International Scholars Journals

International Journal of Medicinal Plants Research ISSN 2169-303X Vol. 12 (2), pp. 001-004, February, 2023. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Studies on the biochemical and haematological effects of an unripe Carica papaya aqueous extract in wistar albino rats

Oduola, T[1*], Adeniyi F. A. A.[2], Ogunyemi E. O.[3], Bello, I. S.[4], Idowu T. O[5] and Subair, H.G[6]

[1]Department of Haematology, Special Investigations Laboratory, Obafemi Awolowo University Teaching Hospitals Complex, Ile-Ife, Nigeria.
[2]Department of Chemical Pathology, University of Ibadan, Ibadan, Nigeria.
[3]Department of Chemical Pathology, Olabisi Onabanjo University, Ago-Iwoye, Nigeria.
[4]Department of General Medical Practice, Obafemi Awolowo University Teaching Hospitals Complex, Ile-Ife, Nigeria.
[5]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife. Nigeria.
[6]Department of Pharmacology, Drug Research and Production unit, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria.

Accepted 21 October, 2022

Aqueous extract of unripe *Carica papaya* is consumed by some sickle cell patients for its antisickling activities in some parts of southern Nigeria without considering its safety. The effects of the extract on functions of the liver, kidney and bone marrow were investigated in 30 wistar albino rats. The rats were in 6 groups of 5 animals per group. The first group is the control and the other five groups were the study groups. The oral acute toxicity of the extract (LD_{50}) was determined, different doses, 50, 100, 150, 200 and 250 mg/kg were administered daily to the study groups (A, B, C, D, E) respectively for 6 weeks. Liver, kidney and bone marrow function tests were assessed using standard techniques. The results obtained for biochemical and haematological parameters for the control and experimental groups showed no statistical significant different (P>0.05). This result showed that the intake of unripe *C. papaya* extract had no adverse effects on the functions of these organs in rats.

Key words: *Carica papaya*, aqueous extract, liver, kidney, bone marrow, antisickling.

INTRODUCTION

In the long history of the world, plants have been used medicinally. A large and increasing number of patients use medicinal herbs or seek the advice of their physician regarding their use ('O' Hara et al., 1998). It has been estimated roughly, that presently more than half of the total population of the world use herbal drugs (Chang, 1987). Increasing interest in medicinal herbs has increased scientific scrutiny of their therapeutic potentials and safety thereby providing physicians with data to help patients make wise decisions about their use ('O' Hara et al, 1998).

Carica papaya (family Caricaceae) originated in central America. It is an interesting tree in that the male and female parts exist in different trees. The fruits, leaves, seeds and latex are used medicinally (Beckstrom-Sternberg et al., 1994). Its main medicinal use is a digestive agent, it is prescribed for people who have difficulty digesting protein and is used to break up blood clots after surgery, this is due to the presence of enzyme papain in the plants latex .The latex from the trunk of the tree is also applied externally to speed the healing of wounds, ulcers, boils and warts. The seed is used to expel worm, the flower may be taken in an infusion to induce menstruation (Reed, 1976; Morton, 1977; Duke, 1984). *Annonaceous acetogenins* derived from the ext-racts of the twigs of the pawpaw tree may be good chemotherapeutic agents for cancer as these compounds inhibit enzymes necessary for metabolism in tumour cells (Rupprecht et al., 1986; Hui et al., 1989a; Hui et al., 1989b; Zhao et al.,

*Corresponding author. E-mail: oduolataofeeq@yahoo.com. Tel: 2348033968785.

Table 1. LD_{50} determination by arithmetic method of Karbar adapted by Aliu and Nwude (1982).

Dose mg/kg	Number of Rats	Number of Deaths	Dose Difference	Mean death	Dose Difference x mean death
Normal Saline	5	0	0	0	0
400	5	0	400	0.5	200
800	5	1	800	1	800
1600	5	1	1600	1.5	2400
3200	5	2	-	-	0

$$LD^{50} = \text{Maximum Dose} - \frac{Y}{\text{Number of rats per group}}$$

1992; Reiser et al., 1992; Zhao et al., 1995).

