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STUDY OF LETHAL DOSE (LD_{50}) DETERMINATION AND BODY/ ORGAN WEIGHT OF RATS ADMINISTERED AQUEOUS EXTRACT OF EQUAL WEIGHT OF PAWPAW (*CARICA PAPAYA*) AND SOURSOP (*ANNONA MURICATA*) LEAVE

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ABSTRACT

A This study determines lethal dose toxicity (LD_{50})and effect on body/organ of rat administered equal weight of pawpaw (*Carica papaya*) and soursop (*Annona muricata*) leaves extra. About 125g each of the sample where mixed, extracted with 200ml of ethanol in triplicate and concentrated. The concentrated sample was used to prepare different dilution for the study. LD_{50} was asserted with a concentration range of 6.67-60.5mg in 150g rats. The effect on body weight and organ weight were studied with concentration range of 27.60mg/kg to 70.43 mg/kg body weight of rats. The LD_{50} was found to be 54.11mg/150g rats (equivalent to 3607.52mg/kg body weight). With exception of rat in group D (i.e., 10% above safe dose), rats administered the respective extract recorded non-significant ($p>0.05$) higher weight compared to rats in control group. The study suggest that the extract is slightly toxic, not convient to transport but had insignificant effect on body/ organ weight.

KEYWORDS

Lethal dose, Toxicity, Concentrated, Body weight, Organ weight.

Introduction

Phytomedicines from time immemorial have been the main stay of health care need for the treatment of various types of diseases. In both developing and developed countries, there appears to be increased awareness of the usefulness of herbs in the management of various disease conditions (Sandhu and Heinrich, 2005). Moreover, the trend of using phytotherapy as alternative medicine has increased the interest for the tropical plants' in Pharmacognosy. In Africa, especially in Nigeria, medicinal plants still play an important role in health care for a large portion of the population (Das *et al.,* 1999).

*Carica papaya*Linn (Papaya) is well known for its exceptional nutritional and medicinal properties throughout the world. Papaya is a juicy and tasty fruit, belonging to family *Caricaceae* and commonly known as Papaya melon tree. Pawpaw or Papau, or Kapaya or Lapaya plant is laticeferous as they contain specialized cells known as laticifers (Anuar *et al.,* 2008). In the historic times, it was considered as an exotic fruit because of its buttery taste and appearance. Papaya was the first genetically modified fruit consumed by human beings for its nutritional and medicinal properties (Parle and Guditta, 2011).

Annona muricata (Soursop) is an evergreen plant that is mostly distributed in tropical and subtropical regions of the world (Moghadamtousi *et al.,* 2015). *A. muricata* is a member of the *Annonaceae* family and is a fruit tree with a long history of traditional usage. A wide array of ethno-medicinal activities is attributed to different parts of *A. muricata* and indigenous communities in Africa and South America extensively use this plant in their folk medicine (Gajalakshmi *et al.,* 2012).

The commonly used term to describe acute toxicity is LD_{50}, where LD means lethal dose deadly amount, and the subscript 50 means that the dose is acutely lethal to 50% of the animals to whom the chemical was administered under controlled laboratory conditions. In other words, LD_{50} is the statistically derived single dose of a substance that produces death in 50% of a population of test animals to which it is administered by any of the methods like oral, dermal, inhalation, or intravenous (Randhawa, 2009). Determination of this test examines the relationship between dose and the most extreme response–death. The more potent or toxic the chemical the lower the LD_{50} and smaller the dose needed to cause death. Therefore, a chemical with an oral LD_{50} of 500 mg/kg would be much less toxic than a pesticide with an LD_{50} of 5 mg/kg (Raj *et al.,* 2013). Normally, LD_{50} is expressed in milligram(s) of test substance per kilogram of animal body weight (mg/kg body weight). It provides information on health hazards likely to arise from short-term exposure.
This data serves as a basis for labeling/classification and also helpful in establishing a dosage regiment in sub-chronic and chronic studies (Raj *et al.,* 2013).

The aim of this study is to assess safe dosage for administration of extract of Pawpaw (*Carica papaya*) and Soursop (*Annona muricata*) leaves equally mixed and also the effect on body/organ weight.
The specific objectives of the study are; to:

i. Extract the leaves of *Carica papaya* and *Annona muricata* with ethanol.
ii. Evaporate the ethanol, find the yield and prepare aqueous sample for the experiment.
iii. Assess the acute toxicity study (LD_{50}) of the extracts.
iv. Determine the final body/organ weight of rats administered different doses.

