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DETERMINATION OF ACUTE TOXICITY AND EFFECTS OF DRIED CARPEL OF *ANNONA SENEGALENSIS* EXTRACT ON ALKALINE PHOSPHATASE, AMINO TRANSFERASES AND BODY WEIGHT IN MICE

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ABSTRACT: *Annona senegalensis is an evergreen shrub that is used for ethnomedicinal purposes and as a source of food. The toxicological potential of dried carpels of Annona senegalensis was investigated by determining the acute toxicity and the effect of the plant extract on body weight, alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) serum level. Cold methanolic extract of the plant was concentrated using steam bath. Acute toxicity testing was carried out to determine LD_{50}. While sub chronic toxicity screening was conducted on the animals grouped in to 3 test groups and a control group comprising of 12 animals per group treated with 100, 300, 500 mg/kg concentrations of the extract and distilled water, respectively. The body weights of the animals were taken at regular intervals and their serum analysed weekly to ascertain the biochemical activities of ALP, ALT and AST. The LD_{50} of the extract indicated a very high safety profile with no mortality. There was no significant ($P > 0.05$) difference in the weights of the animals after treatment except for 500 mg/kg which exhibited a significant ($P < 0.05$) decrease from day 8 up to day 23. Generally, there was a significant increase in the serum level of ALP and ALT with respect to time and concentration in the test groups except for AST which showed a concentration dependent decrease. However, the control had the highest serum level of the analyzed enzymes which were significantly ($P<0.05$) higher than the test groups. The result concludes that dried carpels of A.senegalensis possess no significant toxic effects which justifies why the local dwellers in northern Nigeria have been utilizing it as part of their foodstuff without obvious complaints or toxicity. The consumption dried carpels of Annona senegalensis as part of their food stuff for its hepatoprotective property and hypolipidemic effect should be encouraged.*

KEYWORDS: Annona Senegalensis, Carpels, Enzymes, Methanolic Extract, Toxicity.

INTRODUCTION

Nigeria like other African countries is blessed with variety of nutritional food crops which enhances healthy body development and growth. To secure a healthy Nigeria population that can promote development there is need to reinforce and ensure a standard relationship between food, nutrition and health (Atasie *et al*, 2009). Even with these natural endowments, the problem of malnutrition has remained pronounced in developing countries with Nigeria inclusive especially among infants and children of rural areas (WHF, 2005). This has stirred up lots of research works that is focused on healthy nation building. Richard *et al*, (2007) reported that one possible way of reducing the level of malnutrition is by increasing the intake of foods rich in energy, proteins, iron and especially those from rural environment. In order to redeem the malnourished state of our society there is need to promote the consumption of some nutritious available local resources such as some local indigenous vegetables that are edible. This is due to the fact that the entire human population in Africa according to Achu *et al.*, 2005

have been found to depend solely on the numerous edible vegetable to make up for the limited available minerals and vitamins. The usage of these plants for all purposes is attributed to their relatively easy access and affordability to the people who live mostly in poor rural communities (Adzu *et al.*, 2004).*Annona senegalensis* is one of these wonderful plants it is known as 'gwandar-daaji' by the Hausa people, 'nungbere' by the Nupe's and 'dukuuhi' by the Fulanis. Some other vanacular names of this plant include 'Uburu ocha' (Igbo), 'arere'or 'abo' (Yoruba), 'Ikpokpo' (Igala), 'Uwu' (Idoma), 'ngonowu'(Kanuri) and 'Nkonokono' (Swahili) (Theophine *et al*., 2012).

Knowing the toxicological status of local soup ingredients and foodstuffs is very essential in order to encourage their cultivation and consumption. Dried carpels of *Annona senegalensis* is a cheap and readily available vegetable consumed mainly by the Nupe's in northern Nigeria for its presumed nutritive values, taste and aroma. The neglect and reduction in the consumption of some of our indigenous vegetables prompted this research.The aim of this study is to assess the toxic effect of dried carpels of*Annona senegalensis* in order to ascertain its nutritional suitability and risk factors associated with its use as part of the human diet.

