Scientific Research and Essay Vol.4 (10), pp. 1080-1084, October 2009
Available online at http://www.academicjournals.org/sre
ISSN 1992-2248 © 2009 Academic Journals

Full Length Research Paper

Effect of fermented and unfermented seed extracts of *Carica papaya* on pre-implantation embryo development in female Wistar rats (*Rattus norvegicus*)

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Accepted 21 July, 2009

This study was performed to determine the safety or otherwise of *Carica papaya* seeds used as food condiment on pre-implantation embryo development in female Wistar rats. Rats were divided into 5 major groups: Group I served as control, groups II and III were given the fermented extract at 500 and 1500 mg/kg respectively; groups IV and V were given the unfermented extract at 500 and 1500 mg/kg respectively. They were fed the extracts from GD1 to GD5 and on GD6, they were euthanized. The number of implantation sites (7.13 ± 1.06) and percentage pre-implantation loss (57.75 ± 5.00%) in rats administered aqueous extract of the unfermented seeds at 1500 mg/kg was significantly different (P < 0.05) than the control (12.17 ± 1.01 and 19.00 ± 1.65%, respectively) and other groups. There was no significant difference in the number of *corpora lutea* in all experimental groups compared to the control (15.00 ± 1.21). Results showed an increase in weight during pre-implantation, except those given the unfermented extract at 1500 mg/kg. In conclusion, the unfermented seed extract of *C. papaya* at 1500 mg/kg resulted in higher pre-implantation losses, thus indicating its contraceptive ability, while the fermented extract did not have such effect and may be safe as a food condiment.

Key words: Fermentation, *papaya* seeds, abortifacient, anti-implantation, *Copora lutea*.

INTRODUCTION

Carica papaya is widely cultivated for consumption as a fresh fruit and as ingredient for processing drinks, jams and candies (Bhattacharya and Khuspe, 2001). In India, parts of south-east Asia and Indonesia, the fruit is widely classified as harmful in pregnancy, hence pregnant women are strictly forbidden from eating it for fear of its teratogenic and abortifacient effects (Adebiyi et al., 2002, 2003). Chinoy et al. (2006) proved the anti-fertility, anti-implantation and abortifacient properties of extracts from papaya seeds. It has been established in males that the seeds of *C. papaya* are potential antiferti- lity drugs (Lohiya et al., 1994, 1999, 2005; Udoh and Kehinde, 1999). A study conducted by (Dakare, 2004 showed that papaya seeds are used to produce an) indegenous Nigerian food condiment called 'daddawa', the Hausa word for a fermented food condiment. The condiment is also called "Iru" by the Yorubas; "Ogiri" by Igbos; "Owoh" by Urhobos and Itsekiris, and "Okpiye" among the Igala and Idoma people, depending on the region or area of manufacture and the type of legume or oil seeds used (Achi, 2005). In a recent study, Abdulazeez et al. (2009) demonstrated that the seeds of *C. papaya* when fermented have no effects on litters of rats, whereas, those effects were apparent when the unfermented extract was administered (Abdulazeez, 2008).

Fermentation is one of the oldest methods of food processing. It enhances nutritional quality, improves protein digestibility and degrades antinutritional factors (Rolle, 2006; Sahlin, 1999). It enriches the diet through the production of a diversity of flavours, textures and aromas. It improves the preservation quality and shelf-life of foods,

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reduces cooking times and hence, energy con- sumption required for the preparation of foods (Achi, 2005).

The aim of the present study was to evaluate the effects of fermented pawpaw seeds used as “daddawa” and unfermented seeds on pre-implantation in females, using female Wistar rats.

