ASN-PH-020919
ISSN: 2315-537X

International Journal of Herbs and Pharmacological Research
IJHPR, 2012, 1(2): 48 – 54.
www.antrescentpub.com

RESEARCH PAPER

THE EFFECT OF CARICA PAPAYA SEEDS ON THE HISTOLOGY OF THE LIVER IN WISTAR RATS

[1]Dikibo, E., [2]Okpe, A. C., [1]Turray, A.A., [3]Onodagu, B.O., [4]Ogbodo, L.A., [5]Oyadonghan G.P.

[1]Department of Medical Laboratory Science, Ambrose Alli University, Ekpoma, Edo State, Nigeria. [2]Department of Pharmacology, University of Nigeria, Enugu Campus, Enugu State, Nigeria. Department of [3]Medical Microbiology; [4]Mobid Anatomy, University of Nigeria Teaching Hospital, Ituku-Ozalla, Enugu, Nigeria; [5]Department of Anatomy, Abia State University, Uturu, Abia State.

Corresponding author: elizabethdikibo@gmail.com

Received: 24th April, 2012 Accepted: 30th June, 2012 Published: 31st July, 2012

ABSTRACT

This study investigates the effect of oral ingestion of *Carica papaya* seeds on the liver histology of growing Sprague Dawley rats. The study involved 40 growing rats (95.0±10.0grams). They were divided into eight groups of 5 rats each: A (control; n=5), B (n=10), C (n=10) and D (n=10). Group A1 and A2 served as the acute and chronic control respectively. Group B1, C1 and D1 served as the sub-acute test while group B2, C2 and D2 served as the chronic test. The rats were fed with varying doses of powdered *Carica papaya* seed (6, 8, 10grams). Group A1, B1, C1 and D1 were fed for 3 weeks while Group A2, B2, C2 and D2 were fed for 6 weeks. The results showed test group B1 presented infarction with haemorrhage and exudations. B2 presented pyknosis and eosinophilic cells with cellular degeneration. C1 presented pyknosis, parenchymal erosion and mild vacuolation, haemorrhage and embolism and C2 presented severe vacuolation and pyknosis. D1 presented palour, vacuolation (V) and arterial wall disruption and D2 presented pyknotic cells with transitional phases of karyohexis and karyolysis, parenchymal erosion and severe vacuolation. The observations suggest *Carica papaya* seeds are toxic to the liver and may induce hepatic damages in a dose and duration dependent manner.

Key words: *Carica papaya* seed, Liver, Traditional medicine, Nigeria,

INTRODUCTION

It is a well known fact that as many as 80% of the world's population depend on traditional medicines for their primary health care (WHO, 2000). In this regards, traditional medicines may be said to have been a source of inspiration to the world in general and Nigeria in particular. This is sequel to the fact that medicines derived from plants have made large contributions to human health and well being (Iwu *et al.*, 1999). According to Yinger and Yewhalaw (2007), the reason for this prevalence is due to the facts that traditional medicine has remained the most affordable and easily accessible source of treatment in the primary health care system of resource poor communities.

Consequently, the report that the use of natural plant products for therapeutic purposes, is growing and largely embraced by the general population (Osifo *et al*., 2011) and thus, the shift of attention from synthetic drugs to natural plant products reported by Dada and Ikuerowo (2009). At least 35,000 plant species have been recognized (Kong *et al*., 2003) and large proportion have been used for the treatment of several human ailments for thousands of years (Yakubu *et al*., 2007).

Of interest, are the therapeutic uses of *Carica Papaya* -commonly known as pawpaw. In Nigeria, *Carica papaya* is one of the most popular, cheapest economically important fruit tree grown and consumed for its nutritional content (Baiyewu and Amusa, 2005). According to available literature, every drug is associated with hepatotoxicity almost certainly due to its ability to generate free radicals and to cause disturbance in hepatocyte biochemistry (Fernandez-Checa and Kaplowitz, 2005). Hence, *Carica Papaya* like other drugs is metabolized by the liver and if this be the case, *carica papaya* may subject the liver to a variety of diseases and disorders reference to the fact of Fernandez-Checa and Kaplowitz, (2005) stated above.