Annona senegalensis is a plant that is common and mostly found in the African part of the world. It is referred to as the wild custard apple or wild sour sop in English. Actually, the genus name 'annona' is derived from a Latin word anon which means 'yearly produce'. The Senegal attached to the name ofthis plant signifies the place where the type specie was first collected (Orwa*et al.*, 2009). Even though not much work have been done on the dried carpels 'nungbere' much research activities has been carried out on various parts of the whole plant like the leaves (Onwusonye*et al.*, 2014), stem bark, roots to reveal some of its potentials (Theophine*et al.*, 2012).

Sampling and sample preparation

Dried carpels of *Annona senegalensis* were identified and collected locally from Batati village along Bida road, Niger state. The sample was aired at room temperature for four days and pulverized using mortar and pestle and stored in a labeled air tight polythene bag awaiting extraction.

Collection of animals

Fifty five young mice with an average weight of 23 g were obtained from National Veterinary Research Institute Vom, Jos Plateau State.

Study area

The study was conducted at the animal house of the department of biochemistry, Ibrahim Badamasi Babangida University Lapai, Niger state and the biochemical analysis was carried out in the chemistry laboratory of the Lapai Rural Hospital.

METHODOLOGY

Animal Husbandry

The animals were kept in standard plastic cages and the following conditions were kept at temperature of 23˚ ± 10˚, relative humidity (30-70) %, 12 hours light and dark cycle with

adequate ventilation.The animal care and handling conformed to the accepted guidelines recorded by OECD, (2002).

Acclimatization of Animals

The animals were left to acclimatize to the conditions of the new environment for 14 days. The animals were fed with normal commercial feed and portable drinking water.

Extraction

500 g of the dried powdered sample was weighed and soaked in a solventmixture of 750 ml methanol and 750 ml distilled water for 48 hours with occasional stirring. The solution was filtered and evaporated at 50˚C using a steam bath. The extract was collected into sample bottles and kept in the refrigerator for further use.

Acute Toxicity Testing

The acute toxicity study was conducted to determine the LD_{50} value via intraperitoneal administration of the extract. Nine mice (both male and female) of body weight 20-23 g were selected and coded using laboratory marker after which they were grouped in to three test groups each group comprising of three animals. The groups were labeled A, B and C thereafter feed was withheld from the animals to get them ready for the testing. The extract was prepared by dissolving it with distilled water to obtaindose levels of 1000, 3000 and 5000 mg/kg body weight for the groups A, B and C respectively.From the concentrations prepared the animals were dosed per their body weights via intraperitoneal administration according to their groups.Feed and water was given to the animals thereafter and observations were made at frequent intervals to ascertain the onset of adverse effects, time of death or time to recover as the case may be (Onwusonye *et al*., 2014).

Subchronic Toxicity Studies

The animals were randomized and grouped into four groups labeled A1, A3, A5 and C.100 mg/kg, 300 mg/kg and 500 mg/kg doses were selected to be administered to the test groups A1, A3 and A5 respectively with group C as the control group to be administered the vehicle (distilled water). The extract was prepared by weighing 0.1 g, 0.3 g and 0.5 g of the extract which were dissolved in 10 ml of distilled water separately to obtain 100 mg/ml, 300 mg/ml and 500mg/ml concentrations of the extract respectively.The animals were dosed based on their test groups via intraperitoneal administration every two days for three weeks. Three animals were picked randomly from each of the groups and sacrificed weekly and for each group theblood obtained is pulled together in heparin bottles, centrifuged and the serum collected is subjected to biochemical analysis on weekly basis (Ogbonna *et.al*., 2010).

Detrmination of Animals Body Weight

The study lasted for twenty-four (24) days and the body weights of the animals were determined regularly at intervals throughout the period of study.

Biochemical Analysis

Biochemical analysis was conducted to investigate the serum activities of three enzyme parameters which are *Alkaline phosphatase, Alanine aminotransferase* and *Aspartate aminotransferase.*

Collection of Blood Sample

The animals were inactivated using chloroform thereafter sacrificed. The blood was collected using syringes in to heparin bottles and centrifuged at 3000 roto per 10 minutes. The serum was collected using a micro pipette into a plane bottle.The serum gotten from the animals were subjected to chemical analysis for the verification of the functionality. ALP, AST and ALT were the major liver function tests (LFT) conducted.