The aqueous extract of unripe *C. papaya* was reported to possess both antisickling and reversal of sickling properties (Thomas and Ajani, 1987). The minimum concentration of the aqueous extract of unripe *C. papaya* that achieved both antisickling and reversal of sickling was established to be 1g of unripe *C. papaya* soaked in 1ml of physiological saline, and the antisickling agent was found to be in the ethyl acetate fraction of the extract (Oduola et al., 2006). Popular use of aqueous extract of unripe *C. papaya* for prevention of sickle cell crisis among sickle cell patients makes it necessary for its safety to be determined. Although it has been reported that daily intake of 15 ml of aqueous extract of unripe *C. papaya* at 5 ml three times daily for seven days, in seven-year old sickle cell children, had no adverse effect on the functions of the liver and kidney (Thomas and Ajani, 1987) its safety over a relatively long period on these organs and on bone marrow has not been investigated, hence the present study is designed to determine its LD_{50} and to investigate the effect of intake of aqueous extract of unripe *C. papaya* on the functions of the liver, kidney and bone marrow in rats over a relatively long period.

MATERIALS AND METHODS

Plant authentication and extract preparation

Matured fresh unripe *C. papaya* fruit was obtained in a local garden in Ile-Ife, and was authenticated at the herbarium of the Botany Department, Obafemi Awolowo University, Ile-Ife; the herbarium number is 14729. The fruit was peeled and the cream coloured seeds inside discarded, 100 g of the fruit was soaked in 100ml of distilled water and incubated at room temperature for 72 h. One gram of unripe *C. papaya* fruit soaked in 1ml of physiological saline at room temperature for 72 h has been shown to be the minimum concentration that possessed maximum antisickling activities (Oduola et al., 2006). The extract was sieved into a clean container and kept in the refrigerator until use.

Animals

Male and female wistar albino rats (195 – 225 g) obtained from the animal house of the Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, were used for the study. They were kept in rat cages in well ventilated house, temperature of 27 - 30^{o}C, 12 h natural light and 12 h darkness, with free access to tap water and dry rat pellet (purchased at Ogo Oluwa Enterprises, Ile-Ife). They were allowed to acclimatize for 3 days prior to the experiment.

Acute toxicity studies

Different doses of the aqueous extract corresponding to 400, 800, 1600 and 3,200 mg were administered orally to four groups of five rats per group, another group of five rats served as control and this received 1.0 ml of physiological saline. They were all placed under observation for 24 h after which the number of dead rats was recorded.

The method of Karber (Aliu and Nwude, 1982) was employed in the determination of acute oral median lethal dose (LD_{50}).

Chronic toxicity studies

A convenient range of dosages were then administered to the rats for the experiments. The rats were grouped into six groups of five rats each. The first group is the control and this received 1.0ml of normal saline orally, daily for 42 days. The second, third, fourth, fifth and sixth (A, B, C, D and E) received 50, 100, 150, 200 and 250 mg/kg respectively orally on daily basis for 42days. At the expiration of the 42-day extract exposure, the rats were weighed and blood collected by cardiac puncture under ether anaesthesia. 6 ml of blood were collected from each rat, 2.0 ml dispensed into dipotassium ethylenediamine tetraacetic acid (K_2 EDTA) for haematological analysis and 4.0 ml into lithium heparin for biochemical analysis. After the blood collection, the animals were sacrificed by cervical dislocation. The haematological and biochemical analysis were carried out using standard techniques (Norbert, 1986; Dacie and Lewis, 2001).

STATISTICS

The mean and standard deviation and the level of significance for the differences between means were computed by students test SPSS 6.