MATERIALS AND METHODS

Experimental animals

Apparently healthy and fertile female (n = 40) and male (n = 20) Wistar rats of about 2 months old and purchased from Department of Anatomy, Faculty of Medicine, Ahmadu Bello University, Zaria served as subjects. The animals were housed in cages and fed rat pellet diet (PLS Feeds, Zaria, Kaduna State, Nigeria.). Prior to the administration of the extract, they were kept for 2 weeks during which they were pre-conditioned to experimental procedures. The study was conducted in accordance with the National Institutes of Health standards for the care and use of experimental animals (NIH Publication No. 80-23; revised 1978).

Preparation of the fermented condiment extract

Fermented seeds were prepared using the method described by Dakare (2004). Briefly, fresh seeds of *C. papaya* obtained commercially were sun-dried, cracked and winnowed to obtain the kernel. The seed kernels were boiled for 2 - 3 h, filtered and spread in a lined sack containing fresh pawpaw leaves, while still hot. They were incubated at 37°C and allowed to ferment for 72 h. The fermented seeds were ground into powder using a mortar. To obtain an aqueous suspension of *C. papaya*, 2.5 g of the powder was dissolved in 20 ml of distilled water and allowed to stand for 24 h, before dosage began.

Preparation of unfermented extract

Fresh seeds of *C. papaya* were obtained commercially, authenticated at the herbarium of Ahmadu Bello University and sun-dried. The seeds were ground to powder using a grinding machine (Fabricated at National Research Institute for Chemical Technology, Zaria, Kaduna State, Nigeria). Crude aqueous suspension was obtained by dissolving 2.5 g of the seed in 20 ml of distilled water and allowed to stand for 24 h.

Experimental design

The forty (40) female rats were housed 8 per cage, each cage representing one group, thus, making a total of 5 experimental groups. Animals in group I served as control group and they were given only water; those in groups II and III were given 500 and 1500 mg/kg of the fermented extract, respectively; while rats in groups IV and V were given the unfermented extract at 500 and 1500 mg/kg, respectively. The male rats served as mating partners. Doses were chosen after acute toxicity tests carried out as described by Lorke (1983) showed the seeds were not toxic.

Fertility tests

This was done following the scheme of Farnsworth et al. (1975) and in accordance with the United States Environmental Protection Agency (1996).

Mating ratio

Female Wistar rats were paired at a ratio of 2:1 with males until mating was confirmed by the presence of sperm in vaginal smears examined under a light microscope. The day sperm/vaginal plug was found was taken as the first day of gestation (GD 1).

Gestation length and ovariohysterectomy

Animals were fed the extract from the first to the fifth day of gestation (GD1 to GD5) and on day seven, ovariohysterectomy was performed on the animals, using standard procedure described by Vogel and Vogel (1997).

Determination of implantation sites

This was determined as described by Cavieres et al. (2002) on day seven. Briefly, the uterine horns obtained were dissected, rinsed and kept in phosphate-buffered saline in Petri dishes to prevent drying. The horns were then stained with drops of 10% ammonium sulphide solution for 10 min. Implantation sites were determined by counting the number of dark rings that appeared as a result of staining in the uterine horns of each rat.

Determination of *Corpora Lutea*

This was carried out using the method described by Armanda-Dias et al. (2001). Briefly, the ovaries obtained from the rats were dissected and fixed in Bouin’s fixative overnight. The ovaries were cut into 5 – 7 µm thick sections and stained with haematoxylin and eosin. For each ovary, about 5 sections were selected and total numbers of *C. lutea* were counted under the light microscope.

Determination of pre-implantation loss

The pre-implantation loss was obtained as described by USEPA (1996), using the formula below:

$$\text{Pre-implantation loss (\%)} = \frac{\text{Number of } \textit{corpora lutea} - \text{number of implantation site}}{\text{Number of } \textit{corpora lutea}} \times 100$$

Body weight measurement

The body weights of all rats were determined on the first day of the experiment prior to administration of the extract, throughout gestation and at the end of the experiment using a weighing balance (Ohaus Scale Corporation, U.S.A).

