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Fertility activities of female rats via male rats administered with crude extracts of seed, leaf and pulp of *Carica Papaya* (Linn)

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ABSTRACT

The effect of administering ethanol extracts of seed, leaf and pulp of *C. papaya* on male rats and assessing its effects on the female rats was investigated. 0, 100, 200 and 300mg/kg of each extracts were orally administered to the male rats for 30 days after which they were mated with the female rats in the ratio of 1:1 for the first generation. Conception rate, induced lethal mutation index, litter size and weight at birth and weaning were estimated. The remaining male and female rats were mated again for the second and third generations. Results obtained showed that there were significant effects ($P < 0.05$) of mating treated male rats with the untreated female rats on all the parameters studied. Implicitly though, our results suggest that indiscriminate consumption of herbals by male rats could have devastating effects on the female rats during reproduction. It becomes necessary to carry out in-depth research similar to this works as to make concrete assertion.

Introduction

Fertility is very crucial to living organisms and as such should be given critical attention. On assumption that the female animal is reproductively functional, any dysfunctions that would be observed might be traceable to the male animals. Undoubtedly, some activities engaged by males may have adverse effects on their reproductive pathways, which might directly or indirectly compromise the reproductive integrity of the female.

Carica papaya (Linn), a perennial seed propagated plant that belongs to the family of Caricaceae has been economically and therapeutically exploited (Ikpeme et al., 2007). It's anti-fertility and pharmaceutical potentials have been reported (Udoh and Kehinde, 1999; Lohiya et al., 2000; El-Moussaoui et al., 2001). According to Franco et al. (1993), Udoh and Udoh (2005), Ikpeme et al. (2011), the fruits, leaves, latex, roots and seeds contain novel bioactive compounds that elicit pharmacological effects such as on arthritis, rheumatism, tuberculosis, malaria, cancer, etc. (Adebiyi et al., 2002). Nutritionally, *Carica papaya* fruit is a good source of calcium and an excellent source of vitamins A and C (Nakasone and Paull, 1998). Oloyede (2005) analyzing the chemical and mineral composition of unripe pulp of *Carica papaya* reported that potassium, calcium, and magnesium were in high proportion.

There are reports regarding the disruption of spermatogenic pathways leading to decrease in sperm counts, sperm motility, sperm viability, etc., occasioned by the indiscriminate consumption of herbs or their combinations (Udoh and Ekpenyong, 2001; Ikpeme et al., 2007; Ekaluo et al., 2009; Ikpeme et al., 2010). Lohiya *et al*. (2000) evaluated the contraceptive, toxicological and fertility status of female rats administered with seed extract of *C. papaya*. The toxicological profiles were unaltered whereas there was a remarkable decrease in fertility and contraceptive functions, indicating that the extract was free of toxic effects. In another development, Adebiyi et al. (2002) reported the abortifacient and teratogenic properties of *C. papaya* fruit while Schmidt (1995) observed that unripe papaya fruit, latex and seed extracts have deleterious effects on pregnancy in laboratory animals.

In view of the immense medicinal importance of *C. papaya* as evidenced in the literature, there is strong incentive for further research, especially as regards the ethanol leaf and pulp extracts of *C. papaya* where there is paucity of information. This research stems from the hypothesis that there are possibilities that drugs consumed by the males could affect the conception and the developing embryos during pregnancy. The data in this paper were generated from mating regimen between male albino rats administered with ethanol seed, leaf and pulp extracts of *C. papaya* and their female counterparts (Ikpeme et al., 2010).

