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Research Article

EVALUATION OF ANTIFERTILITY AND TERATOGENIC EFFECTS OF CHROMATOGRAPHIC FRACTIONS OF *PORTULACA OLERACEA* IN MALE AND FEMALE ALBINO RATS

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ABSTRACT

Objective: This study was designed to evaluate the antifertility and teratogenic effect of chromatographic fractions (fractions 1 to 5) of *Portulaca oleracea* at a dose of 3 mg/kg BW in male and female albino rats.

Methods: For male fertility study, 50 days fractions and distilled water (control) treated male rats were cohabitated with untreated female rats. For female fertility and teratogenic study, the fractions were tested at different phases of pregnancy to evaluate its antifertility and teratogenic effects. Data were analysed using ANOVA at $p<0.05$.

Results: Cohabitation of 50 days fraction 1 to fraction 5 treated male rats and untreated female rats for four weeks produced no positive mating, while the cohabitation of the control group male rat with untreated female rats led to positive mating. Treatment of rats from day 1 to 5 of gestation (early pregnancy) with fraction 1 to fraction 5 caused non-significant ($p>0.05$) changes in the number of implantation sites relative to the control. Treatment of rats from day 6 to 15 of gestation (mid-pregnancy) with fraction 1 to fraction 5 caused no significant ($p>0.05$) changes in fetal size relative to the control as well as absence of gross malformations and resorption sites in all the treated and control rats. Treatment of rats from day 16 to 20 of gestation (late pregnancy) with fraction 1 to fraction 5 caused no significant ($p>0.05$) changes in litter size and litter weights relative to their respective controls as well as absence of gross malformations and resorption sites in all the treated and control rats.

These findings probably indicate that the chromatographic fractions of *Portulaca oleracea* have antifertility effects in male albino rats but have no teratogenic and deleterious effects on the fertility of female albino rats.

Keywords: *Portulaca oleracea,* Chromatographic fraction, Implantation sites, Litter size, Albino rats.

INTRODUCTION

Portulaca oleracea belongs to the family of *Portulacaceae*. It is commonly called Purslane in English language, 'Babbajibji' in Hausa language and 'Esan omode' or 'Papasan' in Yoruba language. It is a fleshy annual herb, much-branched and attaining 30 cm long [1].

It is used medicinally in Ghana for heart-palpitations [2]. The plant is used as a diuretic in Nigeria [3]. A tisane of the plant is drunk in Trinidad as a vermifuge [4].

At some areas near Benin City (Nigeria), the plant, along with other ingredients is taken as an aid to the development of the foetus [5].

It has been reported that aqueous and methanolic extracts of *Portulaca oleracea* have contractile effects on isolated intestinal smooth muscle in *in-vitro* preparations [6].

It has also been reported that aqueous and methanolic extracts of *Portulaca oleracea* have some toxic and beneficial potentials on the blood chemistry of albino rats [7].

The extracts of *Portulaca oleracea* have been reported to have protective effects on hypoxic nerve tissue [8], anti-inflammatory effects (Xiang *et al,* 2005) and wound-healing activity [10]. [11] also reported the skeletal muscle relaxant effect of the plant.

This study aims at investigating the antifertility and teratogenic activities of chromatographic fractions of *Portulaca oleracea* in male and female albino rats.

MATERIALS AND METHODS

Experimental Animals

Adult male and female albino rats weighing between 160 g and 180 g bred in the Animal House of Physiology Department, LAUTECH, Ogbomoso were used. They were housed under standard laboratory conditions with a 12 hours daylight cycle and had free access to feed and water; they were acclimatized to laboratory conditions for two weeks before the commencement of the experiments. All experiments were carried out in compliance with the recommendations of Helsinki's declaration on guiding principles on care and use of animals.

Plant Material

Fresh specimens of *Portulaca oleraceae* were collected from the Botanical Garden of the Forestry Research Institute of Nigeria, Jericho, Ibadan, and was authenticated in the above named institute where a voucher specimen (No FHI 108334) was deposited.