Statistical analysis

Data obtained were expressed as mean ± standard error of the mean (mean ± SEM). The significance of the results was evaluated using analysis of variance (ANOVA) and the means were compared using Tukeys test. Values of $P < 0.05$ were considered significant.

RESULTS

Effect of extracts on body weight

The percentage increase in weight of rats in the control

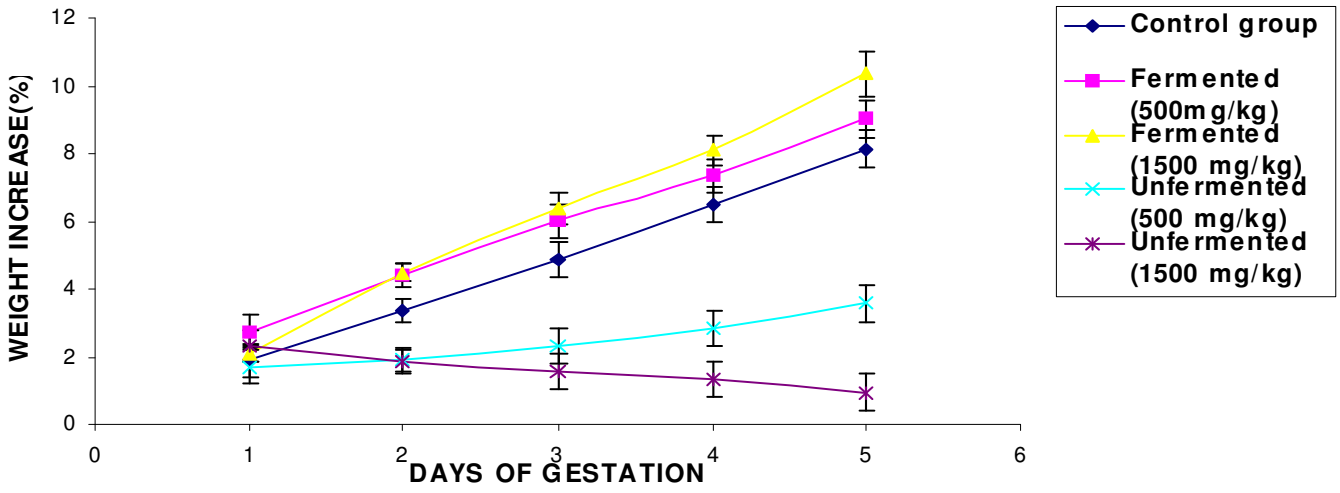

Figure 1: Effect of fermented and unfermented extracts of *C. papaya* seeds on body weight before implantation in female Wistar rats (n = 40).

group increased significantly ($p < 0.05$) from 1.89 ± 0.13% on GD 1 to 8.14 ± 0.38% on GD 5 than those administered with the unfermented extract at a dose of 500 mg/kg (1.70 ± 0.18% to 3.57 ± 0.27%), while the percentage weight of rats dosed with the unfermented extract at 1500 mg/kg (1.98 ± 0.17% to 0.93 ± 0.54%), significantly ($p < 0.05$) decreased when compared to the control group, showing a dose-dependent effect. However, the percentage increase in weight of rats administered with the fermented extract at 500 mg/kg (2.75 ± 0.48% to 9.04 ± 0.55%) and 1500mg/kg (2.10 ± 0.23% to 10.35 ± 0.69%) were not significant when compared with the control group (Figure 1).

Effect of extracts on implantation sites and *C. Lutea*

The number of implantation sites in rats dosed at 1500 mg/kg with the unfermented extract of *C. papaya* seeds (7.13 ± 1.06) was significantly lower ($p < 0.05$) when compared with that obtained in the control group (12.17 ± 1.01), while the administration at a lower dose of 500 mg/kg (11.57 ±0.81), was not significant when compared to the control; showing a dose-effect relationship. There was no significant difference in the number of implantation sites in rats administered 500 mg/kg (10.38 ± 0.56) and those given 1500 mg/kg of the fermented extract of *C. papaya* seeds (11.86 ±1.08), when compared with the control. However, the number of implantation sites in the group treated with the fermented extract of the seeds at a dose of 1500 mg/kg was significantly higher than that obtained in those treated with the unfermented extract at the same dose (Figure 2).

