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Acute and Chronic Toxicity Studies of the aqueous and ethanol leaf extracts of *Carica papaya* Linn in Wistar rats.

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ABSTRACT

In an attempt to mobilize indigenous healthcare knowledge, we carried out an acute and chronic oral toxicity study on the aqueous and ethanol leaf extracts of Carica papaya (CP) in Wistar rats. Pawpaw (Carica papaya L.)is a popular fruit tree of tropical origin. The fruits serve as fruits and vegetables worldwide. Its reported ethnomedical uses include the treatment of anemia, diabetes mellitus, intestinal helminthiasis, malaria, diarrhea, jaundice and wounds. Acute oral toxicity study of the extracts of CPup to a dose of 5gKg^{-1}BW was studied in mice. Sub-acute (aqueous and ethanol extract) and sub-chronic oral toxicity (aqueous only) were carried out on 18 groups of 6 ratseach, at doses of 0.25gKg^{-1}, .5gKg^{-1} and 1gKg^{-1}BW. Control groups received water and corn oil respectively. At the end of the experiments, rats were sacrificed and hematological parameters, plasma biochemical parameters and histopathological examination were carried out. No deaths or signs of acute oral toxicity were recorded. Observations after oral sub-acute and sub-chronic toxicity includedhypoglycemia, hypolipidemiaand hyperglycemia, increased AST, BUN values in aqueous and ethanol extract experimentations respectively. CPshowed no oral acute toxicity. Aq. Extract is hypoglycemic, hypolipidemic whereas the ethanol extract showed signs of liver and kidney toxicity at high doses, which was confirmed after histopathological examination. The aqueous extract showed lesser toxicity than the ethanol extract.

Key words: *Carica papaya, Traditional medicine Africa, toxicity, hypoglycemia, hypolipidemia*

INTRODUCTION

In recent times, focus on plant research has increased all over the world and a large amount of evidence has been collected to show immense potential of medicinal plants used in various traditional systems. More than 13,000 plants have been studied during the last five year period [35] and approximately 10000 of these have documented medicinal use. More than 90% of current therapeutic classes have been derived from a natural product prototype and the discovery of such prototypes has led to significant changes in the practice of medicine [14][9].

The use of African medicinal plants has not been left behind, as ithas received considerable attention despite the observation that the documentation of the continents species that are used in traditional medicine lag behind others in terms of internationally recognized phytochemical standards [28].The review of some medicinal plants though, clearly validates the effectivenessandreliability of ethno-medical knowledge and traditional uses ofthese plant species in managing diseases. Therefore, this requires mobilizing indigenous healthcare knowledge, empowering traditional healers, and fostering the cooperation between traditional and modern healthcare systems [30].Dosage forms, side effects and efficacy of most of these medicinal plant preparations are usually not clearly defined, despite the common and frequent use for therapy, based on the beliefthat they are safe because they are natural [40].

In view of developing Improved Traditional Medicines (ITM's) that are affordable, safe, efficient and user-friendlyor the identification of plant-based drug targets, it is proposed that preclinical testing strategies of botanicals should start with the in vivo examination of extracts in relevant animal models to substantiate the ethnopharmacological/ethnopharmaceutical use. [37].

Paw-paw is of the genus *Carica*of the Caricaceae family and of the species *Carica papaya(CP)*Linn. It is an herbaceous succulent plant that possesses a self-supporting stem [7]. The plant is usually short-lived but can produce fruits for up to 20 years. It can grow up to 10m high. The leaves are alternate, palmate and clustered at the top of the stalk. Petioles are very long and hollow. It possesses male and female white flowers separated on different plants; the male ones are smaller and numerous, clustered in cymes while the female ones are subsessile, larger and less numerous, fixed at the axial of leaves. It bears a big, ovoid berry polysperm fruit which change from green to yellow upon maturity [15].

Apart from being used as food, its reported ethnomedical uses include the use of the fruits to treat anemia, diabetis mellitus[24] and intestinal helminthiasis[32], while the leaves are used for treating malaria[16][12][1][39][31], diarrhea [11], jaundice[18][22] and wounds [34]. The seeds of the unripe fruit are used for the treatment of dysentery [19]. Fresh leaves are also used as an analgesic, emenagogue, febrifuge and as a laxative. In combination with other plants, it is used traditionally for the treatment of malaria [30].

Preliminary phytochemical screening revealed the presence of alkaloids, tannins, saponins, cardiac glycosides [32]. Nutrient evaluation revealed that green plant leaves contained the vitamins, (mg/100g), thiamine (B1): 0.94; riboflavin (B2): 0.13; and ascorbic acid (C). Mineral analysis showed high values of Ca (8612.50mg/Kg), Mg (67.75mg/Kg), Na (1782.00mg/Kg), K (2889.00mg/Kg), Mn (9.50mg/Kg) in the green leaves, and Fe (147.50mg/Kg) in yellow leaves as compared to other elements examined. Therefore pawpaw leaves can be manipulated in the herbal treatment of various diseases and as a potential source of useful elements for drugs formulation [33].