Consequently, this study is aimed to investigate the effect of *Carica papaya* seeds on the histology of the liver (the organ which metabolises substances ingested by man).

MATERIALS AND METHODS

Experimental Animals: Forty (40) Sprague Dawley rats of 7 $\pm$ 1 week old and weights ranging from 95.0 to 105.0g and comparable sizes were procured from the premises of the animal house of the College of Medicine, Ambrose Alli University, Ekpoma, Nigeria. They were moved to the site of the experiment at No. 23 St. Mary Street, Ekpoma where they allowed 2 weeks of acclimatization.

Substance of study: Unripe *Carica papaya* was collected from the premises of the animal house, College of Medicine, Ambrose Alli University, Ekpoma and authenticated by a botanist in the Department of Botany, Faculty of Natural Sciences, Ambrose Alli University, Ekpoma.

Substance preparation: The outer peel of *Carica papaya* was removed and the seeds obtained and sun dried. The dried seeds were then crushed into fine powder using electric blender. The fine powder was measured using Electric Balance (Denver Company, USA, 200398. IREV.CXP-3000) and packaged in small plastic envelopes and then stored pending usage.

For the purpose of this study, pellets were prepared by adding measured quantity of *Carica papaya* powder to feed (grower mesh) to add up to 50grams as described by Nwaopara et al. (2011).

Animal grouping: The experiment involved two stages; stage one (1) which lasted a period of 3 weeks (subacute test) and stage (2) which lasted a period of 6 weeks (chronic test). The animals were assigned into eight groups of 4 rats each: Group A1 and A2 served as the acute and chronic control respectively. Group B1, C1 and D1 served as the acute test while group B2, C2 and D2 served as the chronic test.

Experimental design: The animals were weighed on the first day of the acclimatization period and fed 50grams of feed with water giving *ad libitum*. They were housed in well ventilated labelled wooden cages at the site of the experiment. The cages were designed to secure the animals properly especially from wild animals/insects and cleaned daily.

Substance administration: The preliminary studies, animal acclimatization, ingredients procurement (*Carica papaya* preparation and production), actual animal experiment and evaluation of results, lasted from October, 2011 to February, 2012. However, the actual administration of *Carica Papaya* to the test animals lasted for 6 weeks (acute: 3 weeks; chronic: 6 weeks).

Stage 1 administration: Group A1 (control group) received 50.0g of feed and distilled water alone. Test Groups B1 to D1 received as follows; 44.0g feed, distilled water plus 6g of CP; 42.0g feed, distilled water plus 8g of CP; 40.0g feed, distilled water plus 10g of CP respectively.

Stage 2 administration: All the groups in stage two received as stated for stage 1; the difference is that the feeding period lasted for six weeks unlike stage 1 which lasted for 3 weeks.

Sample collection and analysis: Weight was measured before and after acclimatization, similar weight measurements were done at the end of the acute and chronic treatment periods and the average weight recorded accordingly. The growth performance and feed utilization of the rats were determined at the end of the experiment as described by Dada and Ikuerowo (2009).

Furthermore, the liver of each rats were obtained at the end of each stage under chloroform anaesthesia and fixed in 10% formalin for histological processing.

Processing schedule: The tissues were processed using automatic tissue processor according to the processing schedule used in Obafemi Awolowo University Teaching Hospital Complex (OAUTHC), Ile-ife, Osun State, Nigeria. The fixed plastic cassette tissues in 10% formalin were automatically processed by passing them through different grades of alcohol as follows: 70% alcohol (2hrs), 80% alcohol (2hrs), 90% alcohol (2hrs), 90% alcohol (2hrs), 95% alcohol (2hrs), Absolute (2hrs), Xylene 1 (2hrs), Xylene II (2hrs), Molten paraffin wax 1 (2hrs) and Molten paraffin Wax II (2hrs).