Materials and methods

Plant collection and preparation of extracts

Seeds, leaves and unripe pulp of *C. papaya* (Solo variety) used for the study were collected from State Housing Lane. Ediba, Calabar and certified in the herbarium unit of the Department of Botany, University of Calabar, Calabar, Nigeria. The seeds and leaves were washed and dried at room temperature (25°C) while the pulp was oven dried (Continent, MG800G) at 40˚C for 15 minutes. The dried plant materials were then pulverized with an electric blender (4250 Braun, Germany) to a powdery form. Each plant material was subjected to ethanol extraction in a soxhlet extractor (M & B, UK). The pastry samples were further concentrated in a rotary evaporator (Sigma, USA) at a controlled temperature of about 70˚C for 1 hour. The semi solid residue from the extracts was the stored at 5-6˚C in the refrigerator. One gram of each extract was dissolved in 100 ml of normal saline to give 100 mg/l

Experimental animal and administration of extracts

One hundred and sixty-two clinically healthy albino rats of about two months old weighing between 150-180 g were obtained from the Animal Unit, Zoology and Environmental Biology Department, University of Calabar, Calabar. They were

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housed in cages under standard laboratory conditions of temperature range of 25-29˚C and 12h light/dark cycle throughout the experimental periods. The rats were left to acclimatize for two weeks with free access to water and feed. Four experimental groups of six rats were used for the study in 3x4 factorial experimental layouts using completely randomized design.

The female rats were not treated. Male rats in group 1 served as control and received 1ml of normal saline and normal chow. Rats in group 2 received 100 mg/kg BW while rats in groups 3 and 4 received 200 mg/kg BW and 300 mg/kg BW of each extract, respectively for 30 days through oral gavage. At the end of the treatment regimen, the treated male rats were cohabited with the untreated female rats in the ratio of 1:1. After mating was confirmed through vaginal plug, the male rats were separated from the females. The mated females were observed till the 14^{th} day. Two female rats from each group were sacrificed for induced mutation assay according to the method of Odeigah (1991) while the remaining four were allowed to kindle. Litter sizes and weights at birth and weaning, conception rate and number of runts were recorded according to the method of Ikpeme *et al*. (2007). After the first generation, the four remaining female rats were with the treated males for the two generations.

Data collection and analysis

Data on dominant lethal assay, conception rate, litter sizes and weights at birth and weaning were subjected to the analysis of variance (ANOVA) while Least Significant Difference (LSD) was used in separating significant means (Obi, 2002).

Results

Effect of the seed, leaf and pulp extracts of *C. papaya* on dominant lethal mutation index (ILMI) in female rats

Results on DLMI revealed that there were significant differences ($P < 0.05$) on the female rats after mating with treated male rats. It showed that the female rats mated with the seed extract treated male rats had more effect, especially at 300mg/kg. The trend of effect on the female rats is seed extract treated male rats > leaf extract treated male rats > pulp extract treated male rats (Table 1).

Conception rate of female rats mated with males treated with seed, leaf and pulp extracts of *C. papaya*

The conception rate among the female rats showed that the female rats mated with seed extract treated (200mg/kg and 300mg/kg) male rats had lower conception rate of 66.7%. However, there were no significant differences ($P > 0.05$) observed among the female rats mated with the male rats administered with the leaf and pulp extracts (Table 2).

Effect of seed, leaf and pulp extracts on litter sizes and weights at birth and at weaning

There were significant differences ($P < 0.05$) in the litter sizes at birth and at weaning periods of the female rats mated with the treated males. The effect was dose-dependent. The plant part notwithstanding, as the dose of administration increases, the number of pups reduced. It was also observed that as the generation increased, litter sizes and weights both at birth and weaning increased though the trend was not consistent. There were also significant differences observed in the litter weight at birth and at weaning. The results showed that the effect was plant part- specific and dose- dependent. The females mated with the males treated with the leaf extract had the highest mean litter weight at birth and weaning (leaf extract > pulp extract > seed extract).