There was also no significant difference observed in the number of *c. lutea* in all experimental and control groups (Figure 3).

Effect of the extracts on pre-implantation loss

The percentage pre-implantation loss was significantly ($p < 0.05$) higher in the rats dosed at 1500 mg/kg with the unfermented extract of the seeds of *C. papaya* (57.75 ± 5.00%) when compared with the control group (19.00 ± 1.65%) and those given either of the doses of the fermented extract. Also, the percentage pre-implantation loss recorded in rats administered the unfermented extract of the seed at a lower dose of 500 mg/kg was not significantly different from that of the control group (Figure 4).

The pre-implantation losses observed in rats administered the fermented extract at both doses of 500 mg/kg (20.87 ± 3.00%) and 1500 mg/kg (20.00 ± 1.23%) were significantly lower than the losses in animals treated with the unfermented extract at a dose of 1500 mg/kg (57.75 ± 5.00%), and did not rise with increase in dose (Figure 4).

DISCUSSION

Body weight provides some indication of the general health status of animals. The decrease in body weight of rats treated with the unfermented extract of *C. papaya* seeds at a high dose of 1500 mg/kg may be due to the rejection of food or water caused by reduced palatability, treatment-induced anorexia, or systemic toxicity. The increase in weight of rats dosed with the fermented extract of *C. papaya* seeds may be attributed to pregnancy (USEPA, 1996; Cavieries et al., 2002).

The reduction in implantation sites and loss obtained in the present study may be due to the effect of the unfermented extract of *C. papaya* seeds on blastocysts or the final stage of implantation, probably due to alteration in the endometrial environment or a combination of both (Paria et al., 1993; Carson et al., 2000). Benzylisothiocya-

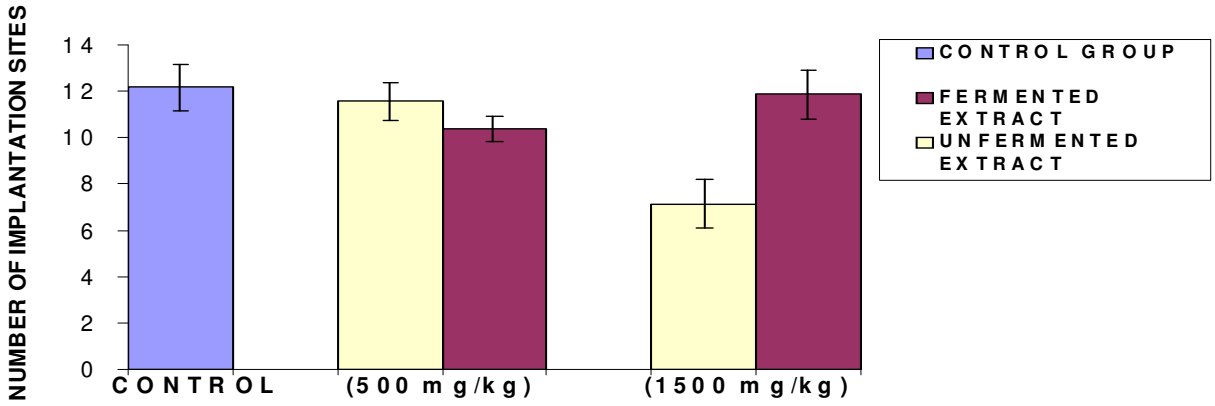

Figure 2. Effect of fermented and unfermented extracts of *C. papaya* seeds on number of implantation sites in female Wistar rats (n = 40).