Further biofractionation of leaf extracts showed that it contains the glycoside, carposide, and the alkaloid, carpaine [38]. Fresh leaf latex contains 75% water, 4.5% caoutchouc-like substances, 7% pectinous matter and salts, 0.44% malic acid, 5.3% papain, 2.4% fat, and 2.9% resin. These active principles in the extracts have shown analgesic,amebicide, antibiotic, antibacterial, cardiotonic, hypotensive, laxative, vermifugeand immunodilatory[25]. Acute and sub-acute oral toxicity of the aqueous extract hasbeen reported [36][42], whereas the leaf latex has been reported to be irritant, dermatogenic, and vesicant externally and internally it causes severe gastritis [17].

This study aims at evaluating the acute, sub-acute and sub-chronic oral toxicity of the aqueous and ethanol leaf extracts of *CP*on some biological and metabolic parameters in rats.

MATERIALS AND METHODS

2.1 Plant Material

Fresh pawpaw leaves were harvested from their natural habitat in the outskirts of Yaoundé, Cameroon in the month of August 2011. Plant identification and voucher specimen No. TN6227 referencing was done at the Institute of Medical Research and Medicinal Plants Studies (IMPM) herbarium in Yaoundé, Cameroon. The freshly harvested leaves were then air dried, pulverized and then weighed quantities were immersed in water and ethanol (80%) respectively for 4 h. Each of the macs was then transferred into a conical percolator for 72 h and then the extracts were filtered with a sieve of 80µm pore size. The ethanol filtrate was first concentrated using a rotary evaporator and

then both filtrateswere concentrated in an air oven at 60^{o}C [43]. The extracts were then weighed and stored in sealed plastic containers at 4^{o}C for subsequent.

2.2 Experimental Animals

Male and female Swiss albino mice (25 – 30g) and Wistar rats (170 – 210g) obtained from the animal house of IMPM were used for the acute, sub-acute and sub-chronic toxicity studies respectively. They were housed in stainless steel wire mesh cages up to a maximum of 6 per cage, in a well-ventilated room with 12 h light/dark cycle, with free access to clean drinking water and food (standard rat feed). They were allowed to acclimatize for one week before experimentation. Plant extracts were administered orally. All animals had regular supply of clean drinking water and food.

2.3 Acute Toxicity Testing

The acute oral toxicity of the aqueous and ethanol extracts of *CP*leafwas evaluated in Swiss albino mice according to the procedures outlined by the Organization for Economic Co-operation and Development [29][36]. Following the fasting period, the mice were weighed and the dose was calculated in reference to the body weight. Volume of the extracts given to the mice was 10mlKg^{-1} Body weight (*BW*) body weight.The crude extract was suspended in a vehicle (distilled water and corn oil for the aqueous and ethanol extracts respectively). For the main test, a single high dose of 5gKg^{-1}*BW* of each crude extract was administered to three male (Test 1) and three female (Test 2) mice in the treatment groups, whereas the control groups received the vehicle by oral route.Food was provided to the mice approximately an hour after treatment. The animals were observed 30min after dosing, followed by hourly observation for 8h and once a day for the next 13 days. All observations were systematically recorded with individual records being maintained for each animal. Surviving animals were weighed and visual observations for mortality, behavioral pattern, changes in physical appearance, injury, pain and signs of illness were conducted daily during the period.

2.4 Sub-acute and Sub-chronic Toxicity Testing

Sub-acute and sub-chronic toxicity of the aqueous and ethanol extracts of *CP* leaf was evaluated in Wistar rats. For the aqueous extract the rats were divided into 4 groups (A, B, C, D) of 12 rats each, while for the ethanol extract the rats were divided into 4 groups (E, F, G, H) of 6 rats each. Groups A and E served as control and received the vehicle only (water and corn oil for aqueous and ethanol extracts respectively), while groups B, C, D and F, G, H served as test (1, 2, 3) groups and were administered graded doses of 0.25, 0.5 and 1gKg^{-1}*BW* of each extract respectively. At the end of 28 days (sub-acute toxicity), 6 rats in each group of A, B, C, D and all the rats of E, F, G, H were sacrificed after an overnight fast, under diethyl ether anaesthesia, whereas the remaining 6 rats of each of groups A, B, C and D were sacrificed in like manner at the end of 90 days (sub-chronic toxicity). Blood was collected for hematological and biochemical analysis through the jugular vein. The liver, kidney and heart were harvested immediately clean of blood using physiological saline and weight. The liver and kidney were then fixed in 10% formalin for histopathological examination.