Extraction and Fractionation of *Portulaca oleracea*

About 3.2 kg of air-dried specimen of *Portulaca oleracea* was cold-extracted in methanol for 72 hours. The mixture was filtered using a wire-guaze and a sieve with tiny pores (0.25 mm) and concentrated at room temperature by exposing the extract for six days. The resulting solution was then placed in the oven at a reduced temperature (50 ^{0}C).

The methanolic extract was then preabsorbed with silical gel and placed in the oven at a reduced temperature (50 ^{0}C) overnight and then subjected to open column chromatography on silical gel (F_{254}, 50-200 mesh, E. Merck) for fractionation. The solvents (mobile phases) were hexane (non-polar), ethylacetate (partially polar) and methanol (polar). The gradients of the mobile phases involved hexane with an increasing percentage of ethylacetate (hexane/ethylacetate mixture) and then ethylacetate with an increasing percentage of methanol (ethylacetate/methanol mixture) as shown in table 1.

Thin Layer Chromatography (TLC)

The 21 fractions were spotted on precoated plates of silica gel GF_{254} (20 x 20, 0.5 mm thick; E. Merck) using capillary tubes. The spotted TLC plates were developed in a tank that contained a mixture of ethylacetate/methanol (9:1) as the mobile phases.

The TLC plates were then examined under the ultraviolet (UV) light at a wavelength of 365 nm and the well-defined spots of the components were then revealed by the UV light. Fractions with

similar relative fronts or retention or retardation factors (R_f value) were then pooled or bulked together, this then reduced the number of fractions to five (fractions 1, 2, 3, 4, 5)

$$R_f = \frac{\text{distance compound has moved from origin}}{\text{distance of solvent front from origin}}$$

The study on the bioactivities of the chromatographic fractions were carried out using these five fractions designated as fraction 1, fraction 2, fraction 3, fraction 4 and fraction 5.

Acute Toxicity Test of Chromatographic Fraction

The acute toxicity test of the five chromatographic fractions of *Portulaca oleracea* was evaluated in albino mice as described by Miller and Tainter (1994). Seventy-five adult male mice weighing between 20-22 g were divided into five mice per group for each fraction. Three doses of each fraction: 1 mg/kg BW, 5 mg/kg BW and 10 mg/kg BW were given orally to the animals. The control group mice (n=5) received 0.2 ml of distilled water. The animals were observed for seven days for behavioural changes and mortality.

Table 1:

Hexane		Ethylacetate		Methanol
100% (50 mL)	:	0% (0 mL)		
90% (45 mL)	:	10% (5 mL)		
80% (40 mL)	:	20% (10 mL)		
70% (35 mL)	:	30% (15 mL)		
60% (30 mL)	:	40% (20 mL)		
50% (25 mL)	:	50% (25 mL)		
40% (20 mL)	:	60% (30 mL)		
30% (15 mL)	:	70% (35 mL)		
20% (10 mL)	:	80% (40 mL)		
10% (5 mL)	:	90% (45 mL)		
0% (0 mL)	:	100% (50 mL)	:	0% (0 mL)
	:	90% (45 mL)	:	10% (5 mL)
		80% (40 mL)	:	20% (10 mL)
		70% (35 mL)	:	30% (15 mL)
		60% (30 mL)	:	40% (20 mL)
		50% (25 mL)	:	50% (25 mL)
		40% (20 mL)	:	60% (30 mL)
		30% (15 mL)	:	70% (35 mL)
		20% (10 mL)	:	80% (40 mL)
		10% (5 mL)	:	90% (45 mL)
		0% (0 mL)	:	100% (50 mL)

Twenty-one fractions were obtained after the column chromatographic procedure.

Experimental Design

(a) Fertility study in male rats (mating experiment)

Six adult male rats (160-180 g) were used. The rats were divided into six groups with each group consisting of one male rat. The first five groups were orally given 3 mg/kg BW of fraction 1, fraction 2, fraction 3, fraction 4 and fraction 5 respectively for 50 days, while the sixth group rat was orally given 0.5 mL of distilled water for the same number of days (control group). On day 51 of the experiment, three untreated female rats of proven fertility (160-180 g) were cohabitated with each of the male rats in the six groups; the cohabitation lasted for four weeks. Vaginal lavages were carried out on daily basis to observe the presence of spermatozoa which normally indicates positive mating.