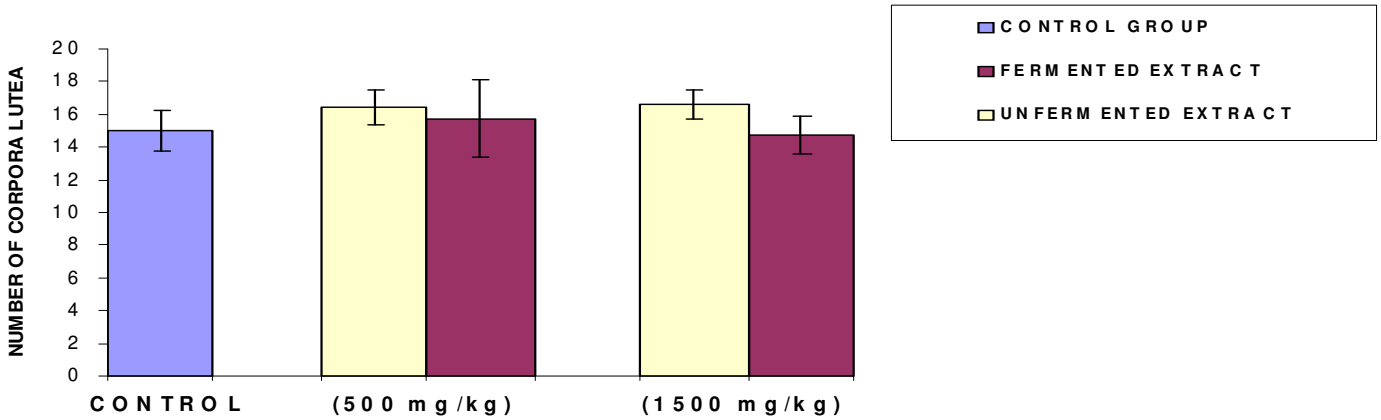

Figure 3. Effect of fermented and unfermented extracts of *C. papaya* seeds on number of corpora lutea in female Wistar rats (n = 40).

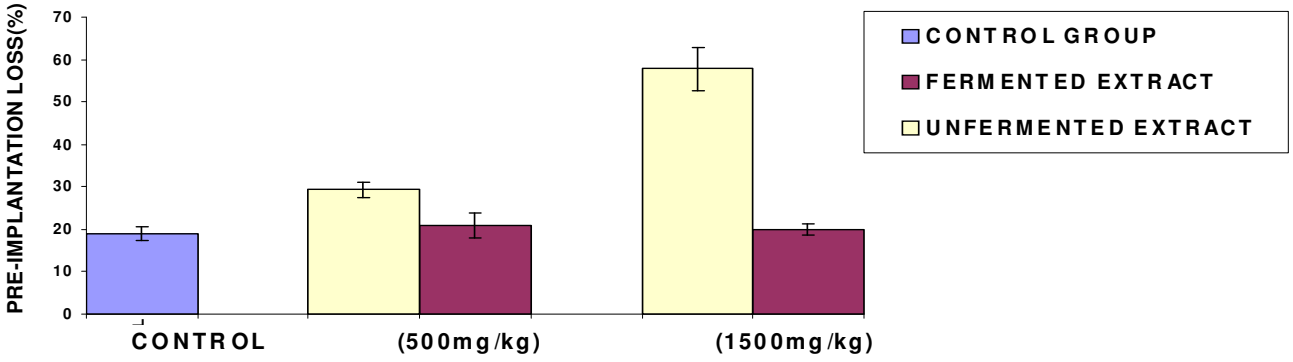

Figure 4. Effect of unfermented and fermented extracts of *C. papaya* seeds on pre-implantation loss in female Wistar rats (n = 40).

nate (BITC), the main bioactive compound in *C. papaya* seeds (Benett et al., 1996; Kermanshai et al., 2001) has been shown to be responsible for the anti-fertility effect (Adebiyi et al., 2003). It has been demonstrated that BITC is capable of damaging the endometrium, making the uterus non- receptive and, thus, affecting adversely the

implantation (Adebiyi et al., 2003). Paria et al. (2001) showed that implantation can only be successful when the activated state of the blastocyst coincides with the receptive state of the uterus.