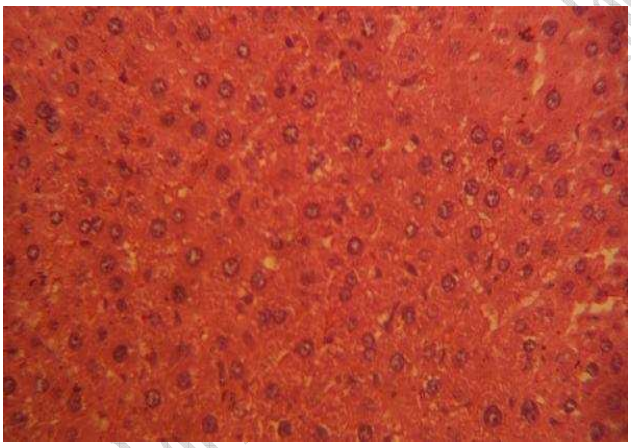
After the last timing, the tissues were removed from their plastic cassettes and placed at the centre of the metallic tissue mould and then filled with molten paraffin wax. They were also left to solidify after which they were now placed in the refrigerator at 5^{o}C for 15 minutes. After the blocks were cool in the refrigerator for the time stated above (15 minutes), the blocks were then removed from the metallic case using a knife and after which the paraffin wax at the side of the blocks were removed.

The blocks were then trimmed and cut serially at 3nm on a rotary microtome. The sections were floated in water bath at 55^{o}C and picked up by the use of a clean frosted end slides. The frosted end slides were now placed on the hot plate for 40 minutes for adequate attachment of the sections on the slides after which the sections were de-waxed, hydrated, air dried and stored in a slide box ready for staining process.

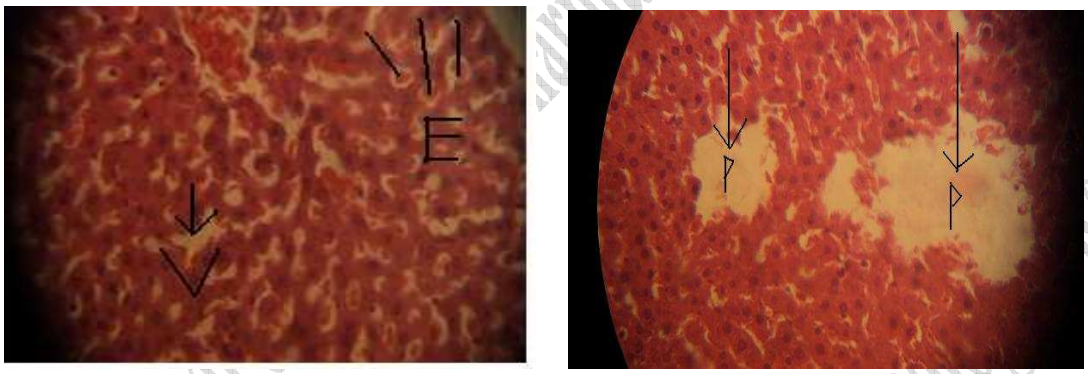
Staining procedure: Sections for general tissue structure were stained by Haematoxylin and Eosin technique. The sections were dewaxed in 3 changes of xylene (5 minutes), the sections were hydrated through descending grades of alcohol (absolute, 95%, 80% and 70%), the sections were stained in Harris haematoxylin (5 minutes), the sections were rinsed in running tap-water to remove excess stain, The sections were differentiated in 1% acid alcohol (3 seconds), the sections were blued in running tap water (10 minutes), the sections were counterstained with 1% eosin (1 minute), sections were finally rinsed in water, dehydrated in ascending grades of alcohol (70%, 80, 95% and absolute), and the sections were cleared in xylene, air-dried and mounted with dibuthylphthalate propylene xylene (DPX). The slides were examined under a light microscope and photomicrographs were taken.

