

Full Length Research Paper

## Effect of fermented seed extract of *Carica papaya* on litters of female wistar rats (*Rattus norvegicus*)

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This study was performed to determine the effects of fermented seeds of *Carica papaya* on litters of female Wistar rats, and ascertain its safety as a food condiment. Animals in group one served as control, and were given only water; those in groups two and three were given the aqueous extract of the fermented seeds of *C. papaya* at 500 and 1500 mg/kg, respectively, from gestation day six to gestation day 15, kept till terms and allowed to litter. Results showed that the percentage increase in weight of rats in the control group was not significant ( $P < 0.05$ ) when compared to those administered the fermented extract at 500 and 1500 mg/kg. There was no significant difference ( $P < 0.05$ ) in litter size and litter body weight in rats within all the groups, compared to the control group. Also, the anogenital distance (AGD) to crown rump length (CRL) ratio in rats dosed with the fermented extract of *C. papaya* seeds at 500 and 1500 mg/kg was not significantly different from the control group. In conclusion, this study has shown that the fermented seeds of *C. papaya* seeds does not affect the litters and may be safe as a food condiment.

**Key words:** Papaya seeds, fermentation, anogenital distance, crown rump length.

### INTRODUCTION

The antifertility properties of papaya have been the subject of significant evaluation using animal models. In males, the seeds of *C. papaya* have been emerging as a potential antifertility drug (Lohiya et al., 1994; Lohiya et al., 1999; Lohiya et al., 2005). Crude extracts from the seeds of *C. papaya* has been shown to induce variable responses depending on the dose, duration, and route of administration in laboratory animals (Udoh and Kehinde, 1999; Kamal et al., 2003; Verma and Chinoy, 2002). 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). Chinoy et al. (2006) proved the anti-fertility, anti-implantation and abortifacient properties of extracts from papaya seeds, which they realized could be restor-

ed after withdrawal of treatment. However, according to Oderinde et al. (2002), this abortifacient property will only occur when high doses of the extract are given.

These studies have shown that papaya seeds are reproductively toxic, and with such anti-fertility effects, it is easy to conclude that papaya seeds cannot be used as source of food. However, a recent study by Dakare (2004) utilized papaya seeds to produce an indigenous 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. These condiments serve as both flavour enhancers and nutritious non-meat protein in rural communities. "Daddawa" is a fermented product of either locust bean (*Parkia filicoidea*) or soybean (*Glycine max*) (Achi, 2005), hence, the use of pawpaw seeds becomes a welcome development if accepted. This will prevent the wastage of pawpaw seeds, while some of the other seeds originally used can be made available as sources of other foods for

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man and animals (Achi, 2005). Soya bean can then be made readily available as source of protein, contributing to a reduction in the incidence of protein energy malnutrition, one of the most complex diseases affecting people in the developing countries of tropical and subtropical zones (Whitehead and Alleyne, 1972).

During reproductive toxicity experiments, apart from studying the dams before, during and after littering, several end points are used to determine the health or otherwise of litters (USEPA, 1996). The litters are first examined physically for any malformations before certain parameters are determined. These include determination of certain hormone levels, duration of male organ development, vaginal opening, birth weight, anogenital distance and crown rump length (USEPA, 1996; Ronis et al., 1998; Henrich et al., 2000; Hotchkiss et al., 2007).

This study therefore aims to determine the health or otherwise of litters of female Wistar rats administered fermented seed extracts of *C. papaya*, in order to certify its safety as food condiment.

## MATERIALS AND METHODS

### Experimental animals

Clinically healthy and fertile female (n=24) and male (n=12) Wistar rats of about two months old and purchased from Department of Anatomy, Faculty of Medicine, Ahmadu Bello University, were used in this research. The animals were housed in cages and fed rat pellet diet (PLS Feeds, Zaria, Kaduna State, Nigeria.). They were allowed to acclimatize for two weeks before the administration of the extract, during which they were preconditioned to experimental procedures. This study was conducted in accordance with the National Institutes of Health standards for the care and use of experimental animals.

