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Acute and Chronic Hepatotoxicity and Nephrotoxicity Study of Orally Administered chloroform extract of *Carica papaya* Seeds in Adult Wistar Rats

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Abstract- *Carica papaya* is a medicinal plant which has been proven to contain substances that are being exploited for medicinal purposes. Some of its uses include anti-ulcerogenic, anti-amoebic, anti-fungal, anti-microbial, anti-tumour, employed in wound-healing activity and antifertility activity. This study was designed to evaluate the acute and chronic hepatotoxic and nephrotoxic effects of orally administered chloroform extracts of *Carica papaya* seed in adult Wistar rat. The OECD, 2003 guideline was used for the acute oral toxicity study to determine LD50 and this was above 2000mg/kg. For the acute and chronic studies five adult wistar rats per group weighing 160-220gm were used. They animals were grouped into two major groups (acute and chronic) of three sub groups each. Group one was the control, group two and three received 100mg and 1000mg/kg body weight of the extract respectively for five days and 60 days respectively. The haematological indices for both acute and chronic studies were normal and so also were the renal function test and liver function test. The histological studies of the liver kidneys and spleen did not reveal any pathologic changes when compared to the control group and this was in line with the organ body weight which was within normal. In Conclusion it can be said that chloroform extract of *Carica papaya* seed extract is not hepatotoxic and nephrotoxic to adult wistar rats in the tested doses and duration.

Index Terms- *Carica papaya,* hepatotoxic, histological studies, kidney, liver and spleen.

I. INTRODUCTION

The history of medicinal plants is intimately connected with the history of civilization. Records of early civilization in all parts of the world reveal that a considerable number of drugs used in modern medicine were in use even in ancient times. According to the world health organization, about 80% of the population in many third world countries still use traditional medicine (medicinal plants) for their primary health care due to poverty and lack of access to modern medicine (Silva, 1997). WHO therefore approved the use of herbal products for national policies and drug regulatory measures in order to strengthen research and evaluation of the safety and efficacy of these products (Saxena, 2001). Farnsworth and his co-worker in1985 reported that of the 119 plant derived drugs listed by WHO study, 74% were discovered as a result of chemical studies to isolate the active compounds responsible for the use of original plant in traditional medicine (Farnsworth *et al*., 1985). *Carica papaya* (Pawpaw) is a tree like herbaceous plant in the family *caricaceae*. It is believed to have its origin from the low lands of Eastern Central America, from Mexico to Panama (Nakasone and Paull, 1998). *Papaya* is cultivated mainly for their ripe fruit used as desert fruit. *Carica papaya* is a medicinal plant in that it contains substances that can be used for therapeutic purposes. These substances are precursors for chemopharmaceutical synthesis as such this plant has been used traditionally in cases of kidney failure, low sperm count, dental care, heart problems, natural memory enhancer, and remedy for fibroids in uterus (Krishna, *et al.*, 2008). This plant has been recommended as an anti-ulcerogenic, anti-amoebic, anti-fungal, anti-microbial, anti-tumour, hypolipidaemic and employ in wound-healing activity, free radical scavenging activity, diuretic activity, uterotonic activity and antifertility activity (Krishna, *et al.*, 2006). The powdered seeds of *Carica papaya* have numerous applications worldwide, some includes its use in Northern India as an antihelminthic and their extract also used as anti-inflammatory and analgesic agents (Villegas 1997) *Carica papaya* seeds is also said to possess Antimicrobial properties (Nester *et al.*, 1998). Some other uses of *Carica papaya* includes, for example, the use of dead leaves of *Carica papaya* that fall off the tree as abortifacient (Sofowora, 1982). *Carica papaya* seed extract is currently being marketed as a nutritional supplement with purported ability "to rejuvenate the body condition and to increase energy". According to Mojica-Henshaw *et al*., (2003), the product is said to improve immunity against common infection and body functioning.

