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Postcoital anticonceptive activities of the Aqueous Methanolic Extract of unripe *Carica papaya* fruits in rats

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Abstract: *This study investigated the postcoital anticonceptive activities of the aqueous methanolic extract of unripe Carica papaya (AMEUCP) fruits in rats. The unripe papaya fruits were extracted by cold marceration. The anticonceptive activity of AMEUCP was studied using mature albino rats (15 females and 8 males). Successfully mated rats were separated and classified into three (3) groups (n=5). Group A (control) received 0.5 ml of distilled water while groups B and C received AMEUCP at 450 and 900 mg/kg respectively from the 1st day to the 7thday of pregnancy. They were sacrificed on their 10th day of pregnancy and the viable and non-viable fetuses, adsorbing sites and corpora lutea were counted. Data obtained from the study were subjected to one-way analysis of variance (ANOVA). Variant means were separated using the Duncan's multiple range test and significance was accepted at $p < 0.05$. The result showed that the extract possess a dose- dependent anticonceptive activity. The number of resorbed embryo in the AMEUCP treated groups were significantly ($p < 0.05$) higher than that of the control while the mean live foetal number (LFN) in the AMEUCP treated groups were significantly lower ($p < 0.05$) than that of the control. In conclusion, the findings from this study lends some credence to the folkloric claim that unripe papaya possesses antifertility properties and may therefore be considered as a potential source of contraceptives with very minimal or no side effects.*

Key words: *Adsorbing sites, Contraceptives, Corpora lutea, Postcoital, Unripe Carica papaya*

I. Introduction

The world population is presently estimated to be about 7 billion and recent estimate has projected the world population to be about 9 billion by the year 2045 [1]. In response to this rapid increment, fertility control has come to the forefront as a topic of global concern with important medical, social and political considerations. Quite a number of contraceptive methods are now available to combat the unchecked rapid growth of the population. However, most of these are not without their peculiarities and associated side effects necessitating the search for biologically active botanical substances or fertility-regulating agents of plant origin which are ecofriendly in approach and interfere with the natural patterns of reproduction [2, 3]. The anti fertility effects of some plants extacts on rodents have been reported [4-7].

Carica papaya, a tree of the *caricaecia* family is one of the indigenous plants which have been attributed with lots of medicinal value. Especially useful are the leaves, the seeds and the latex from the unripe fruits where most of these medicinal qualities are said to reside. Unripe (green) papaya is used as an antihypertensive [8], anti ulcer [9] and is used in south west Nigeria for management of sickle cell anaemia [10]. It may also be incorporated into livestock feed as dietary supplement [11] while in Indian traditional medicine, green papaya is used as an emmenagogue, contraceptive and an abortifacient [12]. The present investigations were undertaken to study the post coital contraceptive effects of aqueous methanolic extract of unripe carica papaya (AMEUCP) as most of the reported effects are based on folklore rather than scientific facts.

II. Materials and methods

2.1 animals

Adult male and female Wistar albino rats were obtained from the Animal House Unit of the Department of Pathology and Microbiology, Faculty of Veterinary Medicine, University of Nigeria, Nsukka. They were kept inside rat cages in a well ventilated house with a temperature of about 27 – 30^{o}C and 12 hour natural light and 12 hour darkness. The animals were provided commercial feed (Grand Cereals and Oil Mills Ltd, Bukuru, Jos, Nigeria) and clean water ad libitum. Protocol for this experiment was in accordance with the guidelines on the care and well being of research animals [13]. They were also allowed to acclimatize for two weeks before the commencement of the experiment.

2.2. Collection of plant material

Fresh sample of mature unripe pawpaw fruits were obtained from Nsukka urban region and authenticated by Mr A. Ozioko, a taxonomist at the International Centre for Ethnomedicine and Drug Development (INTERCEDD), Nsukka. The unripe fruits were cut open and the seeds inside discarded. The

fruits were then sliced into thin sheets and shade-dried after which they were ground into coarse powder using mortar and pestle and then pulverized into a fine powder using a dry grinder. The powder was stored in an air tight container at 23-25°C until required.

2.3 Preparation of Extracts and Determination of Plant Yield

About five hundred (500) grams of the dried sample were subjected to cold maceration with 80% methanol for 48 hours, stirring at intervals. The extract was concentrated to dryness in a hot air oven at a reduced temperature of 40^{o}C and the weight was obtained using a Mettler balance (H_3O Switzerland). The percentage yield of the extracts was calculated using the formular: %Yield (w/w) = (a/b) 100 where a = weight of recovered extract and b = weight of plant material used. The extract was then stored at 4^{o}C throughout the period of the study.

2.4 Acute Toxicity Studies

The up and down method [14] was used for the acute toxicity study of unripe *Carica papaya.* Briefly, 12 mice were used for the experiment and were divided into six groups (n=2). Two mice in each group were orally dosed with 300,750, 1500, 3000, 3500 and 5000mg/kg of the extract. They were allowed access to feed and water ad libitum and were observed for mortality and signs of toxicity for 48hrs.