MATERIALS AND METHODS

Materials/Apparatus

Beaker, centrifuge (Centrifuge 80-3, Lab Science, England), hand gloves, micro pipette (Huma Pette Smart Line, Germany), plastic cages, refrigerator, spectrophotometer (721 visible spectrophotometers, PEC medical, USA), weighing balance (Shimadzu, TX323L, England), conical flask, distiller, syringe, sample containers dissecting set, surgical blades, tissue papers, fuel papers, test tubes, push pin, Analytical balance, gavage, stop watch.

Reagents/Chemicals

All reagents used were of analytical grade including Ethanol (British drug house, poole UK).

Choloform, Distilled Water.

Collection of *Sample*

Sample of Pawpaw (*Carica papaya*) leaf and Soursop (*Annona muricata*) leaf were collected from a local garden in Ekpoma (6.7491^{o}N, 6.0731^{o}E at an elevation of 358.84m), Esan West Local Government Area of Edo State; Nigeria. Proper identification was done in Botany Department, Ambrose Alli University, Ekpoma by a Taxonomist.

Experimental Animals

Albino rats (15) were purchased from Animal House, College of Medicine, Ambrose Alli University, Ekpoma. They were acclimatized for one week in a well-ventilated plastic cage of the Animal House, Biochemistry Department, Ambrose Alli University, Ekpoma. They were kept in condition of diurnal variation in day light and darkness characteristic of tropical rain forest in the month of May. The animal were managed in accordance with guidelines for care and use of laboratory animal (NRC 2011).

Methods

Extraction

The fresh leaves of Pawpaw and Soursop were sun-dried, from which they were blended separately into powdered form using an electric blender. About 125g of each powdered sample (1:1) was weighed and soaked together in 50ml of ethanol (95%) for 12 hours. The solution was then filtered with the aid of a muslin cloth and the filtrate gotten was evaporated by heating to slurry semi liquid and allowed to cool down to room temperature (25^{o}C). The extract (yield) was stored in a sample bottle, labeled and kept in a refrigerator.

Experimental Design

The fifteen (15) rats used in this study were divided into 5 groups of 3 rats each in the control and test groups.

Group A: Control (fed with grower's marsh and water only).
Group B: Safe dose (54.11mg/150g)
Group C: 10 % below safe dose (27.60 mg/150g)
Group D: 10 % above safe dose (65.00 mg/150g)
Group E: 20 % above safe dose (70.34 mg/150g)

The rats were fed for a total of fourteen (14) days.

Measurements of Body Weights

The initial body weight of the animals was recorded individually after randomly distributing them into various groups using a digital weighing balance (Adventurer™ Pro,AV264 ohaus Corporation NJUSA) and labeled "initial body weight". Before the termination of the experiment, the body weight of the rats was also measured and labelled "final body weight".

Acute Toxicity Test (LD_{50})

Modified procedure reported by (Enegide *et al.,* 2013) was used. The initial stage involving three animals were observed. Administration of 1500mg/kg, 1600mg/kg and 2900mg/kg. In the second stage four animals were used, there were respectively administered 5000mg/kg, 6000mg/kg, 72600mg/k and 90750mg/kg.

After due observation the Lorke's method of calculation of LD_{50}(i.e., $LD_{50} = \sqrt{(D_0 \times D_{100})}$) was used and compared with that of Enegide *et al.*, 2013 i.e., $LD_{50}=[D_0 + D_{100}]/2$
D_0 = Highest dose that gave no mortality
D_{100} = Lower dose that produced mortality
The observation for different dose effect on rats during the LD_{50} study, are presented below
Average of the result from the two methods was reported as LD_{50}.

Euthanization of Animals and Excision of Organs

At the end of the treatment period, the animals were humanely sacrificed using dissecting kit after they were anaesthetized with chloroform. Different organs of the rats namely; liver, kidney, lungs, spleen and heart were excised. The organs were blotted on a Whatman filter paper before they were weighed using analytical balance.