RESULTS

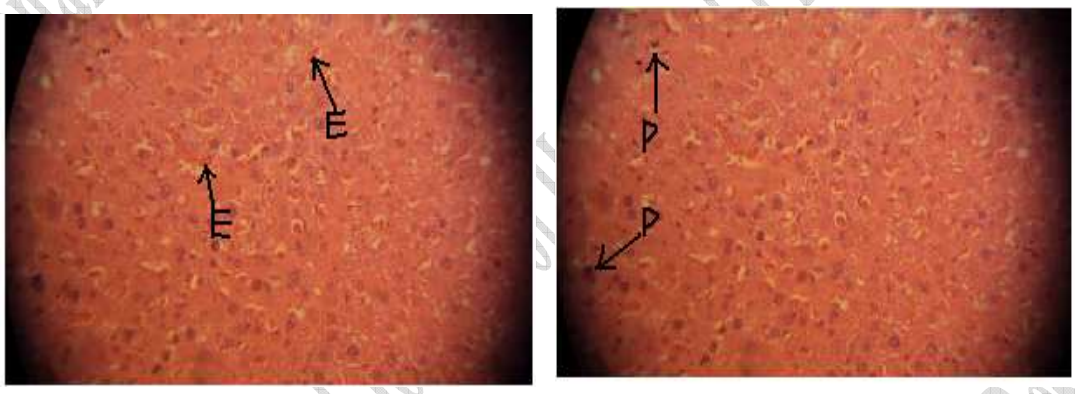
The result of this study has suggest that, oral administration of graded doses of *Carica papaya* seeds over a period of 3weeks(acute) and 6weeks(chronic) presented hepatic damages compare to the control, where normal histological features were observed (figure 4.1). Hepatic damages ranges from hepatic infarction (in group B1 and B2; figure 4.2 and 4.3) to pkynosis and eosinophilic cells with cellular degeneration (in group C1 and C2; figure 4.4 and 4.5 and D1 and D2; figure 4.6 and 4.7). These hepatic changes were observed to be severe with increased dosage (figure 4.2, 4.4 and 4.3) or (figure 4.3, 4.5 and 4.7) and periods of ingestion.



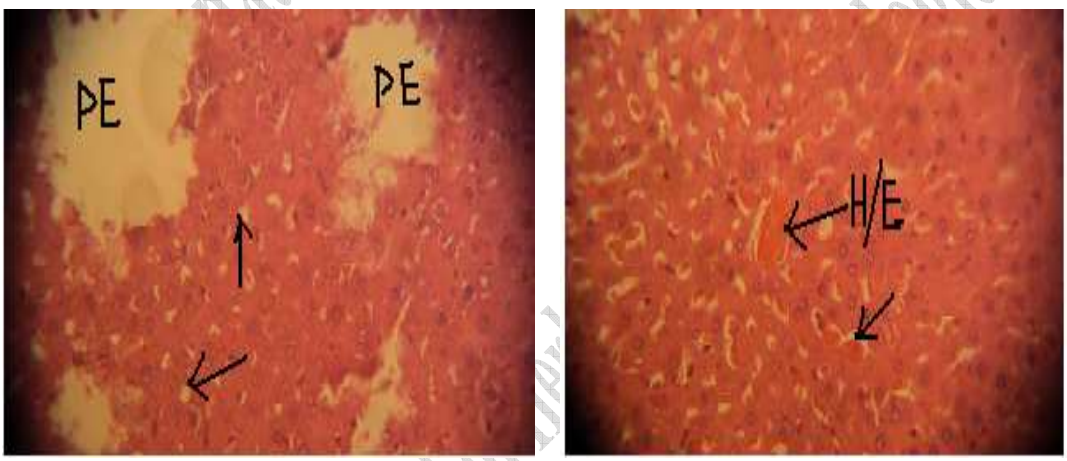
Control group A: A histological representation of control rat liver at X400 magnification (H & E) showing normal hepatocytes



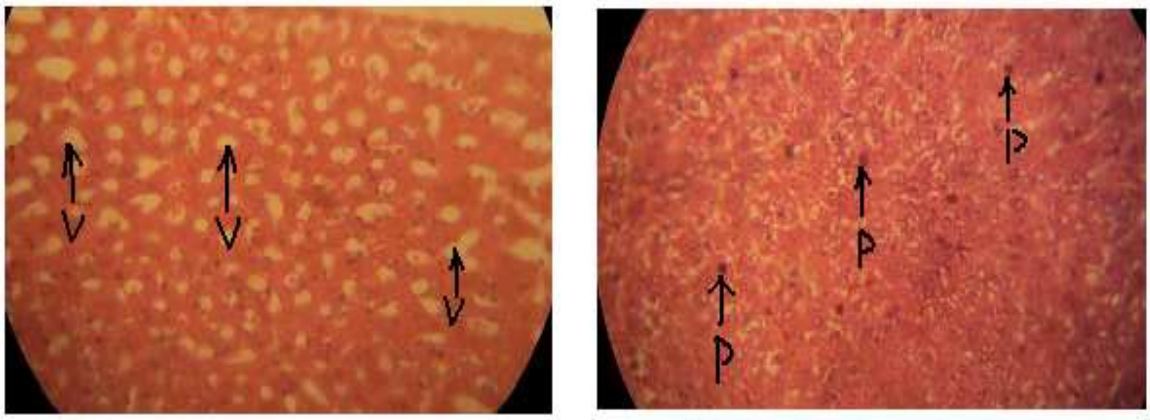
Test group B1: A histological representation at X400 magnification (H & E) of rat liver fed 6 grams/kg/day for 3 weeks [Showing eosinophilic cells with cellular degeneration (E)], vacuolation (V) and parenchymal erosion (P)]