2.5 Evaluation of the anticonceptive effects of AMEUCP

Mature Wister rats (15 females and 8 males) were used for this study. The males were introduced into the female cages during the proestrus phase of the cycle at the ratio of one male to two females. Mating was confirmed by presence of vaginal plug and or positive sperm in vaginal smear [15] and the day of confirmation of mating was designated as day 1 of pregnancy. Successfully mated rats with copulatory plug and or positive vaginal smear were separated and classified into three (3) groups (n=5). Group A (control) received 1ml of distilled water while groups B and C received AMEUCP at 450 and 900 mg/kg respectively starting from day 1 to day 7 of pregnancy. On the 10th day of pregnancy, all the rats were sacrificed and the uterus and ovary units were removed from the animals. The following parameters were evaluated according to Wong [16].

a) Percentage of pregnant female per group (PPF)
b) Mean live foetal number (LFN)
c) Mean corpus luteum number (CLN)
d) Mean resorbed embryo number per pregnant female (REN)

2.6 Statistical analysis

Data obtained from the study were subjected to one-way analysis of variance (ANOVA). Variant means were separated using the Duncan's multiple range test and significance was accepted at $p < 0.05$.

III. Results

3.1 Extraction and acute toxicity test

Following extraction by cold maceration and concentrated to dryness, a dark brown (honey coloured) sticky paste weighing 47g (15.6% yield) was recovered. The extract was well tolerated even at the highest dose of 5000mg/kg as there were neither signs of toxicity (respiratory distress, salivation, weight loss, dull eyes, diarrhea, epistasis, change in the appearance of fur) nor mortality in any of the animal groups for up to 48 hours following its administration.

3.2 Anticonceptive effects of the extract

The result showed that the extract possess anticonceptive activity which was observed to be dose dependent. When autopsied at the 10th day of pregnancy it was observed that the crude extract at doses of 450 mg/kg and 900 mg/kg respectively elicited conception failure in two out of the five (40%) and three out of five (60%) animals administered the fruit extract for seven consecutive days after mating (Table 1). Evidence of foetal resorption was also observed as judged by the mean areas of foetal attachment vis-à-vis the number of live fetuses (Fig 1).

Conversely, all the five animals (100%) in the control group were found with foetuses attached to the endometrium. The body weight of the animals increased progressively for the first five days but began to decrease in animals treated with the extract at about the sixth day (Fig 2). There was however no significant difference in body weight changes among the groups. The mean live foetal number (LFN) in the AMEUCP

treated groups (450 mg/kg and 900 mg/kg) was significantly lower ($p < 0.05$) than that of the control. More so, the number of resorbed embryo in the treated groups (450 mg/kg and 900 mg/kg) was significantly ($p < 0.05$) higher than the number of resorbed embryo number of the control. There was however, no significant difference ($p > 0.05$) in the number of corpora lutea among the groups (Table 2).

Table 1: Anticonceptive Effects of AMEUCP

Group	Number Pregnant / Number Treated	% Conception	% Anticonception
Control	5/5	100	0
450 mg/kg	3/5	60	40
900mg/kg	2/5	40	60

Table 2: Effects of AMEUCP on some reproductive parameters

Groups	BW (g)	LFN	CLN	REN
Control	220.00 ± 19.77	7.80 ± 0.58 [b]	8.4 ± 0.93	1.50 ± 0.97[b]
450 mg/kg	178.38 ± 14.67	5.00 ± 1.51 [a]	9.0 ± 1.00	5.00 ± 1.10[a]
900mg/kg	192.12 ± 05.44	3.60 ± 1.50 [a]	10.4 ± 0.87	6.80 ± 0.37[a]

***a b different superscripts in a column represents significant differences ($p < 0.05$) between groups**

Keys

BW = Body weight in grams
LFN = Live foetal number
CLN = Corpora lutea number
REN = Resorbed embryo number

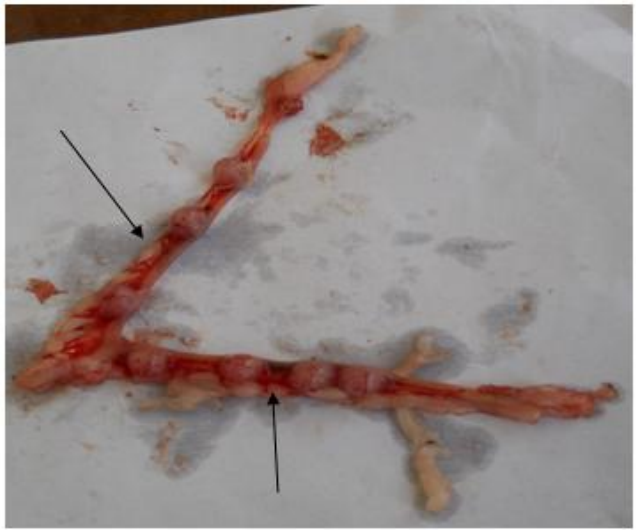

Figure 1: Left and right uterine horns of a pregnant (day 10) rat from the extract treated group. Arrows are showing points of embryo resorption.