### 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. This was incubated at 37°C and allowed to ferment for 72 h. The fermented seeds were ground into powder using a mortar. To obtain the 'aqueous extract', 2.5 g of the powder was dissolved in 20 ml of distilled water and allowed to stand for twenty four hours.

### Experimental design

Twenty four (24) female and twelve (12) male Wistar rats were used in this experiment. The females were housed eight per cage, making a total of three experimental groups. Animals in group 1 served as control group; those in groups 2 and 3 were given 500 and 1500 mg/kg of the fermented extract, respectively. The male rats served as mating partners. Doses were chosen after determination of LD<sub>50</sub> as described by Lorke (1983).

### Fertility tests

This was done using a scheme by 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 found was taken as the first day of gestation (GD 1).

### Gestation length

Animals were fed the extract from the sixth to the fifteenth day of gestation. They were then kept till terms and allowed to litter.

### Body weight measurement

All animals were weighed at the beginning of the experiment, using the weighing balance (Ohaus scale corporation, U.S.A) to determine body weight. They were also weighed before and after birth to obtain birth and post-natal birth weights, respectively. The litters were also weighed at birth.

### Litter examinations

At birth, the litters were examined for any malformations. The anogenital distance (AGD) and crown rump length (CRL) length of litters were also determined by measuring using a measuring tape.

### Statistical analysis

Data obtained were expressed as mean  $\pm$  standard error of mean (mean  $\pm$  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 regarded as statistically significant.

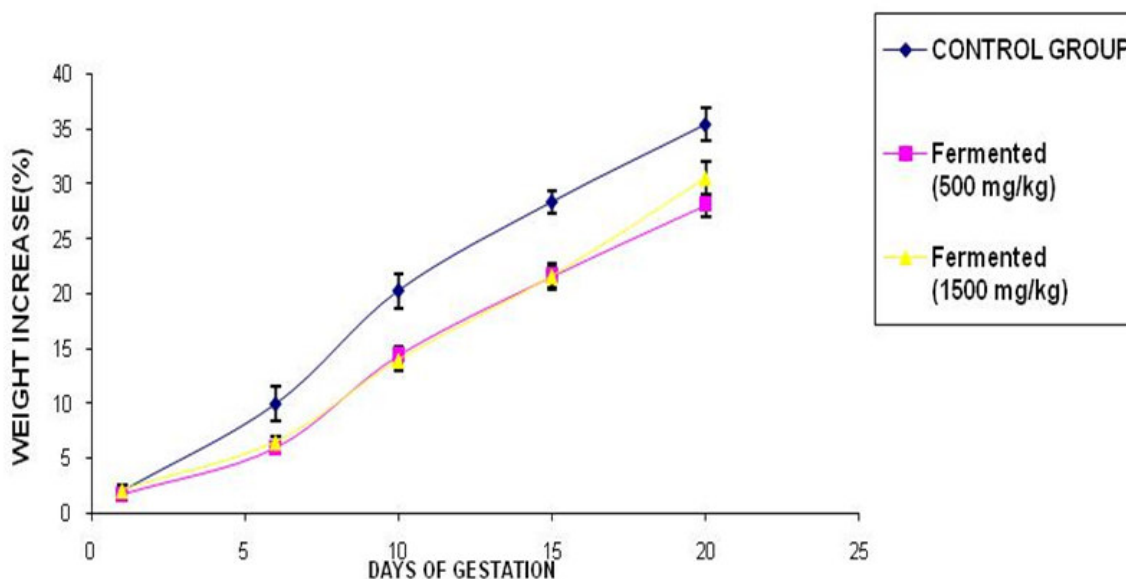
## RESULTS

### Body weight changes during gestation

The body weights of dams during gestation increased as the gestation days progressed in animals of all groups. This increase was positively related to the number of litters in each group, and the litter body weight at birth. The percentage increase in weight in the control group ( $2.04 \pm 0.36\%$  on GD 1 to  $35.4 \pm 1.46\%$  on GD 20) was not significantly different ( $P < 0.05$ ) from those given the fermented extract at 500 mg/kg ( $1.71 \pm 0.27\%$  to  $28.08 \pm 1.02\%$ ) and 1500 mg/kg ( $2.09 \pm 0.43$  to  $30.52 \pm 1.58\%$ ) (Figure 1).

