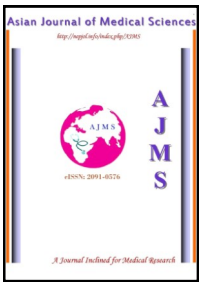
ASIAN JOURNAL OF MEDICAL SCIENCES



Morphometric Malformations In Fetal Rats Following Treatment With Aqueous Leaf Extract Of Carica Papaya

Moses B. Ekong[1*], Mandu U. Akpan[2], Theresa B. Ekanem[2], Mfon I. Akpaso[2]

[1]Department of Anatomy, Faculty of Basic Medical Sciences, University of Uyo, Uyo, Nigeria; [2]Department of Anatomy, Faculty of Basic Medical Sciences, University of Calabar, Calabar, Nigeria.

Abstract

Objective: *Carica papaya* popularly known as papaya or pawpaw is a fruit with diverse nutritional and medicinal properties. The continuous use of the leaf extract as enema by pregnant women necessitated this study on the effect of aqueous extract of the young leaves on the morphometry of the fetuses of pregnant Wistar rats.

Material & Methods: Twenty seven sexually matured Wistar rats consisting of eighteen females and nine males, and weighing 180-200g were used for the study. The female rats were assigned equally into three groups of A, B and C. Pregnancies were obtained after the introduction of the males into the female cages overnight. On day 12 of pregnancy, group A (control) was given sham treatment of tap water, while groups B and C were treated with 60mg/kg and 120mg/kg respectively, of the aqueous leaf extract of *C. papaya* for seven days (days 12-18 of pregnancy). On day 20 of gestation, the animals were anaesthetized using chloroform and euthanized, and the uteri dissected out to study the fetuses.

Results: Results revealed reduced number of viable fetuses, reduced fetal weight, and reduced crown-rump, head, and tail lengths in the group treated with 60mg/kg aqueous leaf extract of *C. papaya*, while there was resorption of all the fetuses in the group treated with 120mg/kg of the aqueous leaf extract of *C. papaya*.

Conclusion: In conclusion, the aqueous leaf extract of *C. papaya* at the given dosage in this study is detrimental to developing fetuses, and these effects were dose dependent. Hence, the use of the extract especially during the period of gestation should be discouraged.

Key Words: *C. papaya;* fetus; morphometry; malformation; rats

1. Introduction

Herbs have been used for centuries in the treatment of different ailments.[1-4] Some of them have unexploited effects which can result in serious unplanned consequences. These consequences could be teratogenic, and as such babies born of such pregnant mothers can be malformed with ignorance of its cause. This study therefore seeks to investigate a commonly used extract, the *Carica papaya* leaf.

Carica papaya (*C. papaya*) commonly known as papaya or pawpaw, is a member of the family, *Caricaceae*.[5] It is popular for its fruits which makes an appetizing food when ripe. All parts of this plant is useful for humans and other animals.[6-9]

Different parts of the plant have effects, some of which have not been studied with the leaf. The unripe seed extract has been shown to have a nephroprotective effect[7] but it has also been reported to have anti-fertility effects.[9,10] It interferes with the pituitary-gonadal axis, and alter male reproductive functions.[9,10] It has also been reported to alter cauda epididymal microenvironment[11], with acute and chronic toxicity[12].

In folk medicine, *C. papaya* leaf decoctions have been used as purgative by parenteral route, abortificient, blennorrhoea, orchitis, chancres and in treatment of hernia and infection of the urogenital system. It has also

***Correspondence:**
Moses B. Ekong, Department of Anatomy, Faculty of Basic Medical Sciences University of Uyo, Uyo, Nigeria. Phone: +2348030868505 E-mail: mbe_flashpoint@yahoo.com

been used as haemostatic and in the promotion of healing, tonic for the heart, analgesia and treatment for stomach ache.[5,13] Other effects of the leaf extract includes; anti-inflammatory and antioxidant properties[9,14] repellent, insecticidal, rodenticidal and fungicidal properties[15]. Indran et al[16], also reported that it is a good therapeutic agent for protection against gastric ulcer and oxidative stress, Imaga et al[17] reported its anti-sickling effect on red blood cells which can be exploited in sickle cell disease therapy.

The leaf of *C. papaya* contain many biologically active constituents. These include; alkaloids, carpaine, dihydrocarpaine, flavonols, tannins, nicotine, cyanogenic glycosides, and papain.[18,19] It has been reported that the young leaves of *C. papaya* have higher quantities of these constituents compared with the matured leaves.[20,21] These compounds, at high concentrations, may cause adverse effects.