II. MATERIALS AND METHODS

Experimental animals: A total of thirty five apparently healthy, adult Wistar rats weighing 155-220gm were acclimatised to the laboratory conditions for 7 days prior to the experiment. 5 animals were used for LD50 while thirty were used the acute and studies. The animals were bred and housed in polypropylene cages in the animal house of the Department of Human Anatomy, Ahmadu Bello University, Zaria - Nigeria. The animals were fed rat pellet diet and layers mesh, exposed to approximately 12 h light: 12 h dark cycle and water was provided ad libitum. Animals were treated humanely; Veterinary

care and supervision were provided throughout the period of study.

Phytochemical Screening Tests

Desirable amount of *Carica papaya* extract was used for phytochemical tests. The extract solution was tested for alkaloids, glycosides, flavonoids, saponins, sugars and tannins according to the protocol described by Trease and Evans (1989).

Extract preparation: Ripe *C. papaya* fruits of Homestead variety were obtained from a local market in Zaria Kaduna State between the months of November and December and authenticated at the Department of Biological Sciences, Ahmadu Bello University, Zaria- Nigeria. The voucher number 0911 was obtained. The seeds were removed, air dried under shade and coarsely powdered. 200grams each of the powdered material was used for extraction. The ground seeds were soxhleted with chloroform respectively in the Department of Pharmacognosy and Drug Development of Ahmadu Bello University, Zaria. The soxhleted material was concentrated under reduced pressure and the obtained residue was weighed to calculate the yield and the extracts were used for the study.

Acute Oral Toxicity Studies (LD50)

The up-and-down method as outlined in the OECD, 2003 guideline for testing of chemicals was used for the acute oral toxicity study. The animals were randomly selected, marked to permit individual identification. They were aged between 8 and 12 weeks and the weights of those used were kept within an interval of 20 % of the mean weight of any previously dosed animals. Five nulliparous, non-pregnant female Wistar rats weighing between 155-180 grams and kept under approximately 12 natural light hours and 12 hours of darkness were used for each extract. The animals were fasted (only food was withheld) for 12 hours and weighed prior to dosing. Varying single doses of the two *Carica papaya* extract was administered orally using intubation cannula to one animal at a time and food withheld for another 4 hours after dosing. The dosing was initiated at 175 mg/kg a 3.2 progression factor was used and the subsequent doses were 550mg/kg and 2000mg/kg. The limit was set at 2000mg/Kg and dosing was stopped after 3 animals survived. This procedure was done for both extracts. After each dose, the animals were observed for 14 days and the toxicity signs checked out included changes in skin and fur, eyes, mucus membranes, tremors, convulsion, salivation, diarrhea, lethargy, sleep and coma (OECD, 2003).

Acute and Chronic studies

The test animals were randomised and five (5) assigned to each of the treatment groups. There were six groups in all; three each for the acute and chronic studies. Group one was the control and received 2ml/100g body weight of normal saline with 2% Tween 80, group two (2) and three (3) had 100mg and 1000mg/kg body weight of chloroform extract respectively for five days while the second set had the same dose and extract administration and lasted for 60 days.. The test extract was dissolved in 2% tween 80. The doses used where 10% and 50% of the limit dose obtained from the LD50 study. After treatment the rats were sacrificed and the blood obtained for haematological indices and biochemical analysis for liver and renal function test. The tissues i.e. liver, kidneys and spleen were carefully dissected out, cleaned of any fats and weighed (absolute weight). The relative organ weight (ROW) of each organ was then calculated according to the following equation

$$\text{ROW} = \frac{\text{Absolute organ weight (g)}}{\text{Body weight of rat (g)}} \times 100$$

The harvested tissues were fixed in 10% formal saline for routine histological processing and stained with haematoxylin and eosin.

Statistical analysis:

The data obtained from the studies are represented as Mean ± SEM. The data obtained were analyzed by one way analysis of variance (ANOVA), 'P' value less than 0.05 was considered as statistically significant. EZAnalyze 3.0 and Microsoft Excel 2007 were used for analysis and production of charts.