RESULTS

The results of the acute oral toxicity study in rats showed the LD_{50} of the aqueous extract of the unripe *C. papaya* to be 2520 mg/kg (Table 1).

Table 2 shows the mean $\pm$ S.D values of liver functions

Table 2. Effect of intake of extract of unripe *Carica papaya* on some liver function tests.

Parameter	Control	Group A	Group B	Group C	Group D	Group E
Total bilirubin (μmol/l)	12.23 ± 1.11	12.96± 1.35	13.03 ±1.29	12.53± 1.62	12.95±1.32	12.47 ±1.15
Conjugated bilirubin (μmol/l)	2.88± 0.71	2.32± 0.94	3.65 ±1.03	3.01± 0.99	4.26± 1.12	3.93 ±1.10
Total protein (g/l)	69.86± 4.34	71.53± 6.30	68.55 ±5.99	69.63± 6.00	70.53± 6.13	70.12 ±4.66
Albumin (g/l)	36.11± 2.37	35.31± 2.86	36.28 ±1.75	38.13 ±1.87	34.81 ±2.11	35.73±2.29
ALT (IU/l)	23.37± 3.11	22.11 ±2.99	23.55 ±2.88	21.99± 2.96	23.00 ± 2.11	22.95 ±3.01
AST (IU/l)	9.96± 0.95	9.32± 1.00	10.12 ± 1.11	8.67 ±0.93	10.36±1.13	9.55± 1.10
ALP (IU/l)	113.36± 7.77	111.57± 6.96	112.63± 7.56	110.36±8.11	113.99± 8.13	112.63± 7.33

n = 6.

Table 3. Effect of intake of extract of unripe *Carica papaya* on renal function tests.

Parameter	Control	Group A	Group B	Group C	Group D	Group E
Na+ (mmol/l)	135.08 ±6.99	134,93 ± 7.00	137.41±8.13	136.25±7.36	133.62± 7.77	134.62 ±7.11
K+ (mmol/l)	4.40± 1.13	4.47±1.15	3.97±1.00	4.13±1.20	3.99± 1.18	4.25± 1.10
Cl- (mmol/l)	104.36± 7.32	102.99± 7.01	105.13± 7.87	102.48±8.11	106.32± 8.11	103.29 ± 7.32
HOC^{-}_{3} (mmol/l)	22.01 ± 4.26	23.44 ± 4.98	22.76 ± 4.13	23.61 ± 4.33	21.13± 3.91	22.94 ± 3.98
Urea (mmol/l)	6.82± 1.11	6.51± 1.83	6.63± 1.70	6.88± 1.36	6.90 ± 1.71	6.79± 1.93
Creatinine (μmol/l)	121.36± 9.36	117. 41± 8.95	120.34± 9.11	119.71±9.22	120.75± 8.35	119.50± 7.92

n =6.

tests. The values of total bilirubin, conjugated bilirubin, total protein, albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) obtained for study groups (A, B, C, D and E) showed no significant difference (P>0.05) from control group. Also, there were no significant differences (P>0.05) between the study groups.

From table 3, there were no statistical significant differences (P>0.05) in the values obtained for sodium (Na^{+}), potassium (K^{+}), bicarbonate (HCO^{-}_{3}), chloride (Cl^{-}), urea and creatinine between the control and study groups, and between the study groups.

Table 4 shows the results of haematology tests. There were no significant differences (P>0.05) in the values obtained for PCV, WBC (total and differential) and platelets counts between the control and study groups, and between the study groups.