Discussion

It has been reported that distortion in the cellular architecture of the testicular cells maligned spermatogenesis (Mohammed et al., 2004), which obviously affect the quality and quantity of sperm cells. Acccording to Ikpeme et al. (2010), the seed, leaf and pulp extracts of *C. papaya* have anti-fertility properties and might also enhance the production of sex hormones, especially the pulp extracts. Induced lethal mutation is reported to be responsible for dysfunction of the gamete, which might be lethal to fertilized egg or developing embryos. Odeigah (1997); Ikpeme et al. (2007); Ekaluo et al. (2009) reported that the frequency of induced lethal mutation is directly proportional to sperm count. Results on lethal mutation index showed that the reduction might be attributed to the effects on embryonic tissues leading to fetal death. This is corroborates with the report of Odeigah (1991).

This also might have led to the reduction in conception rate observed in the current study. It is probable that the induction of a dominant lethal mutation after exposure to the extracts through the male rats must have affected the germinal tissues of the female rats, with the resultant effects on the chromosomes, which could be structural and/or numerical anomalies (Ikpeme *et al*., 2007).

According to Odeigah (1991), induced lethal mutation occurs in the germ cells. This does not cause dysfunction of the gametes, rather lethal to the fertilized eggs or developing embryo, which indicate genotoxicity of the test substance. The low conception rates observed in the current study may not be unconnected with the lethal effect of the extracts on the females via the males. Though this treatment was not directly on the female rats, the effect would have been worse if treated directly on the female rats.

The effects of administering extracts to the males and observing their effects on reproductive performance of females is very elusive as the mechanism of impacts may not be clearly and properly linked and ascertained from the backdrop of extracts' effect on the males on which the extracts were administered (Ikpeme *et al*., 2007). However, it is possible that the observed effects in the litter sizes at birth might be due to the effects of the extracts on sperm count and some histological dysfunctions in male rats. This reduction in sperm count and sperm motility may have affected the number of viable sperm cells that are required for fertilization.

It was also reported that the litter weight at birth and at weaning were significantly affected in female rats mated with male rats administered with the leaf extract of *C. papaya*. This is a confirmation of the previous work of Ikpeme *et al*., (2007). The problem of attributing this effect to the extracts is with the assumption that the administration of the drugs was infused into the females through fertilization and that the extracts have the potency of causing inhibitory actions in the utilization of nutrients by the females.

This might have affected the weights. Attributing this effect to the extract administration will also lead to the understanding that bioactive compound laden sperm cells on achieving fertilization may have emptied their contents in the body of the female rats.

This might probably be the underlying reason for our results in the present study. The possibility of implies that intake of drugs by males might be harmful to reproductive performances of the female rats. Possibly, it might basically be due to factors which might not be very clear from the present study.

Conclusion

Implicitly though, our results suggest that indiscriminate consumption of herbals by male rats could have devastating effects on the female rats during reproduction. To make categorical assertion on this, it becomes necessary to carry out in-depth research similar to this works.

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Table 1: Effects of the seed, leaf and pulp extracts of *C. papaya* on dominant lethal mutation index (ILMI) in female rats

Group (mg/kg)	Extract treatment	Number of females	Implants per female	Average live embryo per female	Average dead embryo per female	DLMI
0	Control	3	9.67±0.33	9.67± 0.33	0	0.0
100		3	9.00± 0.58	7.00± 0.0	2.00± 0.58	0.28^{c}±0.001
200	seed	3	7.33±0.33	5.33±0.33	2.00±0.58	0.45^{f}±0.001
300		3	6.00±0.58	4.00±0.58	2.00±1.16	0.59^{j}±0.003
100		3	9.33±0.33	7.00±0.58	2.33± 0.33	$.28^{c}$±0.002
200	leaf	3	8.00±0.58	6.00± 0.58	2.00± 0.58	$.38^{d}$±0.001
300		3	6.67± 0.33	4.67± 0.33	2.00± 0.00	0.52^{i}±0.001
100		3	9.33 ± 0.33	8.00 ± 0.00	1.33± 0.33	0.18^{a}±0.002
200	Pulp	3	8.67±0.67	7.33 ± 0.88	1.33 ±0.33	0.24^{b}±0.001
300		3	7.00± 0.58	5.67 ± 0.88	1.33 ± 0.31	0.41^{e}±0.001

