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Biosafety evaluation of *Carica papaya* aqueous leaf extract on haematological parameters and organ/body weight ratio in Wistar rats.

***[1]Timothy, O., [1]Okpakpor, E.E. and [2]Iniaghe, L.O.**
[1]Department of Plant Biology and Biotechnology,
Faculty of Life Sciences,
University of Benin,
P.M.B. 1154, Benin City,
Edo State,
Nigeria.

[2]Department of Pharmacology and Toxicology,
Faculty of Pharmacy,
University of Benin,
P.M.B. 1154, Benin City, Edo State,
Nigeria.

Email: odaro.timothy@uniben.edu

Abstract
Carica papaya L. (Caricaceae) is an herbaceous perennial tree crop commonly cultivated for its ripe edible fruit. Locally, the leaf decoction is used for treatment of malaria. This study investigated acute toxicity and sub-acute biosafety of C. papaya aqueous leaf extract on haematological and organ/body weight indices. Matured fresh C. papaya leaves were collected, dried and pulverized before extraction using aqueous decoction technique. The liquid extract was further concentrated to dryness and kept in an air tight bottle until further use. Acute toxicity in mice was appraised according to the method of Lorke, while sub-acute toxicity was determined by assessing haematological and organ/body weight parameters in four groups of Wistar rats pre-treated as follows: Group I (control) received distilled water (ml/kg body weight), while groups II, III and IV were administered single daily doses of 200 mg/kg, 400 mg/kg and 800 mg/kg of the extract for 28 consecutive days. The result from the acute toxicity studies was assessed to be above 5000 mg/kg, while the 28 days sub-acute test revealed that there was no significant difference, P> 0.05, in all haematological parameters and organ/body weight ratios evaluated when compared with the control. This study revealed that orally administered C. papaya aqueous leaf extract was tolerated at a single high dose, LD_{50} above 5000 mg/kg. Also, repeated administration of the test doses did not adversely interfere with any haematological parameter as well as organ/body weight profiles evaluated. The data obtained tend to support the biosafety of the plant extract as reportedly used in herbal home remedies.

Keywords: *Carica papaya*, haematology, organ/body, acute, sub-acute, toxicity.

INTRODUCTION
Carica papaya L. belongs to the family Caricaceae. The species is an herbaceous perennial tree crop mostly grown for its edible fruit. It is commonly known as pawpaw in English, 'Ibepe' in Yoruba, 'Gwanda' in Hausa, 'Ojo' in Igbo, 'Uhoro' in Bini and 'Eto' in Isoko. The origin of

**Author for Correspondence*

the species is ascribed to the lowlands of Eastern-Central America, before spreading across the Caribbeans, South-East Asia, and further distributed across India, the Pacific as well as Africa (Villegas, 1997).

The leaves possess anti-hypertensive properties and anti-tumour properties (Koffi *et al.,* 2009; Jaiswal *et al.,* 2010). In folk medicine, leaf decoctions of *C. papaya* have been used to combat fevers (Ajayi *et al.,* 2020; Deshpande *et al.,* 2021). Whereas, herbal products are often considered to be safe, some have been associated with varying degrees of undesirable or harmful effects (Zhang *et al*., 2015). It is therefore necessary to ascertain the toxicity index of herbal extracts, in order to establish safety limits in their medicinal applications (Bent, 2008). Although, toxicity data of cold leaf extract of *C. papaya* has earlier been reported (Estella *et al.,* 2020), no literature was found on the toxicological profile of decoction extract of the leaf of the species. This study aimed at assessing both acute and sub-acute biosafety of *C. papaya* aqueous leaf decoction on haematological and body/weight indices in rodents.

Methodology

Fresh matured leaves of *C. papaya* were obtained from Ekosodin (6°24'18.1"N/ 5°37'17.3"E), a border community to the University of Benin, Benin City, Nigeria. Authentication of the species was established with the Department of Plant Biology and Biotechnology Herbarium, University of Benin, Nigeria with voucher number UBH-C505. The leaves were shade dried and then pulverized. Weighed portion of the powdered plant, 260 g, was poured into a pyrex conical flask to soak in 1.8 L of distilled water and stirred to mix properly. The flask was then placed on a heating mantle and allowed to boil for 30 minutes. After cooling, a sieve cloth was used to filter the extract. The resulting liquid was further filtered using a 185 mm Whatman filter paper. Concentration to a constant weight was achieved using a laboratory oven at 50 °C (Zakaria *et at*., 2010). The extract was kept in a well-covered bottle in a refrigerator till further use.