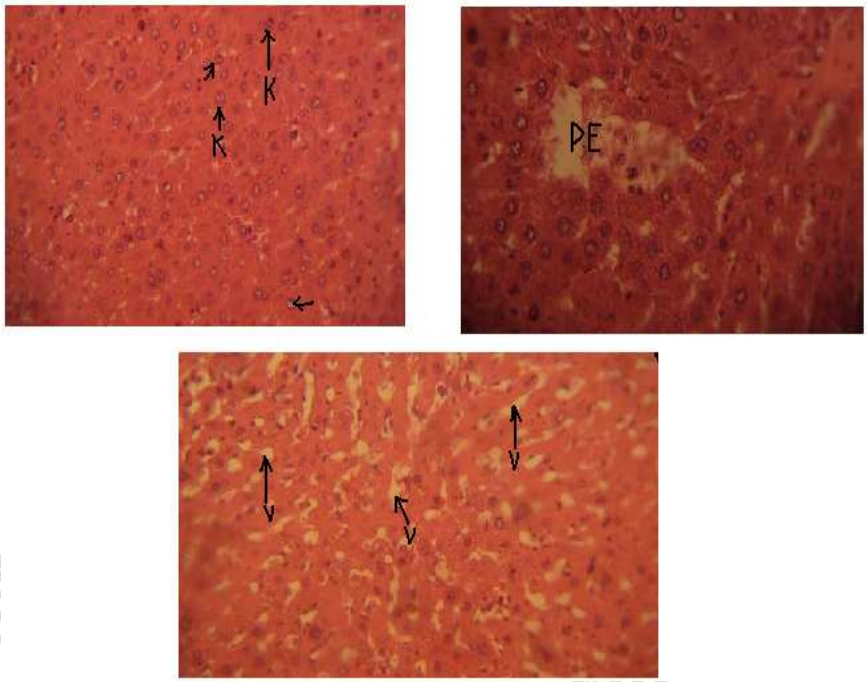
Test group B2: A histological representation at X400 magnification (H & E) of rat liver fed 6 grams/kg/day for 6 weeks [Showing pyknosis (P) and eosinophilic cells with cellular degeneration (E)]



Test group C1: A histological representation at X400 magnification (H & E) of rat kidney fed 8 grams/kg/day for 3 weeks [Showing parenchymal erosion and mild vacuolation (PE), haemorrhage and embolism (H/E)]



