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Ethanol Extract of *Carica papaya* Seeds Induces Reversible Contraception in Adult Male Wistar Rats

[1]Wilson O. Hamman, [1]Sunday A. Musa, [1]Daniel T. Ikyembe, [1]Uduak E. Umana,
[2]Alexander B. Adelaiye, [3]Andrew J. Nok and [4]Samuel A. Ojo
[1]Department of Human Anatomy,
[2]Department of Human Physiology,
[3]Department of Biochemistry,
[4]Department of Veterinary Anatomy, Ahmadu Bello University Zaria-Nigeria

Abstract: The quest for the development of an ideal male contraceptive has led to the discovery of the antifertility property of some species of *C. papaya* seed extracts. This study investigates the antifertility activity and reversibility of ethanol extract of *C. papaya* seeds in sexually matured male Wistar rats. 30 adult males and 60 female Wistar rats weighing between 180 and 220 g were used for the study. The male rats were randomly divided into 3 groups of 10 each. Group I which served as control were orally administered distilled water while groups II and III received 100 and 250 mg/kg/day of ethanol extract of *C. papaya* seeds respectively for a period of 90 days. 5 males from each group were subjected to fertility test before they were sacrificed to harvest the testes for histopathological analysis. The administration of the extract was discontinued for another 90 days period and the remaining 5 males in each group were subjected to fertility test before they were sacrificed to obtain the testes for histopathological analysis. The results showed normal pregnancy outcome in the females paired with the control group, reduced and zero pregnancy outcome in the females paired with the 100 and 250 mg/kg groups respectively after 90-day administration of the extract. After 90 days of discontinued administration of extract, normal pregnancy outcome were recorded in both the control and treated groups. The result of the histopathological analysis showed a moderate and highly depleted germinal epithelium in the 100 and 250 mg/kg groups respectively after 90 day administration of the extract. The germinal epithelium seen in both the control and the experimental groups were normal after 90 days discontinued extract administration. The study concludes that ethanol extract of *C. papaya* seeds induces reversible male contraception in Wistar rats.

Key words: *C. papaya* seeds, male wistar rats, reversible contraception

INTRODUCTION

Overpopulation has become a global problem with grave implications for the future. It is now generally accepted that currently available methods of fertility regulation are inadequate to meet the varied and changing personal needs of couples at different times of their reproductive lives, and in the widely different geographical, cultural and religious settings that exist around the world (Wang, 1999). Contraception, which is the intentional prevention of conception through the use of various devices, sexual practices, chemicals, drugs, or surgical procedures (Stacey, 2008) has taken the center stage in the international war against overpopulation. Today, different chemical, barrier (diaphragm, caps, IUDs condoms), hormonal (progestogen-only pills, combined oral pills, injectables, implants, microspheres and microcapsules, skin patches and gels, LHRH antagonists, anti-progesterone), and sterilization methods are available to women (Dickens, 2000; Marks, 2001; fpa, 2006). However, most of the achievement in male contraception is confined to improving the already existing barrier and sterilization methods (Dickens, 2000; Marks, 2001; Nass and Strauss, 2004; fpa, 2006). Recently, efforts are being devoted to identify a plant-based male contraceptive that is supposedly orally bioactive, non-toxic, and more importantly cost effective, based on ethnomedical information (Lohiya *et al*., 2001). The quest for the development of a male contraceptive particularly from natural sources has led to the discovery of the antifertility efficacy of the seed of some specie of *C. papaya*. The locally available and widely distributed *C. papaya* seed have shown great promise in male contraception in animal models. (Lohiya *et al*., 1999;

Corresponding Author: Dr. W.O. Hamman, Department of Human Anatomy, Ahmadu Bello University Zaria-Nigeria, Tel.: +234-08062303007

Udoh and Kehinde, 1999; Pathak *et al*., 2000; Lohiya *et al.* 2001; Sharma *et al.* 2001; Verma and Chinoy, 2001; Lohiya *et al*., 2002; Lohiya *et al*. 2006). The aim of the present study is to investigate the antifertility property of ethanol extract of the homestead variety of *C. papaya* seeds in adult male Wistar rats.

MATERIALS AND METHODS

Experimental animals: Thirty apparently healthy, proven fertile male Wistar rats weighing between 180 and 220 g and 60 proven fertile non-pregnant female Wistar rats weighing between 180 and 200 g were used in the present study. The animals were bred and housed in polypropylene cages in the animal house of the Department of Human Anatomy, Ahmadu Bello University, Zaria - Nigeria. The animals were fed rat pellet diet and layers mesh, exposed to a 12 h light: 12 h dark cycle and water was provided *ad libitum*. Animals were treated humanely, veterinary care and supervision were provided throughout the period of study.