The decrease in percentage body weight and increase in percentage pre-implantation loss of dams treated with the unfermented extract of *C. papaya* seeds at a high dose of 1500 mg/kg may also be attributed to the levels of oestrogen and progesterone, known to affect both uterine receptivity (Carson et al., 2000; Wang and Dey, 2006), influence food intake and energy expenditure (USEPA, 1996).

The present study, has further confirmed the findings of Adebiyi et al. (2003), Oderinde et al. (2002) and Chinoy et al. (2006) that the unfermented extract of *C. papaya* seeds has adverse effects on female reproduction.

The high number of implantation sites recorded in rats administered with the fermented extract of *C. papaya* seeds, when compared to those administered with the unfermented extract, may be as a result of the fermentation process and the fact that only the of the kernel of the seed was used. The fermentation of the seeds apparently reduced the anti-fertility factor (BITC) and the residual concentration in the kernel may not have the potency to produce any measurable effect. Further studies are required to determine whether the BITC present in *C. papaya* seeds is either in the kernel and/or husk and also to determine the effect of fermentation on the concentration of BITC in the seeds of *C. papaya.*

In conclusion, this study has established that unfermented extract of *C. papaya* seeds, especially at a higher dose of 1500 mg/kg has contraceptive effects, whereas fermented seeds of *C. papaya* does not have contraceptive ability and may be safe for human consumption.

ACKNOWLEDGMENTS

We are grateful to Mr. Monday Dakare of National Research Institute of Chemical Technology (NARICT), Basawa, Zaria for his assistance during the production of fermented seeds of *C. papaya.* Much thanks to Mr. E.N. Kolawale and Mallam M. Bashir of the Department of Biochemistry for the care given to our experimental animals and also all staff of the Department of Physiology and Pharmacology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria.

REFERENCES

Abdulazeez MA (2008). Effect of fermented and unfermented seed extract of *Carica papaya* on implantation in Wistar rats (*Rattus norvegicus*). Thesis submitted to Department of Biochemistry, A.B.U Zaria.

Abdulazeez MA, Ameh DA, Ibrahim S, Ayo JO, Ambali SF (2009). Effect of fermented seed extract of *Carica papaya* on litters of female wistar rats (*Rattus norvegicus*). Afr. J. Biotechnol. 8 (5): 854-857.

Achi OK (2005). Traditional fermented protein condiments in Nigeria. Afr. J. Biotechnol. 4(13): 1612-1621.

Adebiyi A, Adaikan GP, Prasad RN (2002). Papaya (*Carica papaya*) consumption is unsafe in pregnancy: fact or fable? Scientific evaluation of a common belief in some part of Asia using a rat model. Brit. J. Nutr. 88(2): 199-203.

Adebiyi A, Adaikan PG, Prasad RNV (2003). Tocolytic and toxic activity of papaya seed extract on isolated rat uterus. Life Sci. 74: 581-592.

Armanda-Dias L, Carvalho JJ, Breitenbach MM, Franci CR, Moura EG (2001). Is the infertility in hypothyroidism mainly due to ovarian or pituitary functional changes? Braz. J. Med. Biol. Resour. 34(9): 1209-1215.

Benett RN, Kiddle G, Wallsgroove RM (1996). Biosynthesis of benzylglucosinolate, cyanogenic glucosides and phenylpropanoids in *Carica papaya*. Phytochem. 45(1): 59-66.

Bhattacharya J, Khuspe SS (2001). *In vitro* and *in vivo* germination of papaya (*Carica papaya L.*) seeds. Sci. Hortic. 91: 39-49.