The leaf extract of *C. papaya* is commonly used as enema by the locals in treating different ailments, to aid parturition, and in some case, to flush out fecal matter. The deleterious consequences of this when used by pregnant women has often not been weighed. Therefore this study seeks to determine the effect of aqueous extract of *C. papaya* leaf on the morphology of the fetuses of Wistar rats.

2. Material and Methods

Animal care and grouping: Twenty seven sexually matured Wistar rats consisting of eighteen females and nine males, and weighing 180-200g were used for the study. The rats were breed in the animal house of the Department of Anatomy, University of Calabar. They were cared for following the international guidelines for the care of laboratory animals, and ethical approval was obtained from the ethics committee of the institution. The animals were allowed normal rat chow and water *ad libitum*.

The female rats were assigned equally into three groups of A, B and C. Group A constituted the control, while groups B and C were the experimental. Vaginal smears were carried out daily, to determine their estrous cycles. At proestrous, the males were introduced into the females' cages overnight. Coition was confirmed by the presence of tail structures in the vaginal smear the following morning, and thus was called day zero of pregnancy.

Preparation of the extract: Fresh young leaves of *C. papaya* weighing a total of 1000g were obtained from the plant. They were washed clean of dirt and air dried. They were homogenized with 1000ml of tap water and the mixture was filtered with Whatman No. 1 filter paper. This was heated to dryness and the extract weighed and diluted to the given dose with tap water.

Administration of the extract: Group A was given sham treatment of tap water, while groups B and C were treated respectively with 60mg/kg and 120mg/kg of the aqueous extract of *C. papaya* leaf. Treatments was by means of oral gavages, on days 12-18 of gestation (seven days). On day 20 of gestation (two days before complete gestation period), the animals were anaesthetized with chloroform and euthanized. The fetus were obtained by uterectomy, and examined for viable ones. They were then removed, blotted dry and weighed using a Mettler p163 balance. Using a Vernier caliper, the following parameters were also measured; crown rump, head and tail lengths.

Statistical analysis using one way analysis of variance (ANOVA), and a post hoc Tukey's test was carried out. The results are presented as Mean± Standard deviation, and figures of probability level, $p<0.05$ were regarded as significant.

3. Results

Treatment with the leaf extract did not have an outstanding effect on the mothers, but the fecal matter were rather soft in continence compared with the control.

Number of fetuses: The number of viable fetuses was less in the group treated with 60mg/kg of the extract of *C. papaya* leaf which had an average of five fetuses per pregnant rat (thirty viable fetuses in all) compared with the control which had six fetuses per pregnant rat (thirty-three fetuses in all). The size of the fetuses of the group treated with 60mg/kg of the extract of *C. papaya* leaf appeared smaller, and in some cases showed slight deformities. There were no fetus found in the group treated with 120mg/kg of the *C. papaya* leaf extract. Only empty amniotic sacs were seen, and hence, no measurements was done in this group.

Body weight: There was a significant ($p< 0.001$) reduction in the body weights of the fetuses in the group treated with 60mg/kg of the extract of *C. papaya* leaf compared with the control. This is as shown in Table 1.

Crown rump length: There was a significant ($p< 0.001$) reduction in crown-rump lengths of the fetuses in the

group treated with 60mg/kg of the extract of *C. papaya* leaf compared with the control. This is as shown in Table 1.

Table-1: Weights, crown-rump, head and tail lengths of fetuses in the control and the experimental groups

	Body weights of fetuses (g)	Crown-rump lengths (cm)	Head lengths (cm)	Tail lengths (cm)
Control n=33	2.37±0.00	2.20±0.00	2.26±0.00	1.04±0.00
Group B n=30	0.97±0.00***	0.91±0.00***	0.93±0.00**	0.87±0.00 ^{NS}
Group C Nil	NR	NR	NR	NR

Results are presented as Mean ±Standard deviation
***Significantly lower than the control at p<0.001
**Significantly lower than the control at p<0.01
^{NS}Significantly lower than the control at p<0.05
NR: Not recorded

Head length: There was a significant (p< 0.01) reduction in the head lengths of the fetuses in the group treated with 60mg/kg of the extract of *C. papaya* leaf compared with the control. This is as shown in Table 1.

Tail length: There was a slight reduction in the tail lengths of the group treated with 60mg/kg of the extract of *C. papaya* leaf though not significantly (p< 0.05) compared with the control. This is as shown in Table 1.

4. Discussion

In this study, 60mg/kg and 120mg/kg of the leaf extract of *C. papaya* were administered to two experimental groups of pregnant Wistar rats to check the effect on developing fetuses' morphometry. *C. papaya* is known to contain different chemical substance implicated in several adverse effects[22,23], which may likely result in teratogenicity.