White blood cell (WBC), red blood cell (RBC)and platelet (PLT) counts as well as their indices were analyzed using a Hospitex DiagnosticsHema Screen 18 automatic hematology analyzer. Safety endpoints for plasma biochemical analysis included total proteins (TP), aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), blood urea nitrogen (BUN), uric acid (URIC), creatinine (CRE), cholesterol (CHOL), tryglicerides (TGY), glucose (GLU) and these were evaluated usingstandard analytical kits from Fortress Diagnostics Ltd, UK. The fixed organs were dehydrated with 100% ethanol solution and embedded in paraffin. They were then processed into 4-5um thick sections and then stained using hematoxylin-eosin and observed under microscope as earlier described by Gabe [21].

2.5 Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics Version 20 software. Data obtained was expressed as Mean ± SD. The student'st-test was conducted to determine significant differences and p values for significant difference between the mean of control and test groups was considered at $p < 0.05$.

RESULTS

3.1 Acute Toxicity Testing

No mortality was recorded in both male and female mice administered the aqueous and ethanol extracts of *CP* leaf at a dose of $5gKg^{-1}BW$ as shown in Table 1.

Table 1.Potential toxic effects of the crude leaf extracts of *Carica papaya*in mice.

Observation	Aqueous Extract			Ethanol Extract		
	Control (Distilled H_2O)	**Test 1** (Male) ($5gKg^{-1}BW$)	**Test 2** (Female) ($5gKg^{-1}BW$)	Control (Corn oil)	**Test 1** (Male)($5gKg^{-1}BW$)	**Test 2** (Female)($5gKg^{-1}BW$)
Number of Deaths	0/3	0/3	0/3	0/3	0/3	0/3

The test animals did not display any significant changes in behavioral pattern such as trembling, diarrhea, salivation, breathing, impairment in food intake, water consumption, postural abnormalities, hair loss, sleep, lethargy, restlessness, or in physical appearance such as eye colour, mucous membrane, salivation, skin/fur effects, body weight, injury, when compared to the control at the end of 14 days of general observation.

3.2 Sub-Acute and Sub-chronic Toxicity Testing

Effects of oral administration of CP extracts on body weights and organ weights: There were no significant differences in changes in calculated body weights of test animals compared to the control after administration of the aqueous extract for 90 days and ethanol extract for 28 days. All animals exhibited normal change in weight without a marked increase as shown in Table 2.

In same light, there was no significant increase in the weight of the visceral organs observed in test animals after 28 days and 90 days of ethanol and aqueous extract administration at $1gKg^{-1}BW$ when compared to the control groups, as shown in Table 3.

The effect of the aqueous and ethanol extracts of *CP* on the percentage weight gain and relative organ weight (ROW) in experimental animals is presented in Table 4. Administration of *CP* extracts to experimental animals induced an increase in animal organ weights in a dose responsive manner. This increase in organs corresponds to the decreased values in percentage body weight gained in experimental animals compared to the control.

Table 2. Body weight of experimental animals after 28 days and 90 days of oral administration of *Carica papaya* extracts

			Study Groups/Dose			
Extract	**Toxicity (days)**	**Weight (g)**	**Control (A, E)**	**Test 1 (B, F)** ($0.25gKg^{-1}$)	**Test 2 (C, G)** ($0.5gKg^{-1}$)	**Test 3 (D, H)** ($1gKg^{-1}$)
Aqueous	**Sub-acute (28)**	Initial	182.17 ± 3.66	183.50 ± 8.98	182.33 ± 16.17	179.50 ± 7.31
		Final	203.83 ± 3.06	202.83 ± 4.62	202.50 ± 15.11	199.67 ± 7.39
	Sub-chronic (90)	Initial	183.67 ± 4.97	183.50 ± 5.58	184.17 ± 7.68	190.33 ± 9.58
		Final	281.83 ± 10.15	265.17 ± 16.15	268.33 ± 12.18	275.50 ± 16.29
Ethanol	**Sub-acute (28)**	Initial	178.83 ± 3.66	177.33 ± 5.20	177.17 ± 4.54	178.67 ± 6.15
		Final	203.67 ± 3.33	199.67 ± 8.07	199.17 ± 4.71	200.50 ± 7.69

*The data represents the Mean ±SD for each group of rats, n = 6 (number of animals per group). *p<0.05 = significant difference and **p<0.001= highly significant difference compared to controls (groups A&E).*

Table 3: Weight of some visceral organs of experimental animals after 28 days and 90 days of oral administration of *Carica papaya* extracts