DISCUSSION

The result of acute oral toxicity (LD_{50}) of the extract was found to be 2520 mg/kg (Table 1). Presently, the chemical labeling and classification of acute systemic toxicity based on oral LD_{50} values recommended by the Organization for Economic Co-operation and Development (OECD, Paris, France) (Walum, 1998) are as follow: very toxic, ≤ 5 mg/kg; toxic, >5 ≤50 mg/kg; harmful, >50 ≤500 mg/kg; and no label, >500 ≤2000 mg/kg. The aqueous extract obtained from 1.0 g of unripe *C. papaya* soaked in 1.0 ml of physiological saline (corresponding to 50 mg/kg) was reported to be the minimum concentration with maximum antisickling effect in *in vitro* studies (Oduola et al., 2006) and in this study, lethal effect started manifesting from 800mg/kg. Therefore the LD_{50} of 2520 mg/kg of the extract is an indication that the extract is safe, thereby confirming the belief of the users that the extract has no adverse effect since none has been observed in the past. However, LD_{50} has not been regarded as a biological constant because many variables such as animals' species and strain, age, gender, diet, bedding, ambient temperature, caging conditions and time of the day can all affect the LD_{50} value obtained; hence there are considerable uncertainties in extrapolating LD_{50} value obtained for a specie to other species. Consequently, recognizing LD_{50} test as providing, at best, only a ballpark estimate of human lethality has been advocated (Zbinden and Flury-Roversi, 1981).

From the result of liver function tests, the conjugating ability of the liver was intact as revealed by total and conjugated bilirubin levels. The plasma ALT and AST activities are markers of hepatocellular damage, their levels in the rat were not affected by the extract intake; the synthetic ability of the liver was also preserved as shown by the plasma protein and albumin concentrations (Table 2).

The electrolytes, urea and creatinine are markers of kidney function, throughout the study, the plasma levels of Na+, K+, Cl-, HCO^{-}_{3}, urea and creatinine (Table 3) were not affected by the intake of the extract, this is an indication that the extract is not nephrotoxic.

The intake of the extract did not affect the functions of

Table 4. Effect of intake of extract of unripe *Carica papaya* on some haematological parameters.

Parameter	Control	Group A	Group B	Group C	Group D	Group E
PCV (%)	43.29±1.65	42.99±2.00	43.01±1.85	44.12±1.95	43.71±1.86	43.88±2.12
WBC ($X10^3/mm^3$)	3.85± 0.19	3.95± 0.26	3.97± 0.19	3.89±0.20	4.01± 0.16	3.90±0.21
Platelets ($X10^3/mm^3$)	199.86± 11.13	201.11± 11.56	196.34±10.91	200.37±11.10	199.32±11.65	203.22±12.30
Neutrophils (%)	45.02± 4.61	46.13± 4.70	44.91± 4.31	45.49± 4.65	44.95±4.32	45.21±4.31
Lymphocytes (%)	53.23± 5.34	52.05± 5.21	54.00± 5.61	53.61± 5.31	54.01±5.17	53.16± 5.28
Eosinophils (%)	1.31± 0.19	1.56± 0.18	1.06± 0.12	1.00 ±0.10	1.02±0.07	1.14 ±0.09
Monocytes (%)	1.00± 0.13	1.06± 0.10	1.03 ± 0.10	1.01 ± 0.12	1.07± 0.05	1.05 ±0.03
Basophils (%)	0.00	0.00	0.00	0.00	0.00	0.00

n = 6.

the bone marrow as reflected by the values of PCV, WBC, (total and differential) and platelets count; they were neither quantitatively nor qualitatively destroyed. There were no inclusions in the red cells or white cells as observed from the cell morphology.

In conclusion, the results obtained in this study have established that the intake of extract of unripe *Carica papaya* has no adverse effects on the functions of the liver, kidney and bone marrow in rats. However the intake of this extract by sickle cells patients must be standardized through a well controlled human study. The extract is being purified and characterized, and the mechanism of action of its antisickling properties is being studied.

ACKNOWLEDGEMENT

The authors are grateful to Professor K.D Thomas of blessed memory, Department of Chemical Pathology, Obafemi Awolowo University (OAU) Ile- Ife, Nigeria, who initiated this work but did not live to see it to completion. Our appreciation also goes to the staff of animal house, Faculty of Pharmacy, OAU, Ile-Ife, for rendering technical assistance.