Animals

Male Wistar rats weighing between 140-170 g, and albino mice, weighing between 18-33 g were procured from Animal Unit, Pharmacology and Toxicology Department, University of Benin, Nigeria. The animals were kept in polypropylene cages at the Animal house facility of the Department of Pharmacology and Toxicology, University of Benin, Nigeria. They were maintained under standard laboratory conditions (optimum temperature and humidity), and fed with marsh feed and water without restriction in accordance with recommendations of the Faculty of Pharmacy on ethical handling of experimental animals.

Acute toxicity study

Oral median lethal dose (LD_{50}) of *C. papaya* was determined using Lorke's method (1983). In the first phase, 9 mice were randomly allotted into three groups (n=3). They were administered 10 mg/kg, 100 mg/kg and 1000 mg/kg respectively of the extract orally and observed for signs of debility and mortality within 24 hours. Following the outcome of the first phase, fresh batch of 3 mice (n=1) were each orally given 1600 mg/kg, 2900 mg/kg and 5000 mg/kg of the extract and then closely monitored for any evidence of toxicity and death in 24 hours during the second phase.

Assessment of sub-acute toxicity

Twenty-four male rats were allotted randomly into four experimental groups (n=6) and kept in separate cages. They included Group I (control), which received distilled water orally, while Group II-IV were administered single oral doses of the extract at 200 mg/kg, 400 mg/kg and 800 mg/kg respectively for consecutive 28 days (animals were not given more than 0.5

ml of the extract per body weight daily). The animals were closely monitored for signs of morbidity and mortality. At termination of the treatment period, the rats were fasted into the following day, their body weights were recorded before sacrificing under chloroform anesthesia. Blood samples were immediately collected via the abdominal aorta and aspirated into EDTA bottles for haematological analysis. Vital organs were also harvested for the determination of organ/body weight ratio.

RESULTS

Acute toxicity

Table 1 shows there was zero mortality in both phase 1 and phase 2 of the orally administered aqueous extract of *C. papaya* leaf to mice.

Table 1: Oral lethal dose (LD_{50}) of aqueous leaf extract of *Carica papaya* in mice.

Phase	Dose of extract (mg/kg body weight)	Death ratio
Phase 1	10	0/3
(0 - 24 h)	100	0/3
	1000	0/3
Phase 2	1600	0/1
(0- 24 h)	2900	0/1
	5000	0/1

Sub-acute toxicity study

Effect of 28 days oral administration of aqueous extract of *C. papaya* leaf shows no significant alteration, P>0.05, in haematological parameters of Wistar rats compared to the control group that received only distilled water (Table 2).

Table 2: Mean haematological parameters as it relates the effect of consecutive 28 days oral administration of *Carica papaya* aqueous leaf extract.

Haematological parameters	Control (Distilled water)	Extract (mg/kg body weight)		
		200	400	800
WBC (10^3/µl)	10.43±0.55	12.15±1.43	13.52±1.91	11.87±0.85
LYM (%)	80.67±2.27	84.63±2.24	83.61±2.87	87.33±2.21
MON (%)	1.61 ±1.08	1.00 ±0.27	1.50 ±0.46	0.46±0.16
NEU (%)	10.72±3.49	11.68±2.03	11.76±1.93	9.65 ±2.10
RBC (10^3/6µl)	8.20 ±0.13	8.82 ±0.22	8.26 ±0.24	8.20 ±0.34
HGB (g/dl)	15.05±0.39	15.63±0.57	14.94±0.44	14.68±0.53
HCT (%)	46.23±1.26	48.00±1.70	45.37±1.41	45.43±1.68
PLT (10^3/µl)	747.3±16.7	730.7±37.2	735.8±33.1	807.5±67.8

White blood cell (WBC), Lymphocytes (LYM), Monocytes (MON), Neutrophils (NEU), Red blood cell (RBC), Hemoglobin (HGB), Hematocrit (HCT), Platelets (PLT).

Effect on organ/body weight ratio

Daily administration of graded doses of the plant extract for 28 days reveals changes in organ/body weight ratios of the rats compared with the respective controls were not statistically significant, $p>0.05$ (Figures 1-6).