### Effect of extracts on litter size

The control group had the highest litter size of ( $6.16 \pm 0.54$ ), which was not significantly different from the litter sizes of dams treated with the fermented extract at 500



**Figure 1.** Effect of fermented extract of *Carica papaya* seeds on body gestation in female wistar rats.

**Table 1.** Effect of fermented extract of *C. papaya* seeds on litters of Wistar rats.

Parameter	Control Group	Fermented extract (500 mg/kg)	Fermented extract (1500 mg/kg)
Litter size	6.16 ±0.54	5.16±0.31 <sup>a</sup>	5.83 ± 0.31 <sup>a</sup>
Litter body weight (g)	5.57±0.05	5.41±0.12 <sup>a</sup>	5.56±0.08 <sup>a</sup>
AGD/CRL (mm)	1.22±0.02	1.13±0.04 <sup>a</sup>	1.17±0.03 <sup>a</sup>

Values are mean ± S.E.M; <sup>a</sup>Not significant at P<0.05 compared to the control group.

mg/kg (5.16 ± 0.31) and 1500 mg/kg (5.83 ± 0.31) (Table 1).

#### Effect of extracts on litter body weight

The litter body weights recorded in animals dosed with the fermented extracts at 500 mg/kg (5.41 ± 0.12g) and 1500 mg/kg (5.56 ± 0.08g) were not significantly different from the c

#### Effect of extracts on anogenital distance to crown rump length ratio

From the results, the anogenital distance to crown rump length ratio of rats dosed with the fermented extract at 500 mg/kg (1.13 ± 0.04) and 1500 mg/kg (1.17 ± 0.03) were not significantly different from control (1.22 ± 0.02).

#### DISCUSSION

Body weight provides some indication of the general

health status of animals. A decrease may be due to the rejection of food or water caused by reduced palatability, treatment-induced anorexia, or systemic toxicity.

However, this could not have been the case in rats administered with the fermented extract of *C. papaya* seeds, as their weights increased. This increase in weight in animals of all groups can be as a result of pregnancy, which affects the levels of oestrogen and progesterone, known to affect both uterine receptivity (Carson et al., 2000; Cavieres et al., 2002; Wang and Dey, 2006), influence food intake, and energy expenditure (USEPA, 1996).

The similarity in the litter size, litter body weight and AGD/CRL ratio recorded for rats in the control group and those administered the fermented extract of the seeds of *C. papaya*, shows that the fermented seed does not affect foetal growth. This could be as a result of the fermentation process, whereby the husk is removed and only the kernel of the seed was used. Hence, it is possible that any compound with antifertility effect, such as BITC (Benzyl isothiocyanate) which is the suspected compound (Benett et al., 1996; Adebisi et al., 2003), may have been reduced and the residual concentration in the kernel after fermentation may not have the potency to

produce any measurable effect. It is important to note that there has not been any research to determine if the BITC present in *C. papaya* seeds is either in the kernel or husk; or even in both components of the seed. Also, no study has been carried out to determine the effect of fermentation on the concentration of BITC in the seeds of *C. papaya*.

In normal individuals, AGD is shorter in females, but an increase had been observed during maternal exposure to androgens or androgenic substances; while in males maternal exposure to anti-androgens decreases the AGD (Ronis et al., 1998; Ogata et al., 2001). It is also well established that the effect of AGD can be masked by body size (USEPA, 1996; Ronis et al., 1998), hence the need to evaluate the AGD/CRL ratio to determine possible hormonal alterations in animals. Therefore, this study has demonstrated that the fermented extract of *C. papaya* seeds is not androgenic because the AGD/CRL in the neonates was not affected. This implies that the fermented seed extract does not affect sexual development and reproductive function.

In conclusion, this study has established that the fermented seeds of *C. papaya*, which does not have effect on litter size, litter body weight and AGD/CRL ratio, may be safe as a food condiment. However, further studies need to be carried out to determine its safety in adult male and female Wistar rats, before its safety is fully certified.

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