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Evaluation of the Abortifacient Properties of Chloroform Extract of *Carica papaya* L. Seed in Female Albino Rats

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Abstract: This study was carried out to investigate the impact of oral administration of chloroform extract of *Carica papaya* seed (CPE) on implantation and pregnancy in female albino rats. The study was divided into three experimental sections. Each section was subdivided into 4 groups treated, respectively with 25, 50 and 100 mg kg^{-1} b.w CPE and 2.5% tween 80 in normal saline (vehicle for CPE; control). Rats in section 1 were treated with CPE for two weeks before mating (pre-coital). Rats in section 2 were administered CPE from day 1 of pregnancy till term (post coital) while rats in section 3 received the extract for two weeks before mating and thereafter throughout term (pre and post-coital). Implantation sites and resorptions were determined in some of the pregnant rats after laparotomy. The gestation period, litter size and fetal weight were recorded in the remaining rats. The litters were also observed for any morphological alterations. The extract treated rats had significant decreases in litter size and implantation count ($p<0.01$). The percentage resorptions increased in a dose dependent manner while the fetal weight and morphology remain unchanged when compared with the normal untreated control groups. The percentage resorptions were high in CPE treated rats when compared with the control group. None of the 100 mg kg^{-1} b.w CPE treated female rats had litters. The results suggest that the chloroform extract of *Carica papaya* seed has anti-implantation and abortifacient properties in female albino rats.

Key words: *Carica papaya* seed, implantation, pregnancy, abortion

INTRODUCTION

Evidence from several studies suggests that *Carica papaya* fruits (epicarp, endocarp, seeds and leaves) have antifertility properties. The safety of *Carica papaya* endocarp consumption with reference to its common avoidance during pregnancy has been evaluated[1]. According to this study the effect of ripe papaya juice on pregnant and non-pregnant rats uteri showed a significant difference in the number of implantation sites and viable fetuses in the rats that received ripe papaya juice (0.1-0.8 mL) relative to the control. No sign of fetal or maternal toxicity was observed and there was no significant contractile activity of uterine smooth muscles isolated from pregnant and non-pregnant rats treated with the juice. Conversely, crude papaya latex (0.1-3.2 mg mL^{-1}) induced spasmodic contraction of the uterine muscles similar to oxytocin (1 mU mL^{-1} and prostaglandin $F_2\alpha$ (0.028-1.81 μM). In the pregnant uterus (18-19 days), the contractile effect of crude papaya latex was characterized by tetanic spasm. The results of the study suggest that ripe papaya may not pose any danger to pregnancy. However, the unripe or semi-ripe papaya, which contains high concentration of the latex that produced significant uterine contractions, could be unsafe in pregnancy[1]. Studies have also shown that low dose (100 mg kg^{-1} b.w) of the extract did not adversely affect prenatal development while there was an abortifacient effect at high dose (800 mg kg^{-1} b.w)[2].

The toxicity of *Carica papaya* seed products on male reproduction has been investigated in rats and rabbits. Oral administration of aqueous and chloroform extract in rats was found to be neither toxic nor caused impairment to libido[3]. Except the study by Adebiyi *et al.*[1] which reported only on the third trimester of pregnancy using aqueous extract of *Carica papaya* seed and that of Oderinde *et al.*[2] on aqueous extract of *Carica papaya* seed, the impact of this extract on conception and

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pregnancy has not been investigated in detail. The present study was undertaken to investigate the impact of chloroform extract of *Carica papaya* seeds on implantation and pregnancy in female rats during the three phases of pregnancy.

MATERIALS AND METHODS

Animal: This study was conducted between January and September 2004. The study was carried out on young adult female rats of Wistar strain weighing between 130-190 g initially. The animals were kept in the animal house, Physiology department, University of Ibadan, Nigeria with standard environmental conditions of 12 h light and 12 h dark cycles, 24-25°C. The animals were fed with pelleted standard rat diet (Ladokun Feeds Nig. Ltd., Ibadan) and water *ad libitum*. They were acclimated to laboratory conditions for two weeks before the commencement of the study. Oestrous cycles of the rats were established by vaginal smears and those with three regular consecutive oestrous cycles were selected for the study.