			Study Groups/Dose			
Extract	**Toxicity (days)**	**Weight (g)**	**Control (A, E)**	**Test 1 (B, F)** ($0.25gKg^{-1}$)	**Test 2 (C, G)** ($0.5gKg^{-1}$)	**Test 3 (D, H)** ($1gKg^{-1}$)
Aqueous	**Sub-acute (28)**	Heart	0.74±0.04	0.76±0.04	0.78±0.05	0.79±0.02
		Liver	6.68±0.29	6.63±0.35	7.02±0.26	7.28±0.50
		Left Kidney	0.66±0.03	0.68±0.03	0.72±0.11	0.74±0.05
		Right Kidney	0.67±0.03	0.70±0.02	0.71±0.07	0.73±0.04
	Sub-chronic (90)	Heart	0.76±0.08	0.70±0.04	0.73±0.05	0.76±0.05
		Liver	6.80±0.21	6.95±0.47	7.55±0.70	7.16±0.55
		Left Kidney	0.68±0.07	0.67±0.05	0.68±0.06	0.73±0.05
		Right Kidney	0.67±0.05	0.66±0.04	0.69±0.04	0.72±0.03
Ethanol	**Sub-acute (28)**	Heart	0.64±0.03	0.68±0.05	0.69±0.03	0.69±0.04
		Liver	6.90±0.51	6.82±0.41	6.95±0.39	6.87±0.47
		Left Kidney	0.66±0.05	0.66±0.05	0.67±0.07	0.67±0.07
		Right Kidney	0.67±0.06	0.66±0.04	0.65±0.07	0.65±0.06

*The data represents the Mean ±SD for each group of rats, n = 6 (number of animals per group). *p<0.05 = significant difference and **p<0.001= highly significant difference compared to controls (groups A&E).*

Table 4.The relative organ weight (ROW) per 100 g body weight recorded at the end of the study from experimental animals after 28 days and 90 days of oral administration of *Carica papaya* extracts.

			Study Groups/Dose			
Extract	**Toxicity (days)**	**Organ Weight (g)**	**Control (A, E)**	**Test 1 (B, F)** ($0.25gKg^{-1}$)	**Test 2 (C, G)** ($0.5gKg^{-1}$)	**Test 3 (D, H)** ($1gKg^{-1}$)
Aqueous	**Sub-acute (28)**	Heart	0.36 ± 0.01	0.35 ± 0.01	0.38 ± 0.01	0.40 ± 0.01
		Liver	3.27 ± 0.09	3.26 ± 0.07	3.46 ± 0.02	3.46 ± 0.07
		Left Kidney	0.32 ± 0.09	0.33 ± 0.01	0.35 ± 0.01	0.37 ± 0.01
		Right Kidney	0.32 ± 0.01	0.34 ± 0.01	0.35 ± 0.01	0.36 ± 0.01
		% Body wt gained	11.88	10.53	11.06	11.23
	Sub-chronic (90)	Heart	0.27 ± 0.01	0.26 ± 0.01	0.27 ± 0.01	0.27 ± 0.01
		Liver	2.41 ± 0.02	2.65 ± 0.02	2.81 ± 0.05	2.59 ± 0.03
		Left Kidney	0.23 ± 0.01	0.25 ± 0.01	0.25 ± 0.01	0.26 ± 0.01
		Right Kidney	0.23 ± 0.01	0.25 ± 0.01	0.25 ± 0.01	0.26 ± 0.01
		% Body wt gained	53.44	44.50	45.69	44.74
Ethanol	**Sub-acute (28)**	Heart	0.31 ± 0.01	0.34 ± 0.01	0.34 ± 0.01	0.34 ± 0.01
		Liver	3.38 ± 0.01	3.41 ± 0.05	3.48 ± 0.08	3.42 ± 0.06
		Left Kidney	0.32 ± 0.01	0.33 ± 0.01	0.33 ± 0.02	0.33 ± 0.01
		Right Kidney	0.32 ± 0.01	0.33 ± 0.01	0.33 ± 0.02	0.32 ± 0.01
		% Body wt gained	13.89	11.47	12.41	12.21

*The data represents the Mean ±SD for each group of rats, n = 6 (number of animals per group). *p<0.05 = significant difference and **p<0.001= highly significant difference compared to controls (groups A&E).*

Effects of administration of CP extracts on some hematological parameters: The hematological parameters were examined in experimental animals after 28 days and 90 daysof administration of the ethanol and aqueousleaf extracts of *CP* respectively, as shown in Table 5. After 28 days of administration of the extracts, there was a dose-dependent significant increase in white blood cell count ($p<0.05$), lymphocyte number ($p<0.001$), red blood cell count ($p<0.001$), hemoglobin ($p<0.05$), hematocrit ($p<0.05$), as well a highly significant ($p<0.001$) increase (which was decreasing) in platelet count in groups treated with the aqueous extract compared to the control. Meanwhile, in groups treated with the ethanol extract, there was a dose-dependent significant decrease in lymphocyte number ($p<0.05$), red blood cell count ($p<0.001$) and a significant decrease ($p<0.05$) in hematocrit at a dose of $1gKg^{-1}$*BW*. The rest of the parameters did not change significantly.

However, after 90 days of administering the aqueous extract, there was a significant ($p<0.05$) dose-dependent increase in all hematological parameters apart from the platelet count in all treated groups compared to the controls. Platelet countin groups treated with the aqueous extract after 28 daysthat showed significantly high values were observed as normal after 90 days in treated groups compared to the controls.