Calculation of Relative Organ Weights

Different organs namely; liver, kidney, lungs, spleen and heart were carefully dissected out and the weighed in grams (absolute organ weight). The relative organ weight of each animal was then calculated as follows.

$$\text{Relative Organ Weight (\%)} = \frac{\text{Absolute Organ Weight (g)}}{\text{Body weight of rats on the day of sacrifice}} \times 100$$

Statistical Analysis

The data obtained from the experiment was expressed as mean ± standard error of mean. Data was subjected to One-Way Analysis of Variance (ANOVA) and Turkey-krammer multiple comparison post-hoc test using IBM SPSS version 26 software. Significant difference in values were reported at 95% confidence level (P <0.05).

RESULTS

The observable changes in the experimental rats after the administrations of the extract are represented in Table 1, Table 2, Table 3 and Table 4. As show in Table 1 marked sign of toxicity was observed on administration of 484.00mg/150g body weight of rats Death after 48hours was recorded with administration of 605.00mg/150g body weight of rats .The LD50 was calculated from the average from Locke's equation and Enegide equation using the concentration for the last two observation was use to calculate LD_{50}.

Table 1: Observation for Different Dose Effect on Rats During the LD_{50} Studies

Test	Observation
6.67mg/150g	Normal behavior
10.67mg/150g	Normal behavior
19.33mg/150g	Irritated
33.33mg/150g	Irritated
40.00mg/150g	Irritated
484.00mg/150g	Sluggish bulgy eye recovered after 24 hours
605.00mg/150g	Reddish eye sluggish died within 48 hours

Marked sign of toxicity was observed on administration of 484.00mg/150g rats. Death after 48 hours was recorded with the administration of 605.00mg to each rat of the 150g. The calculated LD_{50} was 3618.75 corresponding to safe dosage of 361.88mg/kg body weight .

In the Table 2 the finial body weight of the rats as at the end of the experiment was reported. These were comparable as there was no statistical significant ($p>0.05$) different between those of control group and those of test group.

Table 2: Final Body Weight (g) of Rats Administered Different Dosages of Ethanolic Extract of Equal Mixed of Pawpaw (*Carica papaya*) and Soursop (*Annona muricata*) Leaves

	Treatment				
Sample	Group A	Group B	Group C	Group D	Group E
Weight (g)	168.7±17.56[a]	173.7±32.19[a]	182.5±9.32[a]	158.5±10.69[a]	162.8±12.23[a]

*Data was represented in mean ± standard error of mean. Values with same alphabetic superscripts in row are considered non-significantly different ($p>0.05$).****Group A:** Control; **Group B:** Safe dose (LD_{50}); **Group C:** 10% below safe dose (LD_{50}); **Group D:** 10% above safe dose (LD_{50}); **Group E:** 20% above safe dose (LD_{50}).*

Also respective organ weights recorded values comparable or significantly ($P>0.05$) different from those of control subjects.(Table 3) as shown below.

Table 3: Absolute Organ Weight (AOW) (%) of Kidney, Liver, Heart, Lungs and Spleen of Rats Administered Different Dosages of Ethanolic Extract of Equal Mixed of Pawpaw (*Carica papaya*) and Soursop (*Annona muricata*) Leaves

	Treatment				
AOW (%)	Group A	Group B	Group C	Group D	Group E
Kidney	0.94±0.09[a]	0.93±0.12[a]	0.96±0.18[a]	1.02±0.09[a]	0.95±0.12[a]
Liver	7.14±0.06[a]	7.58±0.93[a]	7.47±0.91[a]	6.35±0.58[a]	7.24±1.76[a]
Heart	0.59±0.12[a]	0.62±0.11[a]	0.73±0.11[a]	0.71±0.03[a]	0.93±0.44[a]
Lungs	1.23±0.03[a]	1.08±0.10[a]	1.39±0.04[a]	1.28±0.01[a]	1.43±0.05[a]
Spleen	0.66±0.02[a]	0.65±0.27[a]	0.59±0.02[a]	0.59±0.06[a]	0.61±0.07[a]

*Data was represented in mean ± standard error of mean. Values with same alphabetic superscripts in row are considered non-significantly different (p>0.05).**Group A:** Control; **Group B:** Safe dose (LD_{50}); **Group C:** 10% below safe dose (LD_{50}); **Group D:** 10% above safe dose (LD_{50}); **Group E:** 20% above safe dose (LD_{50}); **AOW:**Absolute Organ Weight.*

And as shown in Table 4 calculated organ weight recorded non-significant(P>0.05) different when value of test subjects were compared with those of control subject for the respective doses.