Determination of *Alkaline Phosphatase* (ALP)

Alkaline phosphatase (ALP) catalyses the hydrolysis of p-nitrophenyl phosphatase at pH 10.4, liberating p-nitrophenol and phosphate, according to the following reaction:

$$\text{Para-nitrophenyl phosphate} + H_2O \xrightarrow{\text{ALP}} \text{P-nitrophenol} + \text{Inorganic phosphate}$$

ALP = Alkaline Phosphatase

The rate of p-nitrophenol formation, measured photometrically, is proportional to the catalytic concentration of alkaline phosphatase present in the sample (Young, 1995).

Preparation of Working Reagent and procedure

4 volume of reagent 1 (R1)was mixed with 1 volume reagent 2 (R2). The reconstituted reagent is stable for 30 days at 2-8˚C. 1000 µl of the reconstituted working reagent for ALP testing was measured in to a test tube and 20 µl of the sample (serum) was added to it. The mixture was incubated at 37˚C for one minute thereafter the absorbance of the mixture was measured using spectrophotometer at a wavelength of 405 nm. The absorbance of the same mixture was read again using a spectrophotometer after three minute to measure the change in absorbance per minute(ΔOD/min). Three replicates of ΔOD/minwere obtained for each sample drawn from the different test groups and recorded appropriately (Young, 1995).

Calculation

ALP activity (U/L) = (change in optical density/ minute) ×2750

Determination of *Aspartate Aminotransferase* (AST/SGOT)

Aspartate aminotransferase catalyses the transfer of an amino group from L-aspartate to 2-Oxogluterate to form oxaloacetate and L-glutamate. Oxaloacetate spontaneously decarboxylate to form pyruvate under the strongly acidic condition (Young, 2000).

Kinetic determination of Aspartate Aminotransferase (AST) based upon the following reaction

$$\text{L-Aspartate} + \text{alpha} + \text{ketogluterate} \xrightarrow{\text{AST/SGOT}} \text{Oxaloacetate} + \text{L-Glutamate}$$

$$\text{Oxaloacetate} + NADH + H^+ \xrightarrow{\text{MDH}} \text{L-Malate} + NAD^+$$

Preparation of Working Reagent and Procedure

The reagent 2 (R2) was reconstituted in 50 ml volume of the reagent 1 (R1) and left in the container. The reconstituted reagent is stable for 30 days at 2-8˚C. 1000 µl of the reconstituted working reagent for AST testing was measured in to a test tube and 100 µl of the sample (serum) was added to it. The mixture was incubated at 37˚C for one minute thereafter the absorbance of the mixture was measured using spectrophotometer at a wavelength of 340 nm. The absorbance of the same mixture was determined a second time using spectrophotometer after three minute to measure the change in absorbance per minute (ΔOD/min). The procedure was repeated thrice to obtain the ΔOD/min for each sample drawn from the different test groups and recorded appropriately (Young, 2000).

Calculation

AST activity (U/L) = (change in Optical Density/minute) ×1768

Determination of *Alanine Aminotransferase* (ALT/SGPT)

In the presence of alanine, pyruvate is formed by the action of ALT. This in turn is converted to lactate by the enzyme lactate dehydrogenase (LDH) in the presence of NADH which is simultaneously converted to NAD and this is monitored at 340 nm. The action of LDH is inhibited by increasing Lactate Concentration which actually favours the reverse reaction and formation of pyruvate. Also, the assay is monitored by following the initial rate of NAD formation over 3 minutes (Young, 2000).