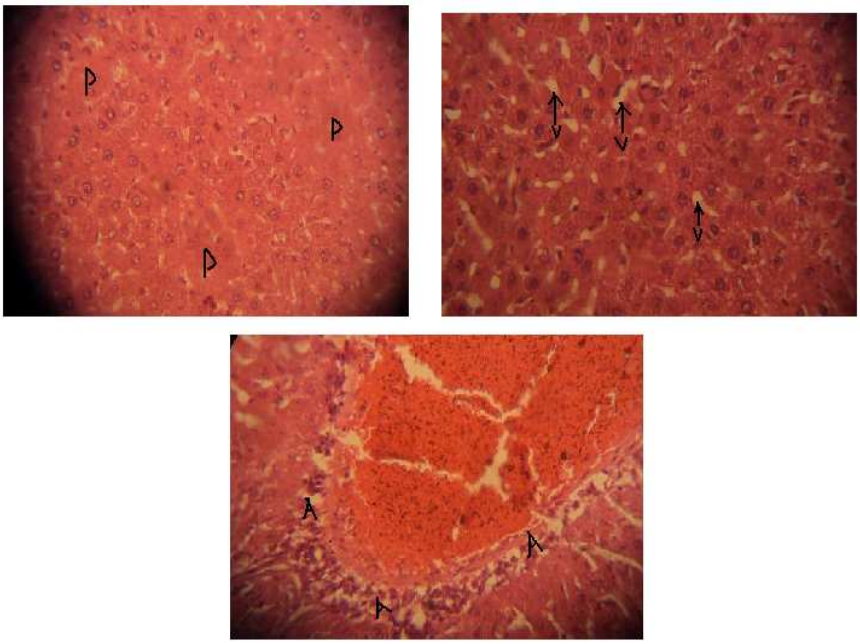
Test group C2: A histological representation at X400 magnification (H & E) of rat kidney fed 8 grams/kg/day for 6 weeks [Showing severe vacuolation (V) and pyknosis (P)]



Test group D1: A histological representation at X400 magnification (H & E) of rat liver fed 10 grams/kg/day for 3 weeks [Showing pyknotic cells with transitional phases of karyohexis and karyolysis (K), parenchymal erosion (PE) and severe vacuolation (V)]

DISCUSSION

The result of this study suggests that *Carica papaya* has hepatotoxic potentials particularly in circumstances of excessive ingestion. Moreover, hepatocytes are especially liable to injury because of their function of taking up and dealing with many metabolites, drugs and other toxic substances as reported by Fernandez-Checa and Kaplowitz (2005) and the results of this study in histological examination support this fact.



Test group D2: A histological representation at X400 magnification (H & E) of rat liver fed 10 grams/kg/day for 6 weeks [showing palour (P), vacuolation (V) and arterial wall disruption (A)]

However, our findings disagrees with the study of Hamman et al. (2011) and several other studies where *Carica papaya* were shown to be hepatoprotective to several hepatotoxins (Vaghela *et al*., 2010) and its aqueous and chloroform extract (Lohiya *et al*., 2006; Goyal *et al*., 2010). On the other hand, *Carica papaya* seeds have been documented to induce mild to severe metaplasia of hepatocytes, proliferation of kupffer cells, hepatic cell cirrhosis and elevation of serum hepatic enzymes (Udoh and Udoh, 2005). Similarly, disarrangement of hepatic cell, necrosis, faded hepatic cell, discolouration of the liver has been reported in fish exposed to *Carica papaya* (Ezekiel and Benedict, 2008; Das, 1980). Papain an active ingredient of *Carica papaya* has also been shown to decrease the absolute and relative weights of the liver in rabbits (Bitto and Gemade, 2001).

It should also be noted that unlike the study by Hamman *et al*, (2011), where 2000mg of *Carica papaya* was used, a dosage choice of 6g, 8g and 10g were used to mimic excessive consumption. This toxic dosage choice for this study, might account for the obvious differences observed between the results of this study and those reported earlier; as well as the use of whole seed as against extracts in other studies. It is recommended therefore, that an excessive/unregulated use of *Carica* papaya seeds for therapeutic purposes amongst others, should avoided.

ACKNOWLEDGEMENT

We sincerely appreciated the efforts of all those that assisted us towards the success of this study.

REFERENCES

Baiyewu, R.A. and Amusa, N.A. (2005). The Effect of temperature and Relative Humidity on pawpaw fruit rot in South- Western Nigeria. *World. J. Agric. Sci*., 1(1): 80-83.
Bitto, I.I. and Gemade, M. (2001). Preliminary investigations on the effect of Pawpaw peel meal on growth, visceral organ and endocrine gland weights, testicular morphometry and the haematology of male rabbits, *Global J.P. & Appl. Sci*. 7(4); 611 – 625.

Dada, A.A. and Ikuerowo, M. (2009). Effects of ethanolic extracts of *Garcinia kola* seeds on growth and haematology of catfish (*Clarias gariepinus*) broodstock. *African Journal of Agricultural Research* Vol. 4 (4), pp. 344-347.