Extract preparation: Ripe *C. papaya* fruits of Homestead variety were commercially obtained from a local market in Zaria and authenticated at the Department of Biological Sciences, Ahmadu Bello University, Zaria-Nigeria with voucher number 0911. The seeds were removed, shade dried and coarsely powdered. The powdered material was soxhleted with ethanol in the Department of Pharmacognosy and Drug Development of Ahmadu Bello University, Zaria. The soxhleted material was concentrated under reduced pressure and the oily residue used in the investigation.

Experimental design: The experiment which was carried out in January, 2010 in the Department of Human Anatomy, Ahmadu Bello University Zaria-Nigeria consisted of three groups of 10 male Wistar rats designated as group I, II, and III. After 14 days period of acclimatization, the animals in groups II and III were orally administered 100 mg and 250 mg/kg/day of ethanol extract of *C. papaya* seed respectively. The animals in group I which served as control were administered same volume of distilled water daily. Due care was taken to ensure that the test groups received the complete dose in the same volume. The period of administration lasted 90 days. At the termination of the extract administration, 5 animals were randomly picked from each group and paired with the fertility proven females in a ratio of 1:2 and fertility test carried out (Lohiya *et al.* 2005). The fertility tested male rats were then sacrifice underanaethesia and their testes obtained and processed for light microscopy while the females were allowed to liter. The remaining male Wistar rats were allowed to recover from extract administration for another period of 90 days. At the termination of 90 days extract withdrawal, the remaining 5 male rats from each group were also paired with fertility proven females in a ratio of 1:2 and fertility test carried out. These rats were also sacrificed under anaesthesia and their testes harvested and processed for light microscopy while the females were allowed liter.

Fertility test: At the termination of the 90 days extract administration and 90 days extract withdrawal, 5 male Wistar rats were randomly selected from each group and paired with 2 proven fertile females Wistar rats each for a period of 5 days. Success of mating was confirmed in vaginal smear and the females were allowed to complete gestation period. Liter sizes were recorded at birth and 4 days after. Percentage fertility was obtained by dividing the number of females delivered by the number of females mated and multiplied by 100 (Lohiya *et al*., 2005).

Percentage fertility = No of females delivered / No of females mated x 100

Viability Index was obtained by dividing the number of viable pups 4 days after delivery by the number of pups at birth and multiply by 100 (Ratnasooria *et al*., 2003)

Viability Index = No of viable pups 4 days after deliver/ No of pups at birth X 100.

RESULTS

Male fertility test after 90 days extract administration: The male fertility test conducted at the end 90 days oral administration of ethanol extract of *C. papaya* seed resulted in normal pregnancy outcome in the control group, reduced pregnancy outcome in the 100 mg/kg (group II) and completely zero pregnancy outcome in the 250 mg/kg group (group III). Nine out of the 10 females paired with the males in the control group obtained a combined litter size of 52 at birth, giving a fertility percentage of 90%. 50 survived up to the 4th day after birth, giving a viability index of 96.12. The pregnancy

Table 1: Fertility test and viability indices of wistar rats after 90-day oral administration and 90-day withdrawal of ethanol extract of *C. papaya* seeds

Parameters	90-day extract administration			90-day extract withdrawal		
group	Control	100 mg/kg	250 mg/kg	Control	100 mg/kg	250 mg/kg
Mating Ratio (M:F)	1:2	1:2	1:2	1:2	1:2	1:2
NFD	9	5	0	10	9	9
NLD	52	28	0	58	50	51
NL on 4th day	50	27	0	55	49	42
Fertility %	90	50	0	100	90	90
Viability index	96.82	96.42	0	94.82	98.00	82.35

NFD: Number of Female Delivered; NLB: Number of Liters at Birth; NL: Number of Liters

outcome among females paired with the males in group II (100 mg/kg) was 5 out of 10 females with a combine litte size of 28 at birth, 27 survived on the 4th day which the a fertility percent of 50% and a viability index of 96.42. No delivery was observed among the females paired with the males in the 250 mg/kg group and therefore fertility percentage and viability index were zero. All the results obtained are as presented in Table 1.