Table 5.Hematological parameters in experimental animals after 28 days and 90 daysof *Carica papaya* administration

	AQUEOUS EXTRACT ADMINISTRATION								ETHANOL EXTRACT ADMINISTRATION			
	SUB-ACUTE TOXICITY (28 days)				SUB-CHRONIC TOXICITY (90 days)				SUB-ACUTE TOXICITY (28 days)			
HEMA PARA.	**A** Control	**B** 0.25gKg^{-1}	**C** 0.5gKg^{-1}	**D** 1gKg^{-1}	**A1** Control	**B1** 0.25gKg^{-1}	**C1** 0.5gKg^{-1}	**D1** 1gKg^{-1}	**E** Control	**F** 0.25gKg^{-1}	**G** 0.5gKg^{-1}	**H** 1gKg^{-1}
WBC (10^3/µl)	11.42 ±2.69	11.90 ±1.93	17.17* ±2.41	20.77** ±2.23	10.75 ±1.54	12.87* ±0.61	13.02* ±0.53	13.48* ±0.61	18.07 ±1.51	19.60 ±2.53	18.18 ±2.45	18.12 ±3.13
LYM # (10^3/µl)	7.05 ±1.03	8.76 ±1.26	12.53** ±1.34	12.85** ±0.58	7.17 ±1.21	9.43* ±0.45	10.49* ±0.69	11.5*8 ±1.37	12.99 ±1.46	17.56* ±2.20	15.48* ±1.52	11.56 ±1.31
LYM % (%)	61.35 ±3.07	70.73 ±2.76	71.38 ±3.44	71.50 ±1.91	64.68 ±8.53	74.51 ±4.61	80.10 ±3.57	80.50 ±4.41	72.73 ±3.16	81.88 ±1.51	78.40 ±1.71	73.60 ±2.00
RBC (10^6/µl)	4.48 ±0.23	8.31** ±0.79	7.71** ±0.75	4.90 ±0.51	6.65 ±0.21	6.10 ±0.55	7.83* ±0.34	7.93* ±0.51	5.73 ±0.28	8.38** ±0.46	7.56* ±0.64	6.51 ±0.73
HGB (g/dl)	12.68 ±1.79	14.18 ±1.38	15.27* ±3.78	14.95* ±1.77	12.02 ±1.76	14.85* ±0.73	15.35* ±0.56	15.92* ±0.47	14.73 ±0.93	14.27 ±1.21	15.73 ±0.46	13.03 ±2.10
HCT (%)	29.87 ±0.82	41.68** ±3.42	39.60** ±2.43	30.75 ±1.43	37.03 ±1.51	40.55* ±2.03	43.58* ±0.53	44.03* ±1.62	36.90 ±2.35	38.05 ±0.98	33.88 ±1.86	32.75* ±1.69
MCV (fl)	58.85 ±0.99	53.50 ±2.74	65.83 ±3.66	71.33 ±3.50	60.00 ±1.79	57.83 ±2.32	56.67 ±2.50	58.33 ±2.80	48.50 ±1.97	47.00 ±1.67	51.67 ±1.97	52.83 ±2.14
MCH (pg)	27.88 ±1.60	19.60 ±2.63	30.12 ±1.91	38.18 ±1.66	22.22 ±0.59	26.22 ±1.15	20.52 ±1.69	20.47 ±1.32	16.83 ±0.92	17.42 ±0.89	20.28 ±1.78	19.62 ±1.75
MCHC (g/dl)	43.35 ±0.87	36.50 ±3.23	42.75 ±3.81	47.03 ±2.71	35.02 ±1.86	46.37 ±1.43	35.67 ±1.49	34.37 ±0.97	33.70 ±1.35	36.52 ±1.40	36.95 ±2.05	37.30 ±1.24
PLT (10^3/µl)	372.00 ±14.89	787.17** ±8.84	546.33** ±14.19	482.33** ±4.00	475.00 ±10.41	237.00±16.36	413.00±10. 07	455.00±1 8.90	490.67 ±9.35	381.33 ±17.76	369.67 ±19.69	344.00 ±12.12

***Hematological Parameters:WBC** (White Blood Cell Count), **LYM #** (Lymphocyte number), **LYM %** (Lymphocyte percentage), **RBC** (Red Blood Cell Count), **HGB** (Hemoglobin), **HCT** (Hematocrit), **MCV** (Mean Corpuscular Volume), **MCH** (Mean Cell Hemoglobin), **MCHC** (Mean Corpuscular Hemoglobin Concentration), **PLT** (Platelet Count).*

The data represents the Mean ± SD for each group of rats, n = 6 (number of animals per group).