* Means followed with the same case letter on each vertical array indicate no significant differences (P > 0.05)

Table 2: Conception rates of female rats mated to male rats treated with seed, leaf and pulp extracts of *C. papaya*

Daily treatment (mg/kg BW)		Number of females mated	Successful mating	Conception Rate (%)
Control	0	6	6	$100.0^{c}\pm0.0$
	100	6	4	$83.8^{b}\pm0.12$
Seed	200	6	5	$66.7^{a}\pm0.23$
	300	6	4	$66.7^{a}\pm0.06$
	100	6	5	$83.8^{b}\pm0.00$
Leaf	200	6	5	$83.8^{b}\pm0.17$
	300	6	5	$83.8^{b}\pm0.12$
	100	6	6	$100.0^{c}\pm0.0$
Pulp	200	6	5	$83.8^{b}\pm0.06$
	300	6	5	$83.8^{b}\pm0.12$

* Means followed with the same case letter on each vertical array indicate no significant differences (P > 0.05)

Table 3: Means ± SE of litter sizes and weights at birth (g) of female rats mated with male rats treated with seed, leaf and pulp extracts of *C. papaya*.

Extract (Mg/kg)	Parity	Group I		Group II		Group III		Group IV	
		Litter size	Birth weight	Litter size	Birth weight	Litter size	Birth weight	Litter size	Birth weight
Seed	1	$6.70^{ef}\pm0.9$	$51.3^{f}\pm0.4$	$6.0^{de}\pm0.6$	$58.4^{f}\pm0.7$	$2.67^{bc}\pm0.8$	$31.5^{d}\pm0.9$	$1.00^{a}\pm0.1$	12.0=±0.3
	2	$9.33^{h}\pm1.2$	$70.2^{g}\pm0.9$	$6.67^{ef}\pm1.3$	$41.4^{e}\pm0.8$	$2.33^{b}\pm0.3$	$25.0^{c}\pm0.5$	$1.33^{b}\pm0.9$	$13.5^{b}\pm0.6$
	3	$9.69^{h}\pm0.9$	$74.0^{h}\pm0.1$	$8.33^{ef}\pm0.9$	$31.8^{cd}\pm0.6$	$4.0^{d}\pm0.8$	$24.8^{c}\pm0.7$	$1.33^{b}\pm0.2$	$18.0^{b}\pm0.4$
Leaf	1	$7.10^{ef}\pm0.9$	$87.0^{i}\pm2.3$	$8.0^{fg}\pm0.6$	$73.0^{gh}\pm1.7$	$6.0^{de}\pm0.2$	$66.5^{g}\pm1.22$	$1.0^{a}\pm0.01$	$22.5^{bc}\pm0.86$
	2	$8.00^{fg}\pm1.0$	$87.5^{i}\pm1.5$	$6.1^{de}\pm0.7$	$73.0^{gh}\pm2.4$	$5.0^{d}\pm0.5$	$71.5^{g}\pm2.31$	$1.0^{a}\pm0.01$	$20.1^{b}\pm1.0$
	3	$9.00^{g}\pm0.8$	$87.5^{i}\pm0.95$	$7.2^{ef}\pm0.32$	$73.0^{gh}\pm3.1$	$4.0^{d}\pm0.7$	$72.0^{g}\pm0.85$	2.5±0.4	$30.6^{cd}\pm1.2$
Pulp	1	$8.0^{fg}\pm0.23$	$89.0^{i}\pm1.5$	$8.0^{fg}\pm0.5$	$70.3^{g}\pm0.2$	$5.0^{d}\pm0.4$	$50.8^{f}\pm1.2$	$1.0^{a}\pm0.01$	$24.1^{c}\pm0.7$
	2	$9.0^{g}\pm0.75$	$78.4^{h}\pm2.1$	$7.0^{ef}\pm0.2$	$71.5^{g}\pm0.7$	$4.0^{d}\pm0.2$	$63.5^{g}\pm0.92$	$0.0^{a}\pm0.0$	$0.0^{a}\pm0.0$
	3	$10.0^{h}\pm0.8$	$80.2^{h}\pm.94$	$7.0^{ef}\pm0.41$	$68.8^{g}\pm0.8$	$5.0^{d}\pm0.6$	$65.2^{g}\pm0.58$	$2.0^{b}\pm0.03$	$28.4^{c}\pm0.8$

*Means followed with the same superscript along each array (horizontal/vertical) per trait indicate no significant difference (P > 0.05).