(b) Fertility and teratogenic study in female albino rats

Vaginal lavages from adult female rats (160-180 g) were monitored on daily basis and animals exhibiting three 4-5 day estrous cycles were used for the studies. Rats found in the proestrous phase of the cycle were caged with males of proven fertility overnight in the ratio of 2:1 and were examined the following morning for evidence of copulation. Vaginal lavages were carried out and rats with motile spermatozoa in their vaginal secretions were separated and the day was designated as the day one pregnancy. The pregnant rats were divided into different groups (with each group consisting of five pregnant rats) for the study of the effects of the fractions on different phases of pregnancy.

(i) Implantation or early pregnancy study (1st-5th Day)

Group I to VI rats were used for the implantation study.

Group I rats received 3 mg/kg BW of fraction 1 from day 1 to 5 of gestation.

Group II rats received 3 mg/kg BW of fraction 2 from day 1 to 5 of gestation.

Group III rats received 3 mg/kg BW of fraction 3 from day 1 to 5 of gestation.

Group IV rats received 3 mg/kg BW of fraction 4 from day 1 to 5 of gestation.

Group V rats received 3 mg/kg BW of fraction 5 from day 1 to 5 of gestation.

Group VI rats received 0.5 mL of distilled water for the same number of days and served as the control group.

On the 6th day gestation, the pregnant rats (groups I-IV) were sacrificed by cervical dislocation to determine the number of implantation sites in both horns of the uterus using a dissecting microscope.

(ii) Mid-pregnancy or period of organogenesis study (6th - 15th day)

Group VII-XII rats were used for the mid-pregnancy study.

Group VII rats received 3 mg/kg BW of fraction 1 from day 6 to 15 of gestation.

Group VIII rats received 3 mg/kg BW of fraction 2 from day 6 to 15 of gestation.

Group IX rats received 3 mg/kg BW of fraction 3 from day 6 to 15 of gestation.

Group X rats received 3 mg/kg BW of fraction 4 from day 6 to 15 of gestation.

Group XI rats received 3 mg/kg BW of fraction 5 from day 6 to 15 of gestation.

Group XII rats received 0.5 mL of distilled water for the same number of days and served as the control group.

On the 16th day of gestation, the pregnant rats (groups VII-XII) were sacrificed by cervical dislocation, fetuses were removed from the rats by ventral laparotomy and examined. The number of fetuses and resorption sites were counted, the fetus were also examined for gross malformations.

(iii) Late pregnancy study (16th -21st Day)

Groups XIII to XVIII rats were used for the late pregnancy study;

Group XIII rats received 3 mg/kg BW of fraction 1 from day 16 to 20 of gestation.

Group XIV rats received 3 mg/kg BW of fraction 2 from day 16 to 20 of gestation.

Group XV rats received 3 mg/kg BW of fraction 3 from day 16 to 20 of gestation.

Group XVI rats received 3 mg/kg BW of fraction 4 from day 16 to 20 of gestation.

Group XVII rats received 3 mg/kg BW of fraction 5 from day 16 to 20 of gestation.

Group XVIII rats received o.5 mL of distilled water for the same number of days and served as the control group.

On the 21st day of gestation, the dams (pregnant rats) (groups XIII to XVIII) were allowed to deliver their litters naturally. At birth, the number of pups (litters) were counted, weighed and examined for gross malformations; the number of resorption sites were also counted.

Statistical Analysis

The mean and standard error of mean (S.E.M) were calculated for all values. Comparison between the control and experimental groups was done using one-way analysis of variance (ANOVA) with least significant difference (LSD). Differences were considered statistically significant at $p < 0.05$.

RESULTS

Acute Toxicity

No mortality and changes in behaviour were observed in all the treated and control groups. Hence lower doses of the fractions were used for this study.

Effects of fraction on fertility of male albino rats

Cohabitation of 50 days fraction 1 to fraction 5 treated male rats and untreated female rats for four weeks produced no positive mating (sterile mating), while cohabitation of the control group male rat with untreated female rats led to positive mating (pregnancy) with 6.25+0.48 litter size and 5.26+0.24g litter weight.