Carson DD, Bagchi I, Dey SK, Enders AC, Fazleabas AT, Lessey BA, Yoshinaga K (2000). Embryo implantation. Dev. Biol. 223: 217-237.

Cavieres FM, Jaeger J, Porter W (2002). Developmental toxicity of a commercial herbicide mixture in mice: Effects on embryo implantation and litter size. Environ. Health Perspect. 110(11): 1081-1085.

Chinoy NJ, Dilip T, Harsha J (2006). Effect of *Carica papaya* seed extract on female rat ovaries and uteri. Phytother. Res. 9(3): 169-165.

Dakare M (2004). Fermentation of *Carica papaya* seeds to be used as "daddawa". An MSc thesis; Department of Biochemistry, A.B.U Zaria.

Farnsworth NR, Bingel AS, Cordell AG, Crane AF, Fong HS (1975). Potential value of plants as source of raw antifertility agents. J. Pharm. Sci. 64(4): 535-592.

Kermanshai R, McCarry BE, Rosenfeld J, Summers PS, Weretilnyk EA, Sorger GJ (2001). Benzylisothiocyanate is the chief or sole anthelmintic in *papaya* seed extracts. Phytochem. 57: 427-435.

Lohiya NK, Goyal RB, Jayaprakash D, Ansari AS, Sharma S (1994). Antifertility effects of the aqueous extracts of *Carica papaya* seeds in male rats. Planta Med. 60(5): 400-404.

Lohiya NK, Pathak N, Mistra PK, Maniovannan B (1999). Reversible contraception with chloroform extract of *Carica papaya Linn*. seeds in male rabbits. Reprod. Toxicol. 13(1): 59-66.

Lohiya NK, Pathak N, Mistra PK, Maniovannan B, Bhande SS, Panneerdoss S, Sriram S (2005). Efficacy trial on the purified compounds of the seeds of *Carica papaya* for male contraception in albino rats. Reproductive Toxicol. 20(1): 135-148.

Lorke D (1983). A new approach to practical acute toxicity testing. Arch. Toxicol. 54(4): 275-278.

Oderinde O, Noronha C, Oremosu A, Kusemiju T, Okanlawon OA (2002). Abortifacient properties of aqueous extract of *Carica papaya* (Linn) seeds on female Sprague- Dawley rats. Nig. Postgraduate Med. J. 9(2): 95- 98.

Paria BC, Huet-Hudson YM, Dey SK (1993). Blastocyst's state of activity determines the ''window'' of implantation in the receptive mouse uterus. Proceedings of the National Academy of Sciences of the United States of America 90: 10159-10162.

Paria BC, Ma W, Tan J, Raja S, Das SK, Dey SK, Hogan LM (2001). Cellular and molecular responses of the uterus to embryo implantation can be elicited by locally applied growth factors. Proc. Natl. Acad. Sci. U. S. A. 98: 1047-1052.

Rolle RS (2006). Review: Technical opportunities and challenges to upgrading food bioprocessing in developing countries. http://www.fao.org.

Sahlin P (1999). Fermentation as a method of food processing production of organic acids, pH-development and microbial growth in fermenting cereals. Licentiate thesis, Lund Institute of Technology, Lund University.

Udoh P, Kehinde A (1999). Studies on antifertility effect of pawpaw seeds (*Carica papaya*) on the gonads of male albino rats. Phytother. Res. 13(3): 226-228.

United States Environmental Protection Agency (USEPA) (1996). Guidelines for reproductive toxicity risk assessment. Federal Register 61(212): 56274-56322.

Vogel GH, Vogel WH (1997). Drug discovery and evaluation: Pharmacological assays. Springer-Verlag, Berlin. Heidelberg.

Wang H, Dey SK (2006). Roadmap to embryo implantation: clues from mouse models. Nat. Rev. Gen. 7: 185-199.