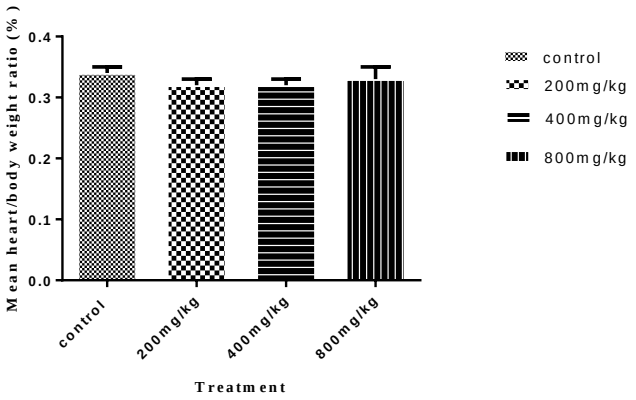

Figure 1: Effect of 28 days daily oral administration of *Carica papaya* aqueous leaf extract on heart/body weight index.

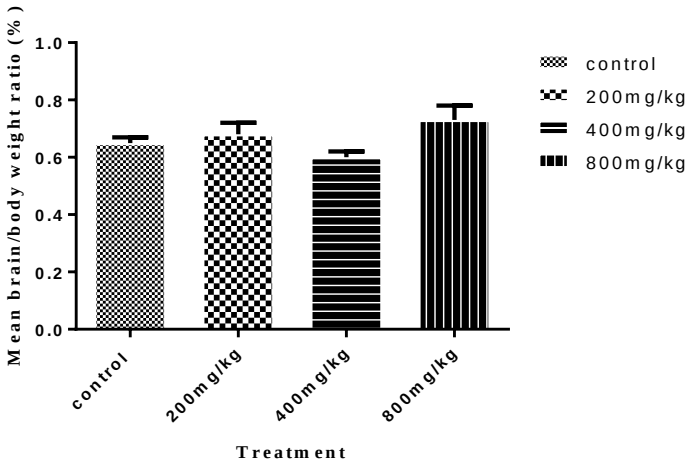

Figure 2: Effect of 28 days daily oral administration of *Carica papaya* aqueous leaf extract on brain/body weight ratio.

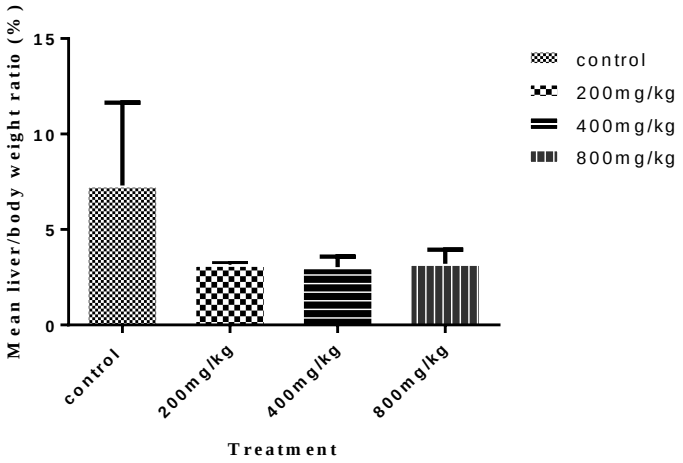

Figure 3: Effect of 28 days daily oral administration of *Carica papaya* aqueous leaf extract on liver/body weight ratio.

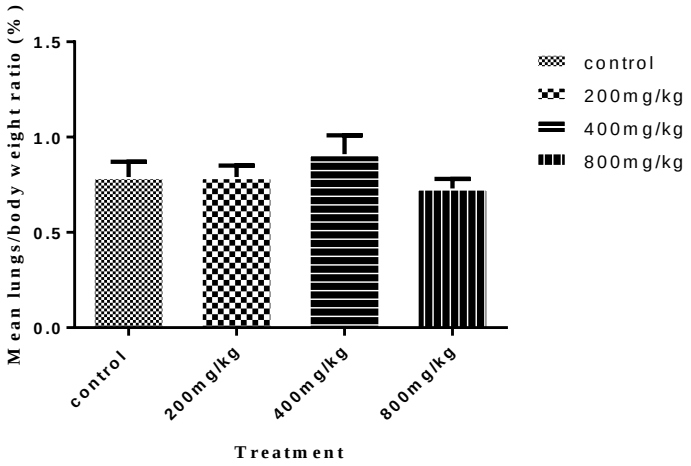

Figure 4: Effect of 28 days daily oral administration of *Carica papaya* aqueous leaf extract on lung/body weight ratio.