Table 4: Relative Organ Weight (%) of Kidney, Liver, Heart, Lungs and Spleen of Rats Administered Different Dosages of Ethanolic Extract of Equal Mixed of Pawpaw (*Carica papaya*) and Soursop (*Annona muricata*) Leaves

	Treatment				
ROW (%)	Group A	Group B	Group C	Group D	Group E
Kidney	0.56±0.01[a]	0.54±0.04[a]	0.53±0.10[a]	0.64±0.07[a]	0.58±0.03[a]
Liver	4.27±0.46[a]	4.38±0.26[a]	4.17±0.68[a]	4.01±0.29[a]	4.51±1.41[a]
Heart	0.35±0.04[a]	0.36±0.02[a]	0.40±0.04[a]	0.45±0.05[a]	0.42±0.05[a]
Lungs	0.74±0.16[a]	0.63±0.05[a]	0.76±0.03[a]	0.81±0.16[a]	0.88±0.07[a]
Spleen	0.39±0.05[a]	0.37±0.08[a]	0.32±0.03[a]	0.37±0.05[a]	0.37±0.07[a]

*Data was represented in mean ± standard error of mean. Values with same alphabetic superscripts in row are considered non-significantly different (p>0.05).**Group A:** Control; **Group B:** Safe dose (LD_{50}); **Group C:** 10% below safe dose (LD_{50}); **Group D:** 10% above safe dose (LD_{50}); **Group E:** 20% above safe dose (LD_{50}); **ROW:**Relative Organ Weight.*

DISCUSSION AND CONCLUSION

Discussion

From this study, extract of equal weight of soursop and pawpaw leaf at 2900mg/kg body weight did not show evidence of toxicity. Toxicity effect was initially observed with administration of 6000mg/kg body weight. However acute toxicity as defined as the occurrence of unwanted effect(s) that occurs either immediately or at a short time interval after a single or multiple administration of substance within 24 hours (Enegide *et al.*,2013) was noticed when 72600mg/kg was administered. It became manifest on administration of 90750mg/kg body weight.

The calculated LD_{50} for the extract was found to 3618.75mg/kg body weight. By Hodge and Sterner toxicity scale the product is slightly toxic (Loomis and Hayes,1996). Substance recording LD_{50} above 1000mg/kg are considered safe or of low toxicity (Clark and Clark 1997).

There is a rule of thumb that a safe upper limit from acute dose is 10% of LD_{50} (Valcke and Bounchard 2009).The safe upper limit from extract of equal weight of soursop and pawpaw leave is 3618.88mg/kg body weight. This is equivalent to the non-observable effect level (NOEL) or lowest observable effect level (LOVEL) for the administered extract. This value when determined with rats or mice tend to have significantly higher value than in human (Valke and Bounchard,2009).Hence safe upper limit as obtain above can be higher for human.

As shown in Table 4, there was no significant ($P<0.05$) different in the relative organ weight index for liver, lung, spleen, lung and heart. This type of change has been reported for toxicity studies in rats feed nature cure bitter (Ingale and Hivrale, 2010).The control subject recorded comparable liver, or lung, weight at the end of the experiment as control subject used in a study of effect of aqueous extract of passiflora edulis in wister Albino rats (Devakiet al., 2012). However, the heart and / or kidney weight were lower than observed in that study. Comparable in rat administered different dosage in this study compared to control suggest that administration of the extract did not have effect on these organs.

Conclusion

Increasing demand for the inclusion of plants extracts and other herbal preparations in both human and animal health necessitated this study, a valuable tool is the LD_{50} investigation. This can provide useful pharmacologic and toxicological information. It also provides practical information relative to worker's protection and in labeling classification for transportation (Adamson 2016). The LD_{50} as determined classified the product as slightly toxic, not convenient to transport and of insignificant ($p>0.05$) low effect on administered over the period of study, as reflected in relative organ weight study/final body weight study. More biochemical evidence will be required to validate these ascertion.

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