Effects of fractions on early pregnancy

The effects of the five fractions on the number of implantation sites during early pregnancy is shown in Figure 1.

Treatment of rats from day 1 to 5 of gestation with 3 mg/kg BW of fraction 1 to fraction 5 caused no significant ($p>0.05$) changes in the number of implantation sites relative to the control.

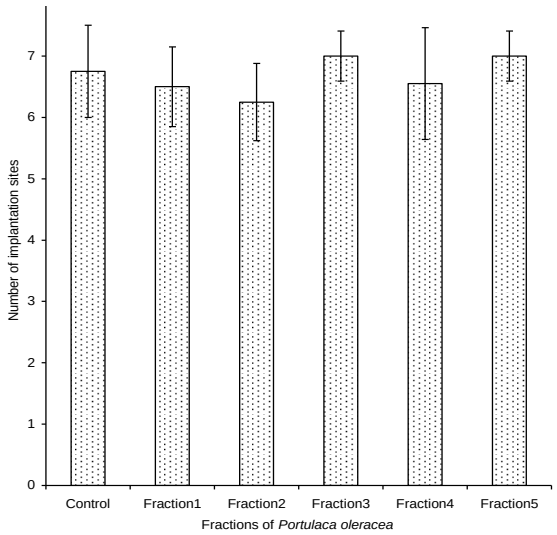

Fig. 1: Effects of the five fractions of *Portulaca oleracea* on the number of implantation sites during early pregnancy (1st -5th day) (n =5).

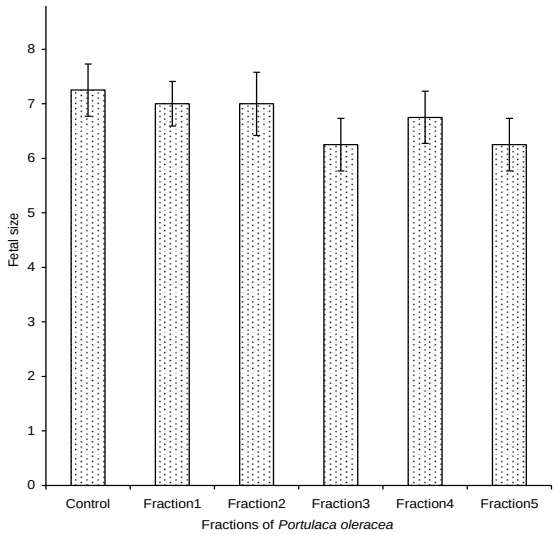

Fig. 2: Effects of the five fractions of *Portulaca oleracea* on the fetal size during mid-pregnancy (6th -15th day, period of organogenesis) (n =5).

Effects of fractions on mid-pregnancy

The effects of the five fractions on fetal size during mid-pregnancy is shown in Figure 2.

Treatment of rats from day 1 to 5 of gestation with 3 mg/kg BW of fraction 1 to fraction 5 caused no significant ($p>0.05$) changes in fetal size relative to the control. Also, there were no resorption sites and no gross malformations (morphological anomalies) in all the treated and control rats.

Effects of fractions on late pregnancy

The effects of the five fractions on litter size and litter weights at birth are shown respectively in Figures 3 and 4.

Treatment of rats from day 16 to 20 of gestation with 3 mg/kg BW of fraction 1 to fraction 5 caused no significant ($p>0.05$) changes in litter size and litter weights relative to their respective controls. Also, there were no resorption sites and no gross malformations (morphological anomalies) in all the treated and control rats.