III. RESULTS

Acute Oral Toxicity Studies (LD50)

No mortality or morbidity was recorded in any of the animals used throughout the 14-day observation period following the oral administration of the different doses of the aqueous and ethanolic extracts of *C. papaya* seeds. There was no significant loss of fur and skin lesions. Nose and eyes appeared clear and normal. There was no diarrhoea, convulsion, salivation, tremors, lethargy, sleep or coma which are signs associated with oral toxicity. Animals did not show any sign of aggression or unusual behaviour during handling. The LD50 of the aqueous and ethanolic extract of *C. Papaya* seed was found to be above 2000 mg/kg.

Phytochemical Screening Tests

The yield obtained was 9.5%. The results of phytochemical screening of chloroform extract of *Carica papaya* revealed the presence of alkaloids, tannins, and flavonoids in the different extracts (Table 1).

Table 1: Phytochemical components seed extracts of *Carica papaya Linn*

Compounds Extracts	Saponin	Alkaloids	Tannins	Glycosides	Flavonoids	Reducing Sugars
Chloroform extract	–	+	+	–	+	–

Acute Oral Administration

The duration of administration was five days at doses of 100mg/kg and 1000mg/kg. No mortality was recorded. There was no significant lose of fur and skin, nose and eyes appeared clear and normal throughout the duration of administration. There was no diarrhoea, convulsion, salivation, respiratory distress, tremors, lethargy, sleep or coma which are signs commonly associated with oral toxicity. The animals were not aggressive and did not exhibit any unusual behaviour during handling.

IV. HAEMATOLOGICAL INDICES

The haematological indices observed after 5 days extract administration is as presented in Table 2. It indicated no significant difference within and between the groups. The WBC values ($X10^6$/ mm^3) obtained were 6.80±0.18, 7.16±0.39 and 6.82±0.51 for groups I, II, and III respectively. The PCV values obtained in percentages were 38.8±0.74, 38.8±0.8 and 38.2.±0.97 for groups I, II and III respectively. The haemoglobin values obtained were 13.1±0.28, 12.9±0.29 and 12.8±0.32 for groups I, II and III respectively. The differential values obtained for the neutrophil; (%) 30.2±1.77, 27.6±2.42 and 29.0±2.67 for groups I, II and III respectively. Lymphocyte (%), 65.6±1.37, 68.8±2.06, and 66.4±2.82 for groups I, II and III respectively. Eosinophils (%) 2.6±0.51, 2.4±0.51 and 3.0±0.55 for groups I, II and III respectively. Monocytes (%) 0.8±0.37, 1.2±0.37 and 1.6±0.25 for groups I, II and III respectively.

V. BIOCHEMICAL STUDIES.

The results obtained in international unit per litre (IU/L) for serum Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Alkaline phosphatase (ALP) after 5 days extract administration as presented in table 3 are as follows; Aspartate aminotransferase (AST); 19.4±3.11, 18.0±0.55 and 21.2±1.72 for groups I, II and III respectively, Alanine aminotransferase (ALT) 50.0±0.74, 49.2±7.87 and 51.8±9.71 for groups I, II and III respectively and serum Alkaline phosphatase (ALP), 47.6±3.28, 66.2±3.65 and 60.6±4.11 for groups I, II and III respectively. The Liver function test results obtained is as presented in Table 3 and shows the differences that exist between the groups are statistically insignificant.

The renal function test results after 5 days of extract administration is as shown in table 3. The urea results are 3.66±0.11, 3.62±0.14 and 3.90±0.10 for groups I, II and III respectively. Sodium results are as follows 139.4±0.51, 140.4±0.51 and 140.6±1.33 for groups I, II and III respectively. Potassium results are 2.6±0.51, 2.4±0.51 and 3.0±0.55 for groups I, II and III respectively. Chloride results are 99.4±1.47, 100±1.41 and 99.80±1.02 for groups I, II and III respectively. The renal function test results obtained shows that the differences that exist between the groups were statistically insignificant.