**p<0.05 = significant difference and **p<0.001= highly significant difference compared to the appropriate control (groups A, A1 and E).*

Table 6.Plasma biochemical parameters in experimental animals after 28 days and 90 daysof *Caricapapaya* administration

	AQUEOUS EXTRACT ADMINISTRATION								ETHANOL EXTRACT ADMINISTRATION			
	SUB-ACUTE TOXICITY (28 days)				SUB-CHRONIC TOXICITY (90 days)				SUB-ACUTE TOXICITY (28 days)			
BIOCH PARA.	**A** Control	**B** 0.25gKg^{-1}	**C** 0.5gKg^{-1}	**D** 1gKg^{-1}	**A1** Control	**B1** 0.25gKg^{-1}	**C1** 0.5gKg^{-1}	**D1** 1gKg^{-1}	**E** Control	**F** 0.25gKg^{-1}	**G** 0.5gKg^{-1}	**H** 1gKg^{-1}
T P (g/dl)	6.28 ±0.16	8.88** ±1.31	8.53** ±0.85	8.64** ±1.04	6.75 ±1.10	5.66 ±0.09	5.89 ±0.25	6.11 ±0.48	8.23 ±0.70	8.01 ±0.60	7.97 ±0.46	8.30 ±0.29
AST (U/l)	126.93 ±5.58	81.49** ±3.82	89.82** ±3.36	97.37** ±12.19	72.89 ±3.63	64.12* ±5.48	74.04 ±3.49	57.54** ±4.35	41.49 ±2.14	44.30 ±2.50	52.81** ±3.01	59.74** ±2.72
ALT (U/l)	14.70 ±1.27	9.36* ±2.13	9.47* ±1.87	14.25 ±1.30	11.93 ±0.90	10.97 ±0.87	11.88 ±0.81	11.65 ±1.29	14.93 ±1.36	15.92 ±1.05	13.34 ±1.66	11.96* ±2.17
ALP (U/l)	210.13 ±6.43	176.45** ±5.76	189.34** ±7.23	206.61 ±4.55	108.81 ±4.11	84.34** ±6.41	87.45** ±8.26	89.55* ±4.61	67.49 ±3.98	76.34* ±3.78	72.49 ±5.61	69.83 ±6.92
BUN (mg/dl)	61.92 ±4.48	56.81* ±2.21	64.95 ±4.94	82.07** ±10.98	105.91 ±8.30	84.29** ±3.67	91.06* ±2.84	96.54* ±2.84	49.48 ±3.32	47.94 ±4.61	62.12** ±2.61	72.54** ±1.60
URIC (mg/dl)	5.43 ±0.24	4.26** ±0.99	4,28** ±0.83	4.35** ±0.47	2.55 ±0.17	4.13** ±0.12	3.43** ±0.21	3.70** ±0.23	4.09 ±0.46	3.43* ±0.38	3.91 ±0.40	4.57 ±0.46
CRE (mg/dl)	0.73 ±0.16	0.80 ±0.04	0.73 ±0.04	0.40 ±0.03	0.56 ±0.17	0.44 ±0.01	0.56 ±0.07	0.56 ±0.01	0.47 ±0.16	0.53 ±0.02	0.40 ±0.01	0.40 ±0.01
TGY (mg/dl)	87.75 ±6.29	74.51 ±10.30	68.63** ±8.46	67.16** ±7.76	63.38 ±9.04	77.00* ±3.85	82.16* ±12.77	101.41**±1 1.82	67.78 ±5.44	54.44* ±8.61	58.89 ±8.07	70.56* ±6.12
CHOL (mg/dl)	59.32 ±5.46	34.09* ±6.50	48.29* ±2.29	48.54* ±2.82	55.05 ±4.63	49.57 ±2.36	54.95 ±1.93	62.47 ±7.36	65.77 ±3.41	52.87** ±1.88	57.98 ±1.58	60.03* ±1.86
GLU (mg/dl)	144.29 ±10.28	104.07* ±13.58	103.23* ±16.83	87.72** ±5.31	128.84 ±8.31	150.59**±6.3 9	130.53 ±5.07	73.57** ±2.83	115.22 ±3.36	117.09 ±5.47	125.37 ±4.19	128.21** ±6.63

***Biochemical parameters:* TP** *(Total Proteins);* **AST** *(Aspartate transaminase);* **ALT** *(Alanine transaminase);* **ALP** *(Alkaline phosphatase);* **BUN** *(Blood urea nitrogen);* **URIC** *(Uric acid);* **CRE** *(Creatinine);* **TGY** *(Triglycerides);* **CHOL** *(Cholesterol);* **GLU** *(Glucose).*

The data represents the Mean ±SD for each group of rats, n = 6 (number of animals per group).