Das, R.P. (1980). Effect of *papaya* seeds on the genital organs and fertility of male rats. *Indian J. Exp Biol*; 18(4): 408-409.

Ezekiel O.A and Benedict O.O (2008). Acute and chronic toxicity of pawpaw (*Carica papaya*) seed powder to adult Nile tilapia (*Oreochromis niloticus* Linne 1757). *African Journal of Biotechnology* Vol. 7 (13): 2265-2274.

Fernandez-Checa J.C. and Kaplowitz N. (2005). Hepatic mitochondrial glutathione: Transport and role in disease and toxicity. *Toxicol*. *Applied Pharm;* 204: 263 – 273.

Goyal, S., Manivannan, B., Ansari, A.S., Jain, S.C. and Lohiya, N.K. (2010). Safety evaluation of long term oral treatment of methanol sub-fraction of the seeds of *Carica papaya* as a male contraceptive in albino rats. *J Ethnopharmacol*; 127(2):286-291.

Hamman, W.O., Musa, S.A., Ikyembe, D.T., Umana, U.E., Adelaiye, A.B., Nok, A.J. and Ojo, S.A. (2011). Acute and Chronic Hepatotoxicity Study of Orally Administered Ethanol Extract of *Carica papaya* Seeds in Adult Wistar Rats**.** *British Journal of Pharmacology and Toxicology* 2(4): 209-212.

Iwu, M.M., Duncan, A.R. and Okunji, C.O. (1999). New antimicrobials of plant origin. In: J. Janick (ed). Perspectives on new crops and new uses. Alexandria, ASHS press. Pp. 457-62.

Kong, J.M., Goh, N.K., Chia, L.S. and Chia, T.F. (2003). Recent advances in traditional plant drugs and orchids. *Acto Pharmacol. Sin*, 24: 7-21.

Lohiya, N.K., Manivannan, B. and Garg, S. (2006). Toxicological investigations on the methanol sub-fraction of the seeds of *Carica papaya* as a male contraceptive in albino rats. *Reprod Toxicol. 22(3):* 461-468.

Nwaopara A.O., Akpamu U, Izunya A.N., Oaikhena G.A., Okhiai O., Anyanwu L.C., Idonije B.O. Oyadonghon G.P. (2011). The effect of *Yaji* meat sauce consumption on cerebellar neurons of white albino rats. *Current Research Journal of Biological Sciences;* 3(4): 308 – 312.

Osifo, U.C., Akpamu, U., Otamere, H.O., and Ekhator, C.N. (2011). A murine model study on the effect of Garcinia kola on body weight. *Archives of Applied Science Research;* 3 (5): 526 – 531.

Udoh, F. and Udoh, P. (2005). Hepatotoxicity of the methanol extract of *carica papaya* seeds in Wistar rats. *Pharm. Biol.;* 43: 349 – 352.

Vaghela, J.S., Rana, A.C. and Sisodia, S.S. (2010). Hepatoprotective activity of *carica papaya* linn. seed extracts against carbon tetrachloride induced hepatic damage in rats. *Journal of Global Pharma Technology*. 2(8): 35-41

Yakubu, M.T., Oladiji, A.T. and Akanji, M.A. (2007). Evaluation of biochemical indices of male reproductive function and testicular histology in wistar rats following chronic administration of aqueous extract of Fadogia agrestis (Schweinf. Ex Heirn) stem. *Afr J Biochem Res* 1: 156–63.

Yinger, H. and Yewhalaw, D. (2007). Traditional medicinal plant knowledge and use by local healers in Sekoru district, Jimmazone, Southwestern Ethiopia. *Journal of Ethnobiology and Ethnomedicine.* 3: 24-30.

World Health Organization (WHO) (2000).Technical report series 894: Obesity: Preventing and managing the global epidemic. Geneva: World Health Organization. http://whqlibdoc.who.int/trs/WHO_TRS_894_(part1).pdf

AUTHORS' CONTRIBUTIONS

All authors (Dikibo, E., Okpe, A.C., Turray, A.A., Onodagu, B.O., Ogbodo, L.A., Oyadonghan G.P.) were actively involved in the successful presentation of this article.