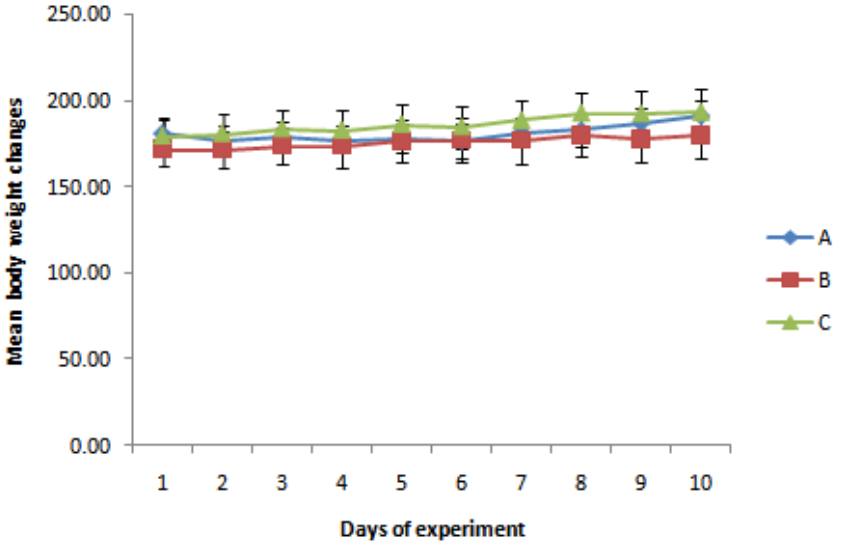

Figure 2: Mean body weight changes in the control and AMEUCP treated groups

IV. Discussion

The absence of respiratory distress, salivation, weight loss, dull eyes, diarrhea, epistasis, change in the appearance of fur, as well as mortality in the extract-treated animals suggests that the extract was not clinically toxic to the animals [17]. The extract was therefore adjudged to possess a wide safety margin (not acutely toxic) according to the WHO acute hazard ranking [14]. This finding is in line with that of Ezike [9] who also reported no mortality in mice following administration of methanoic extract of unripe *Carica papaya* fruit up to 5000mg/kg per os. This study revealed a dose dependent anticonceptive effect of AMEUCP as the administration of the extract at the dose of 450 mg/kg and 900 mg/kg, respectively, elicited conception failure in two out of the five (40%) and three out of five (60%) animals as against 100% conception rate in the control. Similar studies on rats have shown that up to 95% pregnancy rate could be expected when well fed rats exhibiting a fairly regular oestrus cycle are bred with a male of proven fertility [7, 18].

Failure of some of the animals to conceive could be attributed to a direct effect of the extract on uterine cum oviductal muscle function which may have caused accelerated transport of the blastocyst at this period. Phytochemical analysis of unripe extact of *Carica papaya* revealed carbohydrates, terpenoids, glycosides, steroids, flavnoids, saponins and proteins [9]. Some of these may have acted synergistically with substances like prostaglandins ($PGF_{2á}$) and other agonists which modulate oviductal muscle contraction to influence the transport of pre implantation embryos through the ampulla and isthmus of the oviduct resulting in early arrival of the embryo to a non-receptive uterus and failure of the blastocysts to implant [19, 20]. In addition, the extract may have caused modification of the uterine lining function or maternal toxicity which may increase both early resorption and late fetal death [21, 22]. Therefore, a synchronized development of the conceptus and differentiation of the uterus to a receptive state (defined as a hormonally regulated period at which the uterine milieu is conducive for embryo acceptance and implantation) are considered essential to the implantation process. This however is in contrast to the findings of Raji [23] who reported 100% conception rates in both the control and treated rats when administered with aqueous extract of *Allium sativum* (Garlic). Similarly, Dileep and Oommen, [24] did not also observe alteration in the conception rates of Swiss albino mice treated with aqueous extract of Steria rebqudiana bertani

At autopsy, evidence of foetal resorption was seen in the pregnant animals while most of the non-pregnant animals were on the estrus phase of the oestrous cycle. It could therefore be said that the AMEUCP elicited contraception by preventing implantation and may have also caused the death of the already implanted embryo. This may also be attributed to some phytoconstituents of the unripe *Carica papaya* some of which have been incriminated to cause impaired fertility [9, 25, 26]. They may also interfere with normal process of implantations and proper development of the conceptus [27] by causing hormonal imbalances in the systems of the subject concerned.

The non-pregnant rats being in the estrus phase of the oestrus cycle may have resulted from the sudden withdrawal of the extract after administration for seven consecutive days. A similar effect is often observed when standard agents of oestrus synchronization are administered [28]. There is however a need for further research on this.

V. Conclusion

These findings lend some credence to the folkloric claim that unripe papaya possesses antifertility properties. Therefore, consumption of unripe fruits and its products by man and animals should be done with caution. Further research is however ongoing to isolate and identify the active principle(s) associated with the crude fruit extract of this plant and also investigate its interaction with or involvement of the reproductive hormones. Such knowledge will be very useful in the development of new cost effective contraceptives with very minimal side effects.

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