Plant material and preparation of extract: The seeds of *Carica papaya* L. (Caricaceae) from ripe fruits were collected from the University of Ibadan Campus. The plant material was identified and authenticated by the Forestry Research Institute of Nigeria where a voucher specimen with herbarium number F.H.I 106879 was deposited. The air-dried and powdered seeds of *Carica papaya* were extracted with chloroform using Soxhlet extractor at 58°C for 12×3 h. The filtrate obtained was concentrated under reduced pressure and yielded a dark brown semi-solid mass (w/w, 6%) that was used in this experiment.

Experimental design: The study was divided into three experimental sections as follows:

Section 1-Pre-coital PCE administration: Twenty four rats were divided into four groups of 6 rats each and treated daily with 2.5% tween 80 (control), 25, 50 and 100 mg kg^{-1} b.w of CPE, respectively. Administration of the extract was done orally for two weeks before mating by isolated mating technique. Treatment was discontinued as from first day of pregnancy.

Section 2-Post-coital CPE administration: Twenty four rats were divided into four groups of 6 rats each and treated daily with 2.5% tween 80 (control), 25, 50 and 100 mg kg^{-1} b.w of CPE, respectively. Extract administration was done orally as from first day of pregnancy and was continued throughout the gestation period.

Section 3-Pre-and Post-coital CPE administration: Twenty four rats were divided into four groups of 6 rats each and treated daily with 2.5% tween 80 (control), 25, 50 and 100 mg kg^{-1} b.w CPE, respectively. Administration of the extract was done orally for two weeks before mating and thereafter throughout the gestation period.

Assessment of implantation and pregnancy: Rats in various treatment and control groups were introduced to male rats of proven fertility in the ratio of 3: 1. Successful mating in each case was confirmed by the presence of spermatozoa in the vaginal smear and/or presence of vagina plug. The day of appearance of the plug was taken as first day of pregnancy. Three rats in each of the four groups were randomly selected on day 7 of pregnancy and given intravenous injection of 0.2 5% Evans dye blue via the tail vein. About 15 min later, the rats were killed by an over dose of urethane and the uteri opened. The assessment of implantation sites was carried out by counting the number of uterine dye sites in each uterus as described elsewhere[4]. The remaining three rats in each group were allowed to complete the term. The number of litters delivered, their morphology and gestation period were recorded.

Statistical analysis: Values were recorded as X±SEM. Paired and unpaired Student t-test was used in the analysis of the results. Confidence interval of 95% was taken as the least statistically significant level.

RESULTS

Effect of CPE treatment before pregnancy (pre-coital) on implantation sites and pregnancy in rats: The mean implantation sites of CPE treated rats showed a significant dose dependent decrease ($p<0.01$) when compared with the control (Table 1). The percentage viable foetuses in 25 mg kg^{-1} b.w CPE treated rats was 98.3% while that of the 50 mg kg^{-1} b.w CPE treated rats was 97.1%. The 100 mg kg^{-1} b.w CPE treated rats had zero percent viable foetuses. The control rats had 100% viable fetuses and zero percent resorptions. The 25 mg kg^{-1} b.w CPE treated rats had a 17.7% and 50 mg kg^{-1} b.w CPE treated rats had 2.9% resorptions (Table 1). The mean gestation period of all CPE treated rats was not significantly different from that of the control ($p<0.05$). The mean litter size of the 25 mg kg^{-1} b.w CPE treated rats showed an insignificant decrease when compared with the control. The 50 and 100 mg kg^{-1} b.w of CPE doses, respectively exhibited a statistically significant decrease ($p<0.01$) in the litter size when compared with the control (Table 1). The mean fetal weight in the 25, 50 and 100 mg kg^{-1} b.w CPE treated rats was not significantly different from that of the control (Table 1).