Kinetic determination of Alanine Aminotransferase (ALT) according to the following reaction:

$$\text{L-Alanine + a-ketogluterate} \xrightarrow{\text{ALT}} \text{Pyruvate + L-Glutamate}$$

$$\text{Pyruvate + NADH + H}^{+} \xrightarrow{\text{LDH}} \text{L-Lactate + NAD}^{+}$$

LDH = Lactate dehydrogenase

Preparation and Stability of Working Reagents

The reagent 2 labeled (R2) was reconstituted in 50 ml volume of the reagent 1 (R1) and left in the container. The reconstituted reagent is stable fifty (50) days and kept refrigerated at 2-8˚C. 1000 µl of the reconstituted working reagent for ALT testing was measured in to a test tube and 100 µl of the sample (serum) was added to it. The mixture was incubated at 37˚C for one minute thereafter the absorbance of the mixture was measured using spectrophotometer at a wavelength of 340 nm. The absorbance of the same mixture was determined a second time using spectrophotometer after three minute to measure the change in absorbance per minute (ΔOD/min). The procedure was repeated thrice to obtain the ΔOD/min for each sample drawn from the different test groups and recorded appropriately (Young, 2000).

Calculation

ALT activity (U/L) = (change in optical density/minute) × 1768

Statistical analysis

Data were analysed using the prism graph pad and multiple comparison of the mean values was done using One-Way Analysis of Variance (ANOVA) and complemented with the Students t-test and the level of statistical significance was at 5% confidence level.

RESULTS

Acute Toxicity Test Result

The acute lethality and toxicity (LD_{50}) of the dried carpels of *Annona senegalensis* extract showed no mortality at the highest dose (5000 mg/kg body weight) 48 hours after intraperitoneal administration as shown in the table 1 below.

Table 1: Record of Mortality for Acute Toxicity

Extract dose (mg/kg body weight)	Mortality
1000	0/3
3000	0/3
5000	0/3

Number of death per group=0, Number of mice per group=3

Effect of Extract on Animals Body Weight

The result showed there was no significant ($p > 0.05$) difference in the body weights of the mice with respect to their initial weights except for the treated group at 500 mg/kg dose which showed a significant ($p < 0.05$) decrease compared to the control. There was variation in weight change between the treated groups and the control although the result did not express a regular pattern (Table 2).

Table 2: Change in Weight

TWO WAY ANNOVA

	Control	A100 (g)	A300 (g)	A500 (g)
D1	15.52±0.82	25.19±1.41***	31.42±1.46***	24.29±1.15***
D2	15.38±0.73	25.78±1.17***	29.30±1.37***	23.78±1.17***
D4	14.99±0.79	24.33±1.13***	27.68±1.45***	24.00±1.16***
D6	16.99±0.72	24.42±1.21***	28.36±1.51***	22.63±1.08**
D8	17.49±0.78	23.51±1.08***	28.39±1.43***	21.39±1.01
D10	18.24±0.77	24.14±1.61***	27.21±1.62*** [a]	19.80±1.16 [a]
D13	16.73±0.59	23.80±1.47**	27.34±1.77***	19.53±1.19 [a]
D16	17.18±0.72	24.42±1.75**	32.23±1.18***	18.63±1.27 [a]
D18	17.05±0.79	24.38±1.90**	31.20±0.70***	17.95±1.32 [b]
D21	15.93±0.57	22.96±1.71*	30.12±0.99***	16.65±1.23 [c]
D23	15.65±0.61	24.04±2.01**	31.84±1.40***	16.82±1.32 [c]

***P < 0.05 Significantly different from the control**

[a,b,c]p<0.05: Significantly different from D 1

Effect of Extract on Serum Alkaline phosphatase Level (ALP)

The result as represented in table 3 below shows that 300 mg/kg and 500 mg/kg were significantly different from the control at week 1, subsequently in week 2 and 3 500mg/kg was consistently significantly different from the control while 300 mg/kg did not show any significant difference at these weeks.100 mg/kg was significantly different from the control in week 2 but not in weeks 1 and 3.

Table 3: Variation in Serum ALP Level in Different Treatment Group

	Control	A100 (U/L)	A300 (U/L)	A500 (U/L)
WEEK1	56.38±13.47[a]	35.38±6.03[a]	122.49±19.04[b]	164.83±15.79[c]
WEEK2	196.81±0.64[b]	125.13±14.59[a]	174.03±9.02[b]	125.67±17.74[a]
WEEK3	208.08±9.42[a]	232.77±20.73[ab]	194.04±31.43[a]	289.42±50.49[b]

Mean values carrying the same superscript in the same row are not significantly different (P >0.05).

