Normal Ctrl	19.33 $\pm$ 0.67 ^a	36.67 $\pm$ 1.20 ^a	57.67 $\pm$ 4.33 ^a
Group II	<i>C. papaya</i> 200mg/kg	22.00 $\pm$ 1.15 ^{ab}	41.67 $\pm$ 1.20 ^{bc}	65.67 $\pm$ 1.76 ^{bc}
Group III	<i>P. guavaja</i> 200mg/kg	21.00 $\pm$ 1.00 ^a	43.67 $\pm$ 1.26 ^c	60.67 $\pm$ 6.36 ^b
Group IV	<i>A. occidentale</i> 200mg/kg	21.33 $\pm$ 1.33 ^a	40.33 $\pm$ 1.45 ^{bc}	60.33 $\pm$ 5.78 ^b

Values are expressed as mean $\pm$ standard error of mean. Values with same superscript in a row are not significantly (P<0.05) different

Table 2.0: Indices of renal function of rats treated with selected medicinal plants

Groups	Treatment	Urea (mmol/L)	TP (g/L)	Albumin (g/L)
Group I	Normal Ctrl	28.67 $\pm$ 3.17 ^a	69.33 $\pm$ 3.46 ^{ab}	39.67 $\pm$ 1.45 ^b
Group II	<i>C. papaya</i> 200mg/kg	38.67 $\pm$ 0.88 ^b	67.33 $\pm$ 2.40 ^{ab}	39.40 $\pm$ 1.00 ^b
Group III	<i>P. guavaja</i> 200mg/kg	39.00 $\pm$ 2.08 ^b	66.33 $\pm$ 0.88 ^{ab}	40.00 $\pm$ 2.25 ^b
Group IV	<i>A. occidentale</i> 200mg/kg	37.00 $\pm$ 2.08 ^{bc}	66.33 $\pm$ 1.15 ^{ab}	40.00 $\pm$ 2.54 ^b

Values are expressed as mean $\pm$ standard error of mean. Values with same superscript in a row are not significantly (P<0.05) different

4. Discussion

Medicinal plants can be deleterious to human health. This is evident by the fact that about 1,500,000 plants have been reported to contain toxic components which are mainly secondary metabolites [21]. Table 1.0 shows the activity of liver enzymes of rat models sorted into groups and administered with 200 mg/kg b.w methanol leaf extract of *Anacardium occidentale* (cashew) *Carica papaya* (pawpaw) and *Psidium guajava* (guava) leaves accordingly. There was no significant ($P>0.05$) difference in the activity of aspartate aminotransaminase (AST) of treated rats i.e. Groups II-IV compared to that of the normal control group. This may be attributed to the nature or strength of the inherent toxic components, the dose of extract administered, or even the part of the plants ingested [5]. This result is consistent with the finding of Nwiloh and his colleagues which infers that the aqueous leaf extract of the aforementioned plants did not induce significant ($P<0.05$) increase in the activity of aspartate aminotransferase (AST).

However, there was a significant increase in the activities of ALT and ALP of treated groups compared to the control. This result is consistent with the sub-acute toxicity study performed by Charlse and his colleagues [22] which affirms that oral administration of (10-500 mg/kg) methanol leaf extract of the plants being studied for 14 days increased the activities of serum hepatomarkers.

Table 2.0 shows the renal indices of experimental rat models drafted into groups and treated with methanol leaf extract of *Anacardium occidentale* (cashew) *Carica papaya* (pawpaw) and *Psidium guajava* (guava) leaves. The concentration of urea was significantly ($P<0.05$) high in treated groups compared to the normal control. On the other hand, there was no significant ($P>0.05$) difference in the concentration of total protein and albumin in the treated groups compared to normal control group.

This may be as a result as of the nature the secondary metabolites, the dose of extract administered, or even the part of the plants consumed [5]. This result is consistent with the finding of Hallim and his colleagues [23] which shows that urea concentration increased in rat models administered with leaf extract of cashew, pawpaw and guava

5. Conclusion

Findings from this work indicate that there may be inherent compounds capable of inducing liver enzyme which may not necessarily imply a damaged liver.

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