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Investigation of lipophilic and hydrophilic leaf extracts of *Portulaca oleracea* on gestation in albino rats (*Rattus norvegicus*)

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ABSTRACT

Background: *Portulaca oleracea* Linn. is one of the indigenous plants currently gaining ground and recognition globally as both vegetable and medicinal herb. The scarcity of literature on the safety of *P. oleracea* uses in pregnancy prompted this research. **Objective:** This study investigated the effect of the lipophilic and hydrophilic leaf extracts of *P. oleracea* on gestation/fetal indices. **Methods:** The extracts were obtained using two extracting solvents – chloroform and 80% aqueous methanol in succession. Thirty-five successfully mated female rats were divided into seven groups. Group 1 (control) received 0.5 ml 20% Tween 80 (vehicle), Groups 2, 3, and 4 received 125, 250, and 500 mg/kg of the lipophilic extract, respectively, and 5, 6, and 7 received 125, 250, and 500 mg/kg of the hydrophilic extract, respectively, orally from gestational day (GD) 6 to 19. On GD 20, laparotomy was performed to assess the gestation/fetal indices. **Results:** Both extracts did not cause abortion in the pregnant rats or teratogenesis of the fetuses. The extracts had no significant ($P > 0.05$) effect on the indices; however, only hydrophilic extract caused a dose-dependent significant ($P < 0.05$) increase in fetal crown-rump length in comparison with control. **Conclusion:** Leaf extracts of *P. oleracea* as used in this study are safe to fetus and gestating dam, thus gestation friendly.

Keywords: Fetus, Portulaca oleracea, pregnancy, safety

INTRODUCTION

The use of different plant parts as medicine and food supplement is becoming popular in recent times. *Portulaca oleracea*, which is frequently referred to as cosmopolitan weed, is gaining ground and recognition globally as both vegetable and medicinal herb. *P. oleracea* Linn., a member of family Portulacaceae, is commonly called purslane in English language. It is one of the oldest leafy vegetables. It is eaten in many African countries and has wide acceptance in Central Europe, Asia, and the Mediterranean region where it is taken as potherb.[1] It is usually consumed raw as a green salad component or cooked as a sauce. It contains more omega-3 fatty acids, alpha-linolenic acid in particular than any other leafy vegetable.[2] It has high levels of Vitamins E and C and beta-carotene[3] and has been described as a "power food" due to its high nutritive and antioxidant properties.[4] The Chinese folklore described it as "vegetable for long life."[5]

P. oleracea Linn. [Figure 1] is one of the indigenous plants used globally for the management of health challenges and the treatment of diseases. It is used in the treatment of burns, headache, and dysentery; suppressing cough and expelling intestinal worms have made it popular in herbal medicine. In literature, *P. oleracea* is connected with several pharmacological properties: The leaf extracts contracted the smooth muscle of isolated intestine[6] have analgesic and anti-inflammatory action;[7] antifungal activity;[8] antidiabetic effect;[9,10] hepatoprotective activity;[11] antioxidant effects;[1,12] and wound healing properties.[13] In a clinical trial for the management of asthma, the extract exhibited enhancements in pulmonary function tests which were almost the same as those of oral theophylline, the trial control.[14]

No doubt, the use of medicinal plants in pregnancy may pose some danger to the developing fetus just like some orthodox medicines. There is, however, no evidence in literature that substantiates the safety of *P. oleracea* Linn. uses in pregnancy both as vegetable and medicinal herb although the safety of lipophilic and hydrophilic extracts of *P. oleracea* has been demonstrated in male albino rats, exposed for 60 days from our previous studies where the extracts were found to be non-toxic to blood parameters[15,16] and also had no adverse effects on hepatic and renal biochemical profile.[17] This study was, therefore, designed to investigate the effect of lipophilic (chloroform) and hydrophilic (80% aqueous methanol) leaf extracts of *P. oleracea* on gestation.

MATERIALS AND METHODS

Plant Material and Authentication

Fresh leaves of *P. oleracea* were collected from Alakahia axis of Port Harcourt, Nigeria, from December 2017 to February 2018. The plant was identified by Dr. C. Ekeke of the Department of Plant Science and Biotechnology, University of Port Harcourt, Rivers State, Nigeria, and a sample was deposited at the University of Port Harcourt Herbarium with the number UPH/V/1302.

Preparation of Plant Extract

After the collection of the plant, the leaves were shade dried at room temperature to constant weight over a period of 6 weeks. The dried leaves of *P. oleracea* were weighed and ground to a fine powder. The cold maceration method following the successive solvent extraction approach with modifications[18] was used for the extraction. Extraction solvents were used in the ascending order of polarity (chloroform before 80% aqueous methanol). Concisely, a 4.5 kg portion of the powdered leaves of *P. oleracea* was first extracted by maceration in 13.5 L of chloroform for 72 h (with the fresh replacement of solvent every 24 h) to extract the lipophilic constituents of the plant material. The resulting lipophilic constituent free marc after air-drying in a fume cupboard to remove residual chloroform was further macerated by soaking in an equal volume of 80% aqueous methanol for 72 h, with the fresh replacement of solvent every 24 h to extract the hydrophilic constituents of the plant material. The lipophilic and hydrophilic filtrates were each obtained by filtration with Whatman's No. 1 filter paper, concentrated with rotary evaporator (Model No: RE-52A) at 45°C *in vacuo*, later transferred to an evaporating dish, and dried over a water bath (digital thermostatic water bath and Finotech instruments) set at 45°C. The dried lipophilic (chloroform) and hydrophilic (80% aqueous methanol) leaf extracts of *P. oleracea* obtained were stored in a desiccator. All reagents used were of analytical grades.