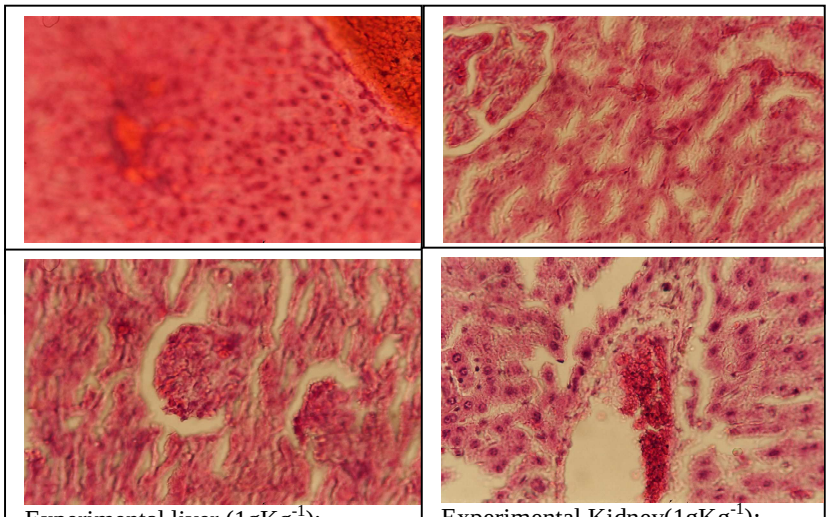
**p<0.05 = significant difference and **p<0.001= highly significant difference compared to the appropriate control (groups A, A1 and E).*

Values of mean corpuscular volume, mean cell hemoglobin and the mean cell hemoglobin concentration of the red blood cells in treated groups remained normal when compared to the controls.

*Effects of administration of CP extracts on some biochemical parameters:*Table 6 presents the effects of administration of the ethanol extracts and aqueous leaf extracts of *CP* after 28 days and 90 days in plasma biochemical parameters in experimental rats. After 28 days, a significant ($p<0.001$) dose-dependent increase in total proteins, a significant increase in BUN ($p<0.05$), significantly low (though increasing with dose) values for AST ($p<0.001$), ALT ($p<0.05$), ALP (though increasing; $p<0.001$), uric acid ($p<0.001$), cholesterol ($p<0.05$) and a significant dose-dependent decrease in TGY ($p<0.001$), glucose ($p<0.05$) were observed in rats treated with the aqueous extract. Meanwhile, in rats treated with the ethanol extract, a significant dose-dependent increase in AST ($p<0.05$), blood urea nitrogen ($p<0.001$), significantly increased ($p<0.05$) values at $1gKg^{-1}$in triglycerides, glucose and significant decreases ($p<0.05$) at 1gKg-1 in ALT and cholesterol were observed. There were no significant changes in the other parameters.

However, after 90 days, significant decreases in AST ($p<0.001$), ALP (though increasing; $p<0.001$), glucose (dose-dependent; $p<0.05$), blood urea nitrogen (though increasing with dose; $p< 0.0.05$) and significant increases in uric acid (though decreasing with dose; $p<0.001$), triglycerides ($p<0.05$) were observed in rats treated with the aqueous extract. The values of total proteins, ALT, creatinine and cholesterol did not show any significant change.

*Effects of administration of CP on some visceral organs (Histopathological examination):*Histopathological examination of the liver and kidney of both control and treated groups did not reveal any morphological differences, after 28 and 90 days of treatment with the aqueous extract at all doses, when compared to the control. However, after 28days of treatment with the ethanol extract, liver vascular congestion and leucocyte infiltration at a dose of $1gKg^{-1}$, as well as kidney vascular congestion, glumerulosclerosis and tubular clarification were observed at a dose of $0.5gKg^{-1}$ and $1gKg^{-1}$when compared to the control, as illustrated in Fig 1.



Experimental liver ($1gKg^{-1}$): Vascular congestion and leucocyte infiltration

Experimental Kidney($1gKg^{-1}$): Glumerosclerosis and tubular clarification

Figure 1.Light micrograph plates of sections from the liver and kidney of experimental animals after 28 days of administration of the ethanol leaf extract of *Carica papaya*, showing abnormal architecture (H & E × 40).

DISCUSSION

In acute toxicity, amongst the male Swiss albino mice treated with aqueous and ethanol leaf extracts of *CP* up to a dose of $5gKg^{-1}BW$, there was no mortality or any signs of toxicity or side effects recorded. In acute toxicity testing, doses higher than $5gKg^{-1}BW$are generally not considered as dose related, which is in accordance with the Organization for Economic Corporation and Development (OECD) Guidance Document for Acute Oral Toxicity Testing [3][29]. Compounds with LD_{50} values lower than $2gKg^{-1}BW$ are generally considered to be relatively safe, since values above this are non-classified. Thus, the aqueous and ethanol extracts of *CP*can be considered to be non-toxic at acute administration since the extracts were well tolerated and there was no observed adverse effect.

The body and organ weights of experimental animals did not show any significant changes after administration of the ethanol and aqueous extract for28 and 90 day respectively, when compared to the various control groups. The increase in weight over time was a normal dose-dependent increase up to $1gKg^{-1}BW$. However, a critical analysis showed that the increase in organ weights of the experimental animals corresponded to a decrease in percentage BW gained when compared to the controls. Comparison of organ weights between treated and control group of animals have conventionally been used to evaluate the toxic or adverse effects of test articles or drugs[20][6].Change in organ and body weight is also used as an assessment of therapeutic response to drugs [41]. In this study, *CP* extracts had a dose-dependent increase effect on the body weight. This means that these extracts did not have any adverse effects on experimental animals that would cause them to loose appetite [26], thereby causing a decrease in food intake and consequently a reduction in weight with increase in dose.The inverse relationship between the ROW of the experimental animals and the percentage *BW* gained when compared to the controls could be indicative of an adaptive response of the organs to the accumulation of the extracts [10]. This was observed in the slightly decreased weight in the liver at $1gKg^{-1}BW$. This signifies that the organ weights did not indicate any toxic or adverse effects from *CP* extracts as earlier observed in the acute toxicity.