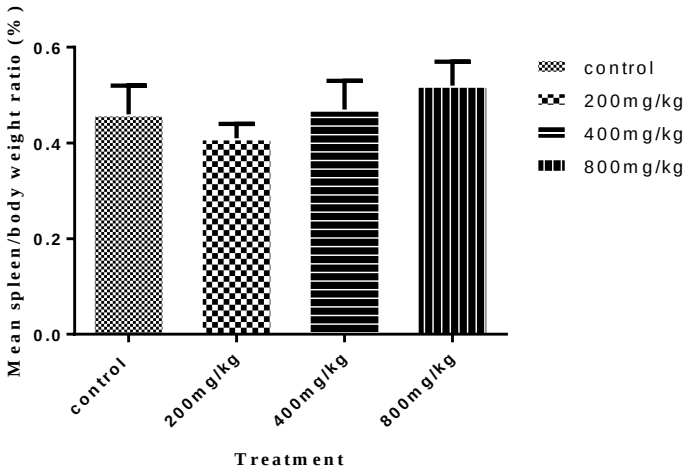

Figure 5: Effect of 28 days daily oral administration of *Carica papaya* aqueous leaf extract on spleen/body weight ratio.

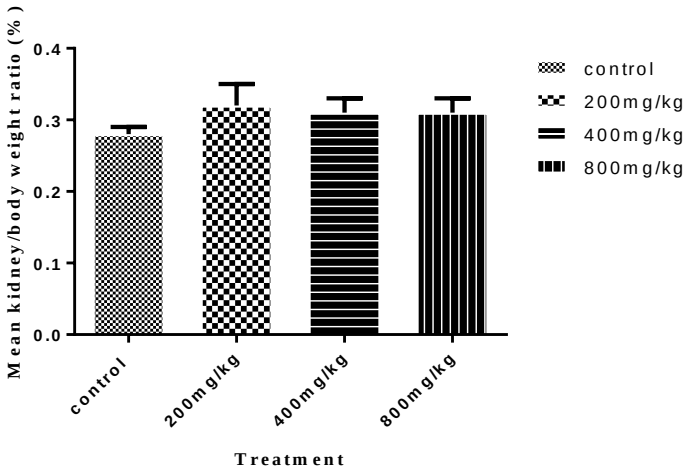

Figure 6: Effect of 28 days daily oral administration of *Carica papaya* aqueous leaf extract on kidney/body weight ratio.

DISCUSSION

Acute toxicity test aids in revealing any adverse effect(s) which is likely to occur within a short interval of time after administration of a single dose of an experimental substance (Loha *et al*., 2019). It has previously been established that any substance having LD_{50} of 5 g/kg ingested orally is safe or essentially non-toxic (OECD, 2001). Results from both phases of acute toxicity test indicated that LD_{50} of the *C. papaya* extract was extrapolated to exceed 5 g/kg, as no mortality was recorded at the highest test dose. Consequently, *C. papaya* extract used in the present study can be said to be relatively safe at a single high dose. Furthermore, sub-acute toxicity studies is not just to characterize the possible toxic effect of a substance or extract, but also to measure how safe a substance can be when used over a protracted period (Arome and Chinedu, 2014). In the present study, both haematological and organ/body weight indices appeared not to be significantly altered, $p>0.05$, due to treatments with the plant extract when compared with the control. Blood parameters act as pathological indicators of status to deleterious substances and underlining disorders (Olafedehan *et al.,* 2010). Also, comparing organ/body weight ratio is an important end point in many toxicity studies to ascertain if there is any alteration in weight due to the effect of the test substance (Nirogi *et al.,* 2014). No significant changes, $p>0.05$, in both blood and organ/body weight indices were observed in this study. Contrarily, cold aqueous leaf extract of *C. papaya* reportedly triggered significant changes in both haematological and organ/body weight indices (Estella *et al.,* 2020). Heat has been shown to breakdown the composition of highly poisonous diester-diterpenoid alkaloids into monoester-diterpenoid alkaloids, which are of low toxicity and almost not toxic (Chen *et al*., 2001). It is possible that the process of decoction might have lowered or eliminated the presence of toxic constituents in the leaf extract of *C. papaya*. Further studies are however required to eliminate the possibility of toxicity in conditions of more chronic exposure or use of the extract. It is also important to investigate the toxicity of the extract on other biological factors, such as biochemical and histopathological paramaters in animal models.

CONCLUSION

In conclusion, aqueous decoction prepared from the leaves of *C. papaya* is tolerable at a single high dose. Furthermore, 28 days accumulated daily doses of the extract have no adverse effect on both haematological and organ/body weight indices in animal model.

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