This study showed decreased morphometry in the group whose mothers were treated with 60mg/kg of the leaf extract of *C. papaya*, while there was total resorption of the fetuses in the group whose mothers were treated with 120mg/kg of the leaf extract of *C. papaya*. The decreased morphometry and resorption in this study indicate adverse effects of some of the constituents of the extract on the developing fetuses. There are no reported teratogenic effects of *C. papaya* leaf extract as in our study.

The leaf of *C. papaya* contains papain, tannins, cyanogenic glycosides and some other components. The presence of these components is believed to be responsible for reported abortificient and teratogenic properties of *C. papaya*.[24,25] Schmidt[25] reported that early studies in India showed that unripe *C. papaya* seed, latex and fruit extracts had deleterious effects on pregnancy in laboratory animals. He stated that papain present in *C. papaya* exhibit anti-implantation activity, increased post-implantation loss and embryotoxicity when administered orally to Wistar rats.

Oderinde[26] reported that aqueous extract of *C. papaya* seeds at oral doses of 100 and 800 mg/kg body weights showed no significant differences in the total body weight of the fetuses exposed to these regimes. However, in the group treated with 100 mg/kg body weight, there was a significant increase (p<0.05) in the implantation sites, and fetal weight was significantly decreased (p < 0.05), with no dead or malformed fetuses found. However, in the group treated with 800 mg/kg body weight, there was complete resorption of about 30% of the fetuses, with the surviving fetuses stunted but without external malformations.

Raji et al[27] reported significant litter reduction with no effect on the fetal weight and morphology with the seed extract, while Abdulazeez et al[28] reported that fermented seed extract did not affect rat litters nor their weights and morphometry.

This result is also in line with a previous study on an individual constituents of the leaf.[29] It was reported that infusion of sodium cyanide into pregnant golden hamsters by subcutaneously implanted osmotic minipumps induced high incidences of resorptions and malformations in the offspring.[29]

5. Conclusion

It is documented that the leaf of *C. papaya* contain the same constituents that cause teratogenicity in the seed and latex, it is likely that the effect of the aqueous leaf extract of *C. papaya* at the dosages seen to be detrimental to developing fetuses may be as a result of these constituents, with these effects being dose dependent. Therefore, the use of this leaf extract especially during the period of gestation should be discouraged.

6. References

1. Siraji D, Islam N, Begum N, Ferdousi S. Effect of Ocimum sanctum Linn (Tulsi) on body weight and some biochemical parameters in restraint stressed albino rats. J Bangl Soc Physiol 2008;(3):29-34.

2. Subaprinya R, Nagini S. Medicinal properties of neem leaf: a review. Curr Med Anti-Conc Agent 2005; 5(2): 146-9.

3. Swan Jutta. Paw Paw Leaf Concentrate. Url: httpwww.naturalhealthcareproducts.comdocumentsPawPawLeafJuice.pdf. 2007. Assessed on 19 Dec 2009.

4. Togun RA, Emma-Okon BO, Aboderin AA. Lectins, mitogenicity and seed germination: a comparative study with the seeds of Telfairia occidentalis (Hook, F.) (Curcurbitaceae), Carica papaya (Linn) (Caricaceae) and Artocarpus communis (J.R. & G. Forst) (Moraceae). Biokemistri 2008; 20(1): 11-5.

5. Burkill HM. The useful plants of West Tropical Africa, vol 1. Families A-D Royal Botanic Gardens, Kew. Library MARC record. 1985. Assessed on November, 2009.

6. Ekanem SB, Okoronkwo TE. Pawpaw seed as fertility control agent on male Nile tilapia. NAGA ICLARM Quarterly 2003; 26(2): 8-10.

7. Olagunjua JA, Adeneyeb AA, Fagbohunkac BS, Bisugac NA, Ketikuc AO, Benebod AS, et al. Nephroprotective activities of the aqueous seed extract of Carica papaya Linn. in carbon tetrachloride induced renal injured Wistar rats: a dose- and time-dependent study. Biol Med 2009; 1(1): 11-9.

8. Owoyele BV, Adebukola OM, Funmilayo AA, Soladoye AO. Anti-inflammatory activities of ethanolic extract of Carica papaya leaves. Inflammopharmacol 2008; 16(4): 168-73. doi:10.1007/s10787-008-7008-0 PMid:18759075

9. Udoh P, Kehinde A. Studies on antifertility effect of pawpaw seeds (Carica papaya) on the gonads of male albino rats. Phytother Res 1999; 13(3), 226-8.doi:10.1002/(SICI)1099-1573(199905)13:3<226::AID-PTR396>3.0.CO;2-E

10. Udoh P, Essien I, Udoh F. Effects of Carica papaya (paw paw) seeds extract on the morphology of pituitary-gonadal axis of male Wistar rats, Phytother Res 2005; 19(12): 1065-8. doi:10.1002/ptr.1388 PMid:16372375

11. Verma RJ, Chinoy NJ. Effect of papaya seed extract on microenvironment of cauda epididymis. Asian J Androl 2001; 3: 143-6. PMid:11404801

12. Ayotunde EO, Ofem BO. Acute and chronic toxicity of pawpaw (Carica papaya) seed powder to adult Nile tilapia (Oreochromis niloticus Linne 1757). Afr J Biotech 2008; 7(13): 265-4.