Male fertility test after 90 days extract withdrawal: The results of the male fertility test conducted after 90 days extract withdrawal is also shown in Table 1. Fertility percentages observed among the control group I, group II and II were 100, 90 and 90, respectively. The combined litter sizes at birth were also 58, 50, and 51, while the viability indices were 94.82, 98.00, and 82.35 respectively for the control group I, group II and group III.

Histopathology of testes: The light microscopic examination of the testes in the control group after 90 days extract administration showed normal histological profile in which the seminiferous tubules were surrounded by intertubular connecting tissues containing the interstitial cells of Leydig and blood vessels. Surrounding the germinal epithelium of the seminiferous tubules is a basement membrane on which rests actively dividing spermatogonia and sparsely distributed Sertoli cells whose apices reach the lumen of the tubules. The primary spermatocytes, secondary spermatocytes, early and late spermatid were seen arranged in a concentric manner from the basal layer of the spermatogonia towards the lumen of the seminiferous tubules as show in Fig. 1. The testes of the Wistar rats treated with 100mg of *C. papaya* extract as shown in Fig. 2 showed depleted germinal epithelium. No late spermatids were seen but a few early spermatids in some sections along with the other spermatogenic cells. The rats that received the 250mg of the extract showed spermatogonia, primary and secondary spermatocytes as the only spermatogenic cells present in the seminiferous tubules as seen in Fig. 3. The transverse sections of the testes of the Wistar rats in all the groups reveals a normal histology after 90 days of extract withdrawal as shown in Fig. 4, 5 and 6.

DISCUSSION

The result obtained from the male fertility study conducted after 90 days administration of ethanol extract of *C. papaya* seeds indicates a dose dependent association between male fertility and pregnancy outcome which implies a positive antifertility efficacy similar to result obtained by Lohiya *et al*. (2005). The 90, 50 and 00 fertility percentages recorded for the control, 100 mg/kg, and 250 mg/kg groups respectively after 90 days oral administration in the present study indicates that ethanol extract of *C. papaya* seeds of the homestead variety is slow acting when compared to the results of other workers

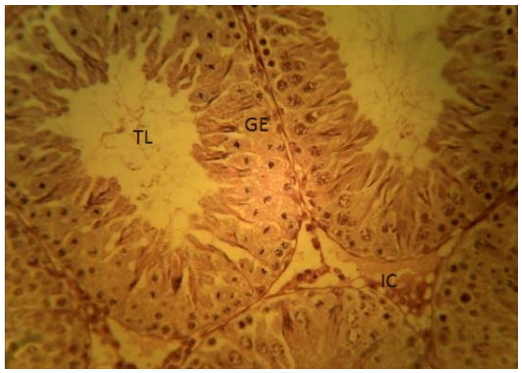

Fig. 1: Transverse section of the Testis of Wistar rats from the Control group showing normal Seminiferous Tubules with normal Germinal Epithelium (GE), Interstitial Cells (IC) and Tubular Lumen (TL) H & E, X250

Fig. 2: Transverse section of the Testis of Wistar rats after 90-day oral administration of 100 mg/kg *C. papaya* seed extract showing a slightly depleted Germinal Epithelium (GE), normal Interstitial Cells (IC) and Tubular lumen (TL), H & E X250

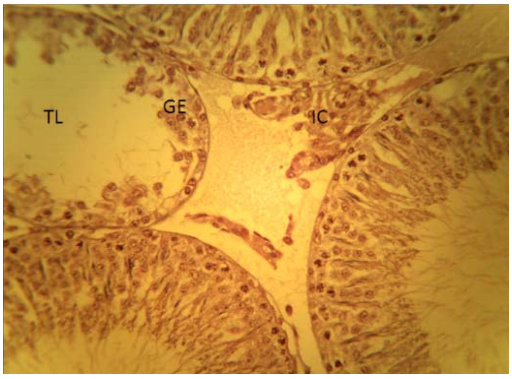

Fig. 3: Transverse section of the Testis of Wistar rats after 90-day oral administration of 250 mg/kg *C. papaya* seed extract showing a severely depleted Germinal Epithelium (GE) and normal Interstitial Cells (IC) H & E, X250