Table 1: Effects of CPE treatment before pregnancy (pre-coital)

	Control	25 (mg kg^{-1} b.w)	50 (mg kg^{-1} b.w)	100 (mg kg^{-1} b.w)
Gestation period (days)	20.33±0.49	20.67±0.42	20.67±0.49	20.50±0.50
Litter size (g)	8.50±0.43	6.83±0.60	2.17±0.98*	0.33±0.21*
Fetal size (g)	5.52±0.06	5.43±0.08	5.51±0.17	5.27±0.12
Implantation site	8.33±0.33	5.67±0.33*	5.00±0.58*	0.98±0.41*
% Viable fetuses	100.00	98.40	97.10	0.00
% Fetal resorptions	0.00	17.70	2.90	0.00

*p<0.01

Table 2: Effects of CPE treatment from first day of pregnancy (post-coital)

	Control	25 (mg kg^{-1} b.w)	50 (mg kg^{-1} b.w)	100 (mg kg^{-1} b.w)
Gestation period (days)	20.67±0.21	20.337±0.21	20.50±0.50	0
Litter size (g)	9.57±0.43	3.67±0.84*	1.17±0.75*	0
Fetal size (g)	5.65±0.11	3.97±0.09*	3.58±0.13*	0
Implantation site	8.00±0.58	7.67±0.33	3.69±0.67	1.52±0.42
% Viable fetuses	100.00	30.40	8.70	0.00
% Fetal resorptions	0.00	69.60	91.30	100.00

*p<0.01

Table 3: Effects of pre and post coital CPE treatment through out gestation period

	Control	25 (mg kg^{-1} b.w)	50 (mg kg^{-1} b.w)	100 (mg kg^{-1} b.w)
Gestation period (days)	20.83±0.17	20.75±0.25	21.00±0.00	0
Litter size (g)	9.17±0.48	2.17±0.70*	0.33±0.33*	0
Fetal size (g)	5.47±0.14	3.61±0.19*	3.23±0.52*	0
Implantation size	9.67±0.33	5.00±0.58*	3.00±0.58*	0
% Viable fetuses	100.00	40.00	33.30	0
%Fetal resorptions	0.00	60.00	66.70	100

*p<0.01

Effect of CPE treatment from first day of pregnancy (post-coital) on implantation sites and pregnancy in rats: The mean gestation periods in all CPE treated rats were not significantly different from those for the control. The mean litter size of CPE treated rats was significantly reduced ($p<0.01$) in a dose dependent manner when compared with the control. The 100 mg kg^{-1} b.w CPE treated rats had no litters (Table 2). The mean fetal weight of the 25 mg kg^{-1} b.w CPE was significantly reduced ($p<0.01$) when compared with the control. There was also a significant decrease ($p<0.01$) in the mean fetal weight of the 50 mg kg^{-1} b.w CPE treated rats. The 100 mg kg^{-1} b.w CPE treated rats had no fetal weight (Table 2). There was no change in mean implantation site in the 25 mg kg^{-1} b.w CPE treated rats when compared with the control. However the 50 and 100 mg kg^{-1} b.w CPE treated rats showed a significant decrease ($p<0.01$) in implantation sites when compared with control. The 100 mg kg^{-1} b.w CPE also showed a significant decrease ($p<0.01$) in the mean implantation sites when compared with the control (Table 2). The control rats had 100% viable fetuses with zero percent resorption. The 25 mg kg^{-1} b.w CPE treated rats had 30.4% viable fetuses and 69.6% resorption. The 50 mg kg^{-1} b.w CPE treated rats had 8.7% viable fetuses and 91.3% resorptions. However, the 100 mg kg^{-1} b.w CPE treated rats had zero percent viable foetuses and 100% resorptions (Table 2).