Table 4: Means ± SE of litter sizes and weights at weaning (g) of female rats mated with male rats treated with seed, leaf and pulp extracts of *C. papaya*

Extract (mg/kg)	Parity	Group I		Group II		Group III		Group IV	
		Litter size	Weaning weight	Litter size	Weaning weight	Litter size	Weaning weight	Litter size	Weaning weight
Seed	1	$9.33^{g}\pm1.2$	$226.26^{fg}\pm0.9$	$8.33^{ef}\pm0.9$	$186.32^{e}\pm0.1$	$4.0^{d}\pm0.8$	$115.1^{cd}\pm0.8$	$1.0^{a}\pm0.1$	$63.72^{b}\pm0.8$
	2	$9.69^{g}\pm0.9$	$251.9^{h}\pm0.1$	$6.67^{e}\pm1.3$	$135.18^{d}\pm0.9$	$2.33^{b}\pm0.3$	$87.2b^{c}\pm0.62$	$1.33^{ab}\pm0.9$	$80.04^{b}\pm0.7$
	3	$6.7^{e}\pm0.9$	$220.1^{f}\pm0.8$	$6.0^{e}\pm0.6$	$134.58^{d}\pm0.9$	$2.67^{bc}\pm0.8$	$108.35^{c}\pm0.7$	$1.33^{ab}\pm0.2$	$66.69^{b}\pm0.8$
Leaf	1	$9.0^{g}\pm0.8$	$342.5^{k}\pm1.2$	$8.0^{f}\pm0.6$	$277.5^{hi}\pm0.82$	$6.0^{e}\pm0.2$	$227.0^{g}\pm2.3$	$1.0^{a}\pm0.01$	$89.6^{c}\pm1.6$
	2	$8.0^{f}\pm1.0$	$362.3^{k}\pm0.95$	$6.1^{e}\pm0.7$	$250.5^{h}\pm1.5$	$5.0^{d}\pm0.5$	$197.0^{ef}\pm0.54$	$1.0^{a}\pm0.01$	$85.2^{b}\pm0.7$
	3	$7.1^{e}\pm0.9$	$313.5^{j}\pm0.78$	$7.2^{e}\pm0.32$	$258.0^{h}\pm0.73$	$4.0^{d}\pm0.7$	$212.5^{f}\pm2.0$	$2.5^{b}\pm0.4$	$90.2^{c}\pm0.75$
Pulp	1	$10.0^{g}\pm0.8$	$306.8^{j}\pm1.3$	$8.0^{f}\pm0.5$	$257.1^{h}\pm1.0$	$5.0^{d}\pm0.4$	$187.2^{e}\pm0.85$	$1.0^{a}\pm0.01$	$89.1^{c}\pm0.7$
	2	$8.0^{f}\pm0.23$	$300.0^{i}\pm.94$	$7.0^{e}\pm0.2$	$220.3^{f}\pm0.84$	$4.0^{d}\pm0.2$	$180.9^{e}\pm2.1$	$0.0^{a}\pm0.0$	$0.0^{a}\pm0.0$
	3	$9.0^{g}\pm0.75$	$289.4^{i}\pm1.6$	$7.0^{e}\pm0.41$	$210.7^{f}\pm0.83$	$5.0^{d}\pm0.6$	$173.4^{e}\pm0.79$	$2.0^{b}\pm0.03$	$103.4^{c}\pm0.8$