13. Giove Nakazawa RA. Traditional medicine in the treatment of enteroparasitosis. Rev Gastroenterol Peru 1996; 16: 197–202.PMid:12165783

14. Rahmat A, Abu Bakar MF, Faezah N, Hambali Z. The effect of consumption of guava (Psidium guajaya) or Carica papaya on total antioxidant and lipid profile in normal male youth. Asia Pac J Clin Nutr 2004; 13: S106.

15. Sridhar S, Arumugasamy S, Saraswathy H, Vijayalakshmi K. Organic vegetable gardening. Chennai, Center for Indian Knowledge Systems. p. 34. 2002.

16. Indran M, Mahmood AA, Kuppusamy UR. Protective effect of Carica papaya L. leaf extract against alcohol induced acute gastric damage and blood oxidative stress in rats. West Indian Med J 2008; 57: 4: 323-6.PMid:19566009

17. Imaga NOA, Gbenle GO, Okochi VI, Akanbi SO, Edeoghon SO, Oigbochie V, et al. Antisickling property of Carica papaya leaf extract. Afr J Biochem Res 2009; 3(4): 102-6.

18. Chillemi S. Papaya Leaf. Url: http://www.authorsden.com/categories/article_top.asp?catid=16&id=26530. Assessed on December 12, 2009.

19. Duke J (2007). Carica papaya L. Caricaceae. http://sun.ars-grin.gov:8080/npgspub/xsql/dukeplantdisp.xsql?taxon=209. Assessed on October 20, 2010.

20. Bennett RN, Kiddle G, Wallsgrove RM. Biosynthesis of benzylglucosinolate, cyanogenic glucosides and phenylpropanoids in Carica papaya. Phytochem 1997; 45 (1): 59-66.doi:10.1016/S0031-9422(96)00787-X

21. Brocklehurst K Salih E. Fresh non-fruit latex of Carica papaya contains papain, multiple forms of chymopapain A and papaya proteinase OMEGA. Biochem J 1985; 228 (2): 525-7. PMid:4015629 PMCid:1145013

22. EPA. Summary Review of Health Effects Associated with Hydrogen Cyanide, Health Issue Assessment Environmental Criteria and Assessment Office. Office of Health and Environmental Assessment Office of Research and Development. US Environmental Protection Agency Research Triangle Park, North Carolina, USA. 1990.

23. World Health Organization (WHO). Toxicological evaluation of certain food additives and naturally occurring toxicants. WHO Food Additive Series: 30. World Health Organization, Geneva. 1993.

24. Eno AE, Owo OI, Itam EH, Konya RS. Blood pressure depression by the fruit juice of Carica papaya (L.) in renal and DOCA-induced hypertension in rat. Phytother Res 2000; 14: 235-9.doi:10.1002/1099-1573(200006)14:4<235::AID-PTR574>3.0.CO;2-G

25. Schmidt H. Effect of papain on different phases of prenatal ontogenesis in rats. Reprod Toxicol 1995; 9: 49-55.doi:10.1016/0890-6238(94)00055-2

26. Oderinde O, Noronha C, Oremosu A, Kusemiju T, Okanlawon OA. Abortificient properties of aqueous extract of Carica papaya (Linn) seeds on female Sprague-Dawley rats. Nig Postgrad Med J 2002; 9: 95-8.PMid:12163882

27. Raji Y, Morakinyo AO, Oloyo AK, Akinsomisoye OS, Olufadekemi T, Kunle-Alabi T, et al. Impart of the chloroform extract of Carica papaya seed on oestrous cycle and fertility in female albino rats. J Med Sci 2005; 5(4): 337-43.

28. Abdulazeez MA, Ameh AD, Ibrahim S, Ayo JO, Ambali SF. Effect of fermented seed extract of Carica papaya on litters of female Wistar rats (Rattus norvegicus). Afr J Biotech 2009; 8(5): 854-7.

29. Doherty PA, Fern VH, Smith RP. Congenital malformations induced by infusion of sodium cyanide in the Golden hamster. Toxicol Appl Pharmacol 1982; 64: 456-64. doi:10.1016/0041-008X(82)90242-3