Acute Oral Toxicity Study

The acute toxicity of the extracts was evaluated according to the method of Lorke[19] using a total of 36 female albino rats, 18 animals for each extract. This aspect of the study was done to determine the LD_{50} of the extracts, which is vital in identifying its clinical effects following oral administration and also to determine the doses to be employed in the study.

For the lipophilic extract, in the first phase, three groups of three rats each were administered with the extract at doses of 10 mg/kg, 100 mg/kg, and 1000 mg/kg body weight by oral gavage and observed for 24 h. In the second phase, three groups of three rats each were administered with the extract at doses of 1600 mg/kg, 2900 mg/kg, and 5000 mg/kg by oral gavage. They were observed for 24 h for mortality and other signs of toxicity. The same procedure was repeated for the hydrophilic extract.

Animals

Thirty-five mature female albino rats (*Rattus norvegicus*) with an average weight of 190 g and 18 male rats acquired from the Animal House of Pharmacology Department, University of Port Harcourt, Nigeria, were used for the study. Before the study, the animals were acclimatized for 2 weeks. The commercial feed from top feeds Nigeria and clean drinking water were given to them *ad libitum*. The male rats served as mating partners. Following acclimatization, two females were put together with a male overnight in a cage. Mating was established the next day by the presence of sperms in vaginal aliquot and/or vaginal plug [Figure 2] and referred to as gestational day (GD) 0.[20,21]

The mated rats were randomly divided into the seven groups of five each for the following treatment:

- Group 1 (control) – 0.5 ml 20% Tween 80 (vehicle)
- Group 2 – 125 mg/kg of lipophilic extract
- Group 3 – 250 mg/kg of lipophilic extract
- Group 4 – 500 mg/kg of lipophilic extract
- Group 5 – 125 mg/kg of hydrophilic extract
- Group 6 – 250 mg/kg of hydrophilic extract
- Group 7 – 500 mg/kg of hydrophilic extract.

Figure 1: A photograph of *Portulaca oleracea* Linn. showing the leaves, stem, seed pods, and yellow flower

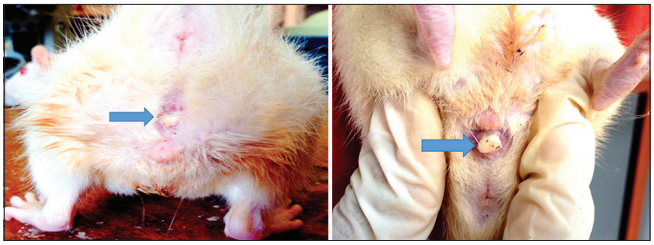

Figure 2: Presence of vaginal plug (arrow) in female albino rats after mating

All treatments were by oral gavage daily from GD 6 to 19. Animals' weights were taken daily and the doses adjusted accordingly. On day 20 of gestation, the rats were anesthetized and dissected; the uteri were excised and incised at the greater curvature of the horns. The gestation and fetal indices were evaluated using the following parameters according to the methods of Assayed *et al*.[20] and Kagbo and Obinna.[22]

1. The total uterine implants and resorptions
2. The dead and living fetuses
3. The mean fetal weight at GD 20
4. The mean fetal crown-rump length (FCRL)
5. Post-implantation deaths (%) = ([Total number of implants–Total number of living fetuses] ÷ Total number of implants) × 100
6. Birth index (%) = ([Number of live pups ÷ Number of implants] × 100).

The fetuses were also assessed for gross peripheral abnormalities with some fixed in Bouin's solution for internal organ assessment.

Statistical Analyses

Statistical analyses were done with SPSS 21; the data were represented as mean ± standard deviation and analyzed using one-way analysis of variance and Tukey *post hoc* test. The significance level was set at $P < 0.05$.

RESULTS

Acute Toxicity Study

The animals did not show any mortality, morbidity, or other obvious signs of toxicity such as locomotor alterations, diarrhea, or piloerection at the doses used in the acute toxicity test. This was a proof that both extracts were not noxious at the maximum dose of 5000 mg/kg. Hence, 1/40th, 1/20th, and 1/10th of this maximum dose (5000 mg/kg) were adopted for the studies which gave rise to 125, 250, and 500 mg/kg doses of Lipophilic (chloroform) and Hydrophilic (aqueous methanol) leaf extracts of *P. oleracea* treatment groups.

Gestation/Fetal Indices

Tables 1 and 2 summarize the effects of lipophilic and hydrophilic leaf extracts of *P. oleracea* on the