Hematological parameters analyzed included the complete blood count of experimental and control group animals. Analysis of blood parameters in animal studies is relevant to evaluate the risk of alterations of the hematopoietic system in toxicity studies, for necessary application to humans [13]. Hematopoiesis is the process of blood cell formation. All blood cells are believed to be derived from the pluripotential stem cell, an immature cell with the capability of becoming an erythrocyte (RBC), a leukocyte (WBC), or a thrombocyte (platelet). In healthy adults, stem cells in hematopoietic sites undergo a series of divisions and maturational changes to form the mature cells found in the blood [4]. In this study, administration of the aqueous extract of *CP* after 28 and 90 days up to a dose of $1gKg^{-1}BW$induces a dose-dependent significant increase in all hematological parameters. This is as a result of the stimulation of the hematopoietic system, leading to the production of WBC (leukopoiesis), RBC (erythropoiesis) and platelets [2].The WBC protect the body from infection by foreign organisms, the RBC boost the immune system and the platelets protect blood vessels from endothelial damage as well as initiate repair of these vessels. This therefore suggests a strong immuno-modulatory, antioxidant and endothelial protection and repair activity of *CP* extracts. Earlier studies had reported the membrane-protective activity, protection against hemolysis of the RBC [27] and wound healing potential of *CP* [34]. The Mean corpuscular hemoglobin (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC), which are RBC indices used in classifying types of anemia did not show any significant changes in experimental animals when compared to the controls, again validating the RBC-protection by the extract from oxidative damage.

However, after 28days of administration of the ethanol extract at $1gKg^{-1}BW$, the significant decrease in hematologic parameters suggests an eventual decrease or loss of the protective nature of this extract at higher doses.

Assay of biochemical parameters was performed in order to evaluate the liver, renal, lipid and glycemic profiles of experimental compared to control animals, in order to give insight into pathological changes and nature of disease. In this study, assay of the liver profile parameters (TP, AST, ALT, ALP), revealed normal functioning of the liver after 90 days of administration of the aqueous extract, with reduced to normal values in experimental animals when compared to the controls. The significant dose-dependent increase in AST in experimental animals after administration of the ethanol extract for 28 days, indicated cellular damage to the liver [4].The renal profile parameters (BUN, CRE, URIC) showed increased values in BUN after administration of the aqueous and ethanol extracts for 28 days. However, after prolonged administration (90 days), of the aqueous extract, this value reduces significantly. Uric acid significantly increases after 90 days of aqueous extract administration. Creatinine remains

normal in all experimental animals. BUN and creatinine are indicators of glomerular filtration rate (GFR), which is an indicator of the renal function [8]. Therefore, the increased URIC after aqueous extract administration might be as a result of tissue damage but since it is dose-dependently decreasing, it might not be very indicative whereas the increased BUN after ethanol extract might be indicative of tissue necrosis. The lipid profile parameters (TGY, CHOL) are indications that these extracts do not present any risk of hypercholesterolemia or artherosclerosis at low doses. The water extracts show great hypoglycemic activity, contrary to the hyperglycemic activity shown by the ethanol extracts. This might be due to impaired insulin action or inadequate insulin secretion [5].

Histopathological examinations of the liver and kidney did not reveal any morphological changes after administration of the aqueous extracts. However, after administration of the ethanol extracts at a dose of $1gKg^{-1}BW$, we noticed vascular congestion and leucocyte infiltration of the liver and glumerulosclerosis and tubular clarification of the kidneys. This observation was confirmed by the increased AST and BUN values.

CONCLUSION

The administration of the aqueous leaf extract of *CP* did not show any signs of acute or chronic toxicity on the weight of experimental animals, organ weights, liver, renal, lipid, glycemic and histopathological profiles, at a dose of $1gKg^{-1}$ *BW*. However, administration of the ethanol extract showed signs ofliver and kidneytoxicity at a dose of $1gKg^{-1}BW$.

The aqueous extract showed hypoglycemic and hypolipidemic activities, and therefore could have an application in blood sugar control in diabetic conditions,weight control and artherosclerotic disease manegment, pending further and appropriate research.

Despite the fact that the sub-chronic toxicity of the aqueous leaf extract was not significantly different from the sub-acute toxicity apart from significant increases in uric acid and triglycerides at high doses, prolonged use should be discouraged and low doses encouraged.

A comparative analysis of the aqueous and ethanol extracts reveals that the aqueous extract is less toxic than the ethanol extract, as earlier reported by Wilcox [23] and definitely would be more efficient.

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