Table .2: Mean Haematological Indices of Adult Wistar Rats after 5 Days of *C. Papaya* Seed Extracts Administration

GROUPS PARAMETERS	I Control normal saline	VI Chloroform 100mg/kg	VII Chloroform 1000mg/kg
WBC ($x10^3/mm^3$)	6.80±0.18	7.16±0.39	6.82±0.51
PCV (%)	38.8±0.74	38.8±0.80	38.2±0.970
HB (g/100ml)	13.1±0.277	12.94±0. 291	12.76±0.317
NEUTROPHIL(%)	30.2±1.77	27.6±2.42	29.0±2.67
LYMPHOCTE(%)	65.6±1.37	68.8±2.06	66.4±2.82
EOSINOPHIL(%)	2.6±0.51	2.4±0.51	3.0±0.55
MONOCYTE(%)	0.8±0.37	1.2±0.37	1.6±0.25

N per group =5 Data expressed as Mean±SEM

Table .3: Mean Serum ALAT, ASAT, ALP and Urea and Electrolytes of Wistar Rats after 5 Days Of *C. Papaya* Seed Extracts Administration

GROUPS PARAMETERS	I Control normal saline	VI Chloroform 100mg/kg	VII Chloroform 1000mg/kg
ASAT (IU/L)	19.4±3.11	18.0±0.55	21.2±1.72
ALAT (IU/L)	50.0±0.74	49.2±7.87	51.8±9.71
ALP(IU/L)	47.6±3.28	66.2±3.65	60.6±4.11
UREA	3.66±0.11	3.62±0.14	3.90±0.10
SODIUM	139.4±0.51	140.4±0.51	140.6±1.33
POTASSIUM	2.6±0.51	2.4±0.51	3.0±0.55
CHLORIDE	99.4±1.47	100±1.41	99.80±1.02

N per group =5 Data expressed as Mean±SEM

Chronic Oral Toxicity

During the 60-day oral administration of both extracts of *C. Papaya* seed, no mortalities were recorded in the groups. There was no morbidity evident in all the treated animals throughout the period of administration. There was also no significant lose of fur and skin, nose and eyes appeared clear and normal. There was no diarrhoea, convulsion, salivation, tremors, lethargy, sleep or coma which are signs associated with oral toxicity. Animals did not show any sign of aggression or unusual behaviour during handling.

ORGAN BODY WEIGHT RATIO AFTER 60 DAYS EXTRACT ADMINISTRATION.

After 60 days of daily oral administration of the chloroform extract of *C. papaya*, there was general increase in body weight in all the groups as illustrated in Table 4. The mean increase in body weight of the animals were 31.80±1.59, 31.20±1.46 and 30.80±1.38 for groups I, II and III respectively. The weights difference per 100g observed after 60 days treatment were 17.04±0.76, 16.42±1.03 and 16.78±0.65 for groups I, II and III respectively (Table 4). The body weight changes observed between the groups were not statistically significant when compared to the control. There were no changes were observed in gross observation of systemic organs of both control and treated groups as all appeared normal. The organ body weight ratio were as follows; the liver, 5.48±0.075, 5.59±0.13 and 5.65±0.037 for groups I, II and III, respectively, the spleen had 0.58±0.014, 0.57±0.007 and 0.57±0.014 for groups I, II and III, respectively, while the kidney had 0.87±0.017, 0.85±0.010 and 0.84±0.012 for groups I, II and III, respectively (Table 5). The organ body weight ratios were also not significantly different from between the treatment groups and the control.

Table 4: Mean Difference in Body Weight of Wistar Rats After 60 Days Of *C. Papaya* Seed Extract Administration

GROUPS PARAMETERS	I Control normal saline	II Chloroform 100mg/kg	III Chloroform 1000mg/kg
Weight Before	192.4±12.90	189.4±11.82	189±12.98
Weight After	224.2±14.74	220.4±10.53	219.8±11.92

Mean difference	31.80±1.59	31.20±1.46	30.80±1.38
Mean difference/100g	17.04±0.76	16.42±1.03	16.78±0. 65

N per group =5 Data expressed as Mean±SEM

Table 5: Mean Organ Body weight of Liver, Spleen and Kidneys Of Wistar Rats After 60 Days Of *C. Papaya* Seed Extract Administration

GROUPS PARAMETERS	I Control normal saline	II Chloroform 100mg/kg	III Chloroform 1000mg/kg
LIVER (g/100)	5.48±0.075	5.59±0.13	5.65±0.037
SPLEEN (g/100)	0.58±0.014	0.57±0.007	0.57±0.014
KIDNEY (g/100)	0.87±0.017	0.85±0.010	0.84±0.012