REFERENCES

Aliu YO, Nwude N (1982). Veterinary Pharmacology and Toxicology Experiments, 1st Edition Abu Press, pp:104-110.

Beckstrom-sternberg, Stephen M, James A, Duke, Wain KK (1994) The Ethnobotany Database'. http://probe.nalusda.gov. 8300kg:-bin//browse/ethnobotdb. (ACEDB version 4.3-data version.

Chang IM, (1987). Toxicity of herbal drugs, International Forum on Research and Development for Procedures involving Risk Assessment of Toxic Chemicals. In Korean Soc. Toxicol (Chang IM , ParkCW eds). pp: 243-257

Dacie JV, Lewis SM (2001). Practical Haematology. Churchill Livingstone, London, 9th ed. pp. 19-46

Duke JA (1984). Borderline herbs CRS Press. Boca raton FL.

Hui YH; Rupprecht JK; Anderson JE; Liu YM; Smith DL, Chang CJ and Mclaughlin JL. (1989a) Bullatalicin, a novel bioactive acetogenin from Annona bullata (Annonacae), Tetrahedron. 45:6948

Hui YH; Rupprecht JK; Liu YM; Anderson JE: Smith DL; Chang CJ and McLaughlin JL. (1989b). Bullatacin and bullatacinone: two highly potent bioactive acetogenins from Annona bullata" J. Nat. Prod. 52:463-77

Morton JF. (1977). Major Medicinal plants. C.C Thomas Springfield, IL.

Norbert WT. (1986). Textbook of Clinical Chemistry. Philadelphia, WB. Saunders Company. pp. 1373-1430.

'O' Hara M, Kiefer D, Farrel K, Kemper K (1998). A review of 12 commonly used medicinal herbs. Archives of Family Medicine. 7: 523-536

Oduola T, Adeniyi FAA, Ogunyemi EO, Bello IS and Idowu TO (2006). Antisickling agent in an extract of unripe pawpaw (*Carica papaya*): Is it real? African J. Biotechnol, 5(20): 1947 – 1949.

Reed CF. (1976). Information Summaries on 1000 Economic Plants. Typescripts submitted to the USDA.

Reiser MJ, Hui YH, Rupprecht JK, Kozlowski JF, Wood KV, McLaughlin JL, Hoye T, Hanson PR and Zhuang ZP.(1992) Determination of absolute configuration of stereogenic carbinol centres in annonaceous acetogenins by IH and 19F-NMR analysis of Mosherester derivatives",114:10203-10213.

Rupprecht JK, Chang CJ, Cassady JM, McLaughlin JL, Mikolajezak KL, Weisleder D. (1986) "Astimicin, a new cytotoxic and pesticidal acetogenin from the pawpaw, Asimina triloba Annonaceae), " Heterocycles, 24: 1197-1201.

Thomas KD, Ajani B (1987). Antisickling agent in an extract of unripe pawpaw fruit (Carica papaya). Transaction of the Royal Society of Tropical Medicine and Hygiene. 81: 510-511.

Walum E (1998). Acute oral toxicity. Environ. Health Perspect. 106: 497 – 503.

Zbinden G, Flury-Roversi M (1981). Significance of the LD_{50} test for the toxicological evaluation of chemical substances. Arch. Toxicol, 4792: 77.

Zhao GX, Gu ZM, Zeng L, Chao JF, Wood KU, Kozlowski JK and Mclaughlin JL.(1995). The absolute configuration of trilobacin and trilobin, a novel highly potent acetogenin from the stem bark of Asimina triloba (Annonaceae). Tetrahedron, 51:7149-7160.

Zhao GX, Hui YH, Repprecht JK, Mclaughlin JL, Wood KV (1992). "Additional bioactive compounds and trilobacin, a novel highly cytotoxic acetogenin from the bark of Asimina triloba, "J. Nat. Prod. 52:347-56.