Effect of Extract on Serum Alanine Aminotransferases (ALT)

At the first week, the test groups were significantly different from the control but there was no significant difference between 300 mg/kg and 500 mg/kg. At week two there was a significant difference between the test groups and the control except for 500 mg/kg. Also no significant difference was noticed between 100mg/kg and 300 mg/kg, the same pattern was expressed in week three except that 500 mg/kg was significantly different from the controlas shown in table 4 below.

Table 4: Variation in Serum ALT Level in Different Treatment Groups

	Control	A100 (U/L)	A300 (U/L)	A500 (U/L)
Week 1	99.4495±1.78[b]	27.58±4.53[a]	44.92±17.10[a]	47.76±14.82[a]
Week2	224.89±1.83[b]	75.58±10.46[a]	106.96±2.55[a]	214.29±8.31[b]
Week3	366.88±25.93[c]	143.33±22.03[a]	122.05±3.69[a]	239.50±14.47[b]

Mean values carrying the same superscript in the same row are not significantly different (P >0.05).

Effect of Extract on Serum Aspartate Aminotransferases (AST)

At the 1st and 2nd week there was no significant difference between 100mg/kg from the controland 300mg/kg from 500 mg/kg. At week 3 the values for control, 100 mg/kg and 500 mg/kg were not significantly different but higher than the value for 300 mg/kg. This is shown in table 5 below:

Table 5: Variation in Serum AST Level in Different Treatment Group

	Control	A100 (U/L)	A300 (U/L)	A500 (U/L)
Week 1	113.72±2.61^{b}	126.11±63.46^{b}	38.34±10.39^{a}	25.76±5.52^{a}
Week 2	193.29±2.89^{b}	180.23±32.21^{b}	96.66±1.27^{a}	55.90±2.16^{a}
Week 3	136.039±6.16^{b}	181.54±24.31^{b}	86.935±6.69^{a}	192.79±71.25^{b}

Mean values carrying the same superscript in the same row are not significantly different (P >0.05)

DISCUSSION

The acute lethality (LD_{50}) of the extract indicated a very high safety profile with no mortality recorded which is in conformity with the finding of Theophine *et al*.(2012) who observed that the root bark extract and fractions of *Annona senegalensis* does not show acute toxicity.

There was no significant variation in the weights of the animals after treatment from their initial weights except those treated with 500 mg/kg of the extract. The significant decrease observed in 500 mg/kg body weight reveals a wasting effect of the extract which is likely to be due to the presence of saponin in the plant as reported by Yisa *et al*. (2010) which agrees with the submission of Bureau *et al*. (1998).

The biochemical parameters analysed (alkaline phosphatase and aminotransferase) did not follow a regular pattern but showed a time dependent increase in their serum level. There was a general increase in the activities of the ALP, ALT and AST that was significantly ($P < 0.05$) higher in the control compared to the treated groups and this contradicts the findings of Musa *et al*. (2005) that adminisstration of ethanolic extract of *Khaya senegalensis* stem bark to rats led to an increased alkaline phosphatase and aminotransferases activities that is higher than those of the control. This may be as a result of variation in the chemical composition of the different species of the plants which might be responsible for the hepatoprotective property causing the decreased level of enzyme activities observed in the treated animals (Tijani *et al*., 2013). The anomalous pattern exhibited by the result on enzyme assay could be due to the influence of environmental factor and response of the animals to the feed (Yisa *et al*., 2010).

CONCLUSION

Conclusively, the dried carpels of *Annona senegalensis* possess no obvious significant toxic effects and the doses treated even at 500 mg/kg dose exhibit no significant acute lethality on the treated animals. Therefore this study justifies why the local dwellers in different part of the savanna and rainforest zones have been utilizing different parts of *Annona senegalensis* for ethnomedicinal purposes and the dried carpels as part of their foodstuff without obvious complaints or toxicity and thus validates the hepatoprotective property of the extract.

Implication to research and practice

From the result of this study people are encouraged to include dried carpels of *Annona senegalensis* as part of their food stuff for its hepatoprotective property and hypolipidemic effects.

Future research

Further studies should be conducted on dried carpel of *A. senegalensis* to investigate its effect on vital organs (histology) of the body.

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