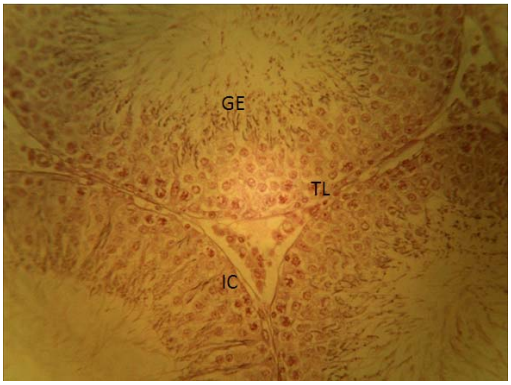

Fig. 4: Transverse section of the Testis of Wistar rats from the Control group showing normal Seminiferous Tubules with normal Germinal Epithelium (GE), Interstitial Cells (IC) and Tubular Lumen (TL) H & E, X250

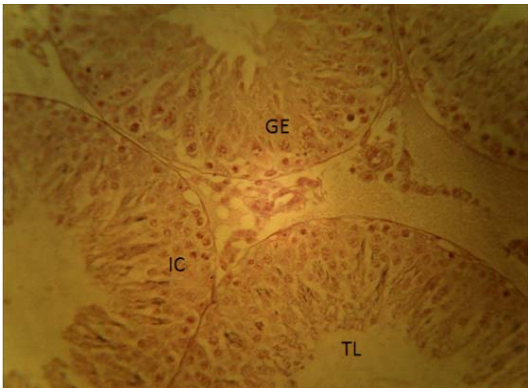

Fig. 5: Transverse section of the Testis of Wistar rats after 90-day withdrawal of 100 mg/kg *C. papaya* seed extract showing a normal Germinal Epithelium (GE), normal Interstitial Cells (IC) and Tubular Lumen (TL). H & E, X250

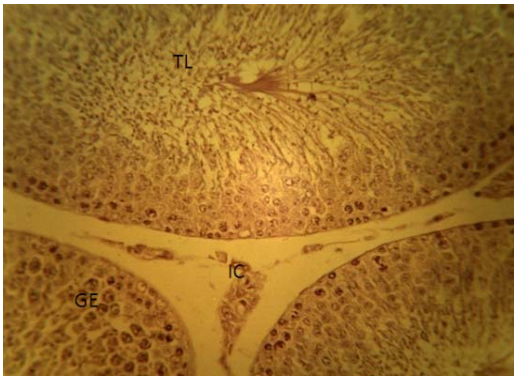

Fig. 6: Transverse section of the Testis of Wistar rats after 90-day withdrawal of 250 mg/kg *C. papaya* seed extract showing a normal Germinal Epithelium (GE), normal Interstitial Cells (IC) and Tubular Lumen (TL). H & E, X250

(Lohiya *et al*., 1994; Lohiya *et al*., 1999; Udoh and Kehinde, 1999; Lohiya *et al*., 2000; Verma and Chinoy, 2001; Lohiya *et al*., 2002; Lohiya *et al*., 2005) that worked on the honey dew and other species of *C. papaya* seed extracts in different experimental animals. The viability indices obtained particularly that of the 100 mg/kg group indicates that the quality of sperms produced before the effect of the extract was exerted has not been completely compromised. However, after 90 days extract withdrawal, insignificant differences were observed in the pregnancy outcome as reflected in the fertility percentages recorded as well as the viability indices. This may be attributed to the systemic clearance of the extract and its effects from the body of the experimental rats and by extension allows for normal fertility recovery. The result of the histopathological examination of the testis sections is indicative of a dose dependent gradual spermatogenic arrest as the number of cell types in the germinal epithelium decreases with increased extract dose. This may either be as a result of direct effect of the extract on the germinal epithelium or indirect effect through its action on the hormone that influence normal spermatogenesis. The moderate and highly depleted germinal epithelium observed in the 100 and 250 mg/kg/day groups respectively is in agreement with the result of the fertility test carried out in the present study since fertility in male is dependent on a normal germinal epithelium among other factors. The depleted germinal epithelium observed in the present study is also in agreement with the findings of other researchers who worked with different species of *C. papaya* seeds on different animal models (Lohiya *et al*., 1994; Lohiya *et al*., 1999; Pathak *et al*., 2000; Lohiya *et al*., 2000)

CONCLUSION

The present study has established 100% antifertility efficacy of ethanol extract of the homestead variety of *C.papaya* seeds in male Wistar rats. However, the activity seems to be slow when compared with other species of *C.papaya* and extraction methods. The reversibility of all the effects of the ethanol extract of the homestead variety of *C. papaya* seed in male Wistar rats have also been confirmed through the present study. The antifertility action being clearly evident on the testicular germinal epithelium of the treated male Wistar rats.

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