Effect of CPE treatment before pregnancy and throughout gestation period (pre and post-coital) on implantation sites and pregnancy in rats: The mean gestation period in all the treated rats except those on 100 mg kg^{-1} b.w CPE was unchanged compared with the control. The 100 mg kg^{-1} b.w CPE treated rats had no litter. The mean litter sizes of the 25 and 50 mg kg^{-1} b.w CPE treated rats were significantly reduced ($p<0.01$) in a dose dependent manner when compared with the control (Table 3). The mean implantation sites of the 25 and 50 mg kg^{-1} b.w CPE treated rats were significantly reduced ($p<0.01$) when compared with the control. The 100 mg kg^{-1} b.w CPE treated rats had zero implantation sites. The percentage viable foetuses were 100% in the control rats with zero percent resorptions. The 25 mg kg^{-1} b.w CPE treated rats had 40 and 60% viable fetuses and resorptions, respectively. The 50 mg kg^{-1} b.w CPE treated rats had 33.30 and 66.70% viable fetuses resorptions, respectively. The percentage viable fetuses and resorptions were 0 and 100%, respectively, in the 100 mg kg^{-1} b.w CPE treated rats (Table 3).

DISCUSSION

The results obtained from this study showed that chloroform extract of *Carica papaya* seed possess anti-implantation and abortifacient properties in female

rats. All rats that received the extract irrespective of period and duration had a significantly reduced implantation site in a dose-dependent manner. This finding is similar to that by Oderinde *et al.*[2] where aqueous extract of *Carica papaya* seeds showed a dose dependent decrease in the implantation site in rats. However, while Oderinde *et al.*[2] employed oral doses of 100 and 800 mg kg^{-1} b.w of aqueous extract of *Carica papaya* seeds administered once daily on days 1-10 post-coitus, in this study three doses were used (25, 50 and 100 mg kg^{-1} b.w) of chloroform extract of *Carica papaya* seeds before pregnancy alone, from first day of pregnancy and before and during pregnancy till term.

The fetal weights were not adversely affected when the extract was administered to the female rats before pregnancy indicating probable non-toxicity of the chloroform extract of *Carica papaya* seed on fetal development if administered before conception. However, the extract produced deleterious effect on fetal weight of rats when administered during pregnancy (post-coital). This finding is consistent with earlier report that aqueous extract of *Carica papaya* seeds at a dose of 800 mg kg^{-1} b.w administered during pregnancy significantly decreased fetal weight in rats[2]. CPE treatment during pregnancy produced a greater decrease in the litter size and implantation site, which is suggestive of the extract adverse effects on post-implantation fetal development. This assumption corroborates the finding of Cherian[5] where maximum contractile activity of *Carica papaya* latex on the uterus at later stages of pregnancy was reported. These effects could have led to a decrease in percentage viable foetuses and an increase in percentage resorptions observed in the present study.

Phytochemical analysis revealed that *Carica papaya* seed extract contained mainly papain, a sulfhydryl protease and chymopapain, which have lysozomal action[6,7]. Smith *et al.* [8] proposed that papain broke down the inter-cellular matrix of cartilage while Arnon and Shapira[9] reported that it degraded protein substrates more extensively than the pancreatic protease. In essence, the increased resorptions in the present study could be correlated with the papain and chymopapain components in *Carica papaya* seeds. Moreover, it has been documented that crude *Carica papaya* latex contain a uterotonic principle which might be a combination of enzymes, alkaloids and other substances that evoke sustained contraction of the uterus[5]. The increased tonicity of the uterus could possibly increase the percentage resorption in the extract treated rats. However, the exact principles that caused the inhibition of implantation, infertility and induced abortion are not known. Characterization of the extract with biological activity is expected to unravel the principle with these activities.

In conclusion, it is suggested that chloroform extract of *Carica papaya* seed possesses anti-implantation and abortifacient properties and therefore may be unsafe during pregnancy.

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