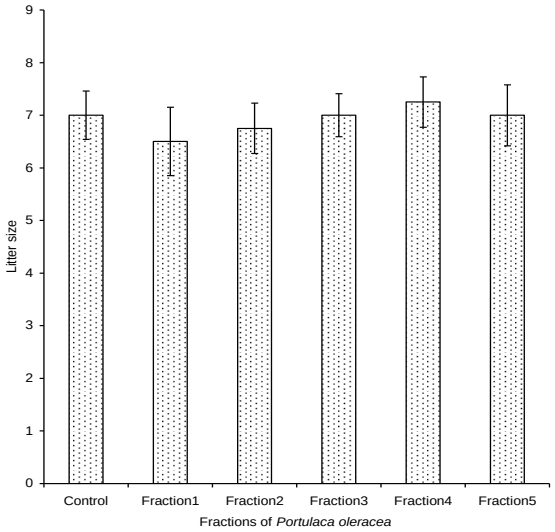

Fig. 3: Effects of the five fractions of *Portulaca oleracea* on the litter size at birth (21st -23rd day) (n =5).

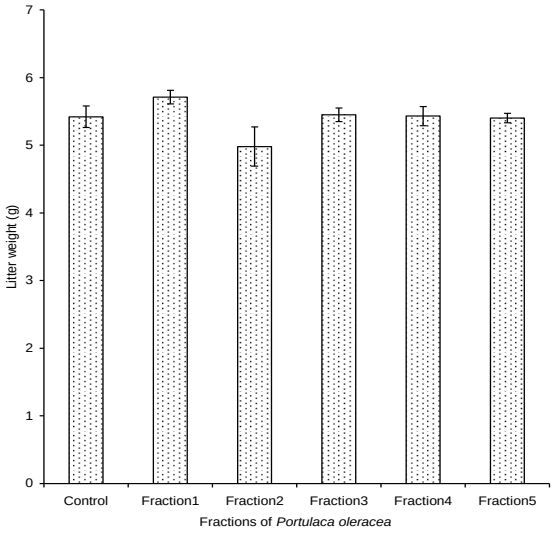

Fig. 4: Effects of the five fractions of *Portulaca oleracea* on the litter weight at birth (21st -23rd day) (n =5).

DISCUSSION

It was observed that the highest doses of the fractions caused no mortality or behavioural changes in all the treated animals which indicates that the fractions have wide safety margins.

Cohabitation of the fractions treated male rats and untreated female rats for four weeks produced no positive (sterile mating). Similar results were reported in rats treated with *Barleria prionitis* extracts [13], *Carica papaya* extract [14] and *C. officinalis* [15]. This observation could be due to reductions in sperm motility, sperm counts, sperm viability and increase in the percentage of abnormal sperm cells induced by the crude extracts as reported by [16].

Treatment of pregnant rats with the fractions during early pregnancy caused non-significant changes in the number of implantation sites relative to the control. Contrary result was reported by [17] in *Hibiscus rosa-sinensis* extract treated rats. This could indicate that the extracts did not cause the disturbance of endocrine-endometrial synchrony which is dependent on estrogen and progesterone balance, since it has been reported that for implantation to take, exact equilibrium of estrogen and progesterone is essential and any disturbance in the level of these hormones may cause infertility [18].

Treatment of pregnant rats with the fractions during mid-pregnancy caused no significant changes in fetal size relative to the control; also there were no resorption sites and no gross malformations (morphological anomalies) of fetuses in all the treated and control rats which probably indicates that the fractions have no teratogenic and deleterious effects on the fertility of female albino rats at this phase of pregnancy.

During late pregnancy, the fractions treated pregnant rats were delivered normally with no evidence of prematurity or abortion or death, suggesting that the fractions were not abortifacients or having prostaglandin-like activities on the uterus. Contrary report was given by [19] in *Dolichandrone falcata* extract treated rats. Also, treatment of pregnant rats with the fractions during late pregnancy caused no significant changes in litter size and litter weights relative to the control as well as absence of gross malformations which probably indicates that the fractions have no teratogenic and deleterious effects on the fertility of female albino rats at this phase of pregnancy.

In conclusion, this study has shown that the chromatographic fractions of *Portulaca oleracea* have antifertility effects in male albino rats. However, these fractions have no teratogenic and deleterious effects on the fertility of female albino rats, this could be the reason why the plant (*Portulaca oleracea)* along with other ingredients is taken as an aid to the development of fetus in some areas in Edo State, Nigeria [5] and to prevent miscarriage in Tanganyika, Tanzania [1].

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