N per group =5 Data expressed as Mean±SEM

HAEMATOLOGICAL INDICES

The haematological indices observed after 60 days extract administration is as presented in Table 6 indicated no significant difference within and between the groups. The WBC values ($X10^6$/ mm^3) obtained after 60 days extract administration were 6.68±0.21, 7.11±0.38 and 6.72±0.50 for groups I, II and III respectively. These values are not significantly different from the control group. The PCV in percentages were 39.0±0.84, 38.7±0.81 and 38.0±0.90 for groups I, II and III respectively, while haemoglobin concentrations obtained after 60 days were 12.96±0.26, 12.9±0. 28 and 12.74±0.25 for groups I, II and III respectively. These values are not significantly different from the control group. The differential values obtained for the neutrophil; (%) 29.8±1.46, 27.5±1.42 and 28.0±2.72 for groups I, II and III respectively. Lymphocyte (%), 65.6±1.37, 68.8±2.06, and 66.4±2.82 for groups I, II and III, respectively, Eosinophils (%) 2.6±0.51, 2.4±0.51 and 3.0±0.55 for groups I, II and III respectively, Monocytes (%) 1.40±0.40, 1.2±0.40 and 1.6±0.37 for groups I, II and III respectively. The haematological indices results obtained after 60 days of administration of the different extract as presented in Table 6 showed the differences that exist between and within the groups are statistically insignificant.

Biochemical analysis.

The results obtained in international unit per litre (IU/L) for serum Aspartate aminotransferase (AST), after 60 days extract administration were 21.6±2.54, 18.400±0..51 and 21.60±1.50 for groups I, II and III respectively (table 7). The results obtained in international unit per liter (IU/L) for serum, Alanine aminotransferase (ALT) were 49.8±3.32, 51.2±7.55 and 48.0±6.04 for groups I, II and III respectively. The results obtained for serum Alkaline phosphatase (ALP) after 60 days extract administration were; 47.8±3.37, 64.2±3.28 and 59.8±3.48. The Liver function test results obtained after 60 days of administration of the different extract showed the differences that exist between the groups are statically insignificant (table 7). The renal function test results after 60 days of extract administration is as shown in table 7 are as follows; urea 3.66±0.11, 3.62±0.14 and 3.90±0.10 for groups I, II and III respectively. Sodium 139.4±0.51, 140.4±0.51 and 140.6±1.33 for groups I, II and III respectively, potassium 2.6±0.51, 2.4±0.51 and 3.0±0.55 for groups I, II and III respectively and chloride 99.4±1.47, 100±1.41 and 99.80±1.02 for groups I, II and III. The renal function test results obtained after 60 days of administration of the different extracts as presented showed the differences that exist between the groups are statically insignificant.

Table 6: Mean Haematological Indices of Male Wistar Rats after 60 Days of *C. Papaya* Seed Extracts Administration

GROUPS PARAMETERS	I Control normal saline	VI Chloroform 100mg/kg	VII Chloroform 1000mg/kg
WBC ($x10^3/mm^3$)	6.68±0..21	7.16±0.38	6.82±0.50
PCV (%)	39.0±0..84	38.7±0.81	38.0±0.90
HB (g/100ml)	12.96±0.26	12.9±0. 28	12.74±0.25
NEUTROPHIL(%)	29.8±1.46	27.6±1.42	29.0±2.72
LYMPHOCTE(%)	65.6±1.36	68.8±2.10	66.4±2.73
EOSINOPHIL(%)	2.6±0.51	2.4±0.51	3.0±0.55
MONOCYTE(%)	1.40±0.40	1.2±0.40	1.6±0.37

N per group =5 Data expressed as Mean±SEM

Table 7: Mean Serum ALAT, ASAT, ALP and Urea And Electrolytes Of Wistar Rats After 60 Days Of *C. Papaya* Seed Extracts Administration

GROUPS PARAMETERS	I Control normal saline	VI Chloroform 100mg/kg	VII Chloroform 1000mg/kg
ASAT (IU/L)	21.6±2.54	18.400±0..51	21.600±1.50
ALAT (IU/L)	49.8±3.32	51.2±7.55	48.0±6.04
ALP(IU/L)	47.8±3.37	64.2±3.28	59.8±3.48
UREA	29.8±1.46	27.5±1.42	28.0±2.72
SODIUM	65.6±1.37	68.8±2.06	66.4±2.82
POTASSIUM	2.6±0.51	2.4±0.51	3.0±0.55
CHLORIDE	0.8±0.37	1.2±0.37	1.6±0.25

N per group =5 Data expressed as Mean±SEM

VI. DISCUSSION

Assessment of haematological parameters can be used to determine the extent of deleterious effect of extracts on the blood of an animal. It can also be used to explain blood relating functions of a plant extract or its products (Yakubu *et al*., 2007). Such analysis is relevant to risk evaluation as changes in the haematological system have higher predictive value for human toxicity, when the data are translated from animal studies for use in man (Olson *et al.,* 2000). According to Kohnke (2009), blood tests can also be used to determine the dehydration state, degree of anaemia, infection and immune challenge, physical stress as well as metabolic conditions. Some of these are undesirable effects that may be attributable to xenobiotics such as plant extracts. The result of this study showed that, the levels of RBC, Hb, PCV and WBC (Lymphocytes, neutrophils, monocytes, eosinophils and basophils) at all the doses were not altered in doses and duration used, again this may suggest therefore, the extracts at doses was not toxicologically significant on these parameter. The absence of significant effect of the extract on RBC, Hb and PCV, could also mean that neither the incorporation of haemoglobin into red blood cells nor the morphology and osmotic fragility of the red blood cells was altered (Adebayo *et al.,* 2005). The non-significant effect of the extract on the RBC may also be an indication that the balance between the rate of production and destruction of the blood corpuscles (erythropoiesis) was not altered. This shows that it is relatively safe when used for long term in both high and low doses as done in the study. Organ weight can be the most sensitive indicator of an effect of an experimental compound, as significant differences in organ weight between treated and untreated (control) animals may occur in the absence of any morphological changes (Bailey *et al*., 2004). In this study, there were no changes observed in gross examination of the organs of both control and treated groups. The liver and kidney and spleen body weight ratio of the Wistar rats in the treated groups compared favourably with those of the controls at all the doses of administration of the extract investigated. This may imply that the extract did not affect the secretory ability of the organs (Schmidt *et al.,* 2007). It is also possible that the extract did not cause any cellular constriction and/or inflammation of the organs which would have resulted in swelling and increase in weight (Schmidt *et al.,* 2007). This finding is collaborated by the histological findings which did not show any pathological changes in the liver kidneys and spleen of the treated animals. The result of the renal function test indicated that none of the assayed parameters was significantly different from the control group. It can thus be said that the different extracts are not nephrotoxic at the dose and duration used in the study. Serum enzyme measurements are valuable tool in clinical diagnosis, providing information on the effect and nature of pathological damage to any tissue (Wills, 1985). Therefore, the increase in serum Alanine aminotransferase (ALT), alkaline phosphatase and Aspartate aminotransferase (AST) activities may indicate liver tissue damage probably by altered cell membrane permeability leading to the leakage of the enzymes from the tissues to the serum. Alanine and aspartate aminotransaminases are considered to be sensitive indicators of hepatocellular damage and within limit can provide a quantitative evaluation of the degree of damage to the liver (Al-Habori *et al.,* 2002). In the study all three enzymes were assayed for and where all within normal range and did not significantly differ from the control group, it could there for be interpreted that the extract was not injurious to the hepatocytes and the secondary organs which produce some of these enzymes.

In conclusion, this study has been able to establish that the chloroform extract of *C. papaya* of the homestead variety is non toxic in Wistar rats following acute and chronic oral administration. The result of the renal and liver function test has also established that the extract lacks nephrotoxic and hepatotoxic effects in the doses and duration of administration tested. Its consumption in various forms for medicinal purposes maybe is indeed harmless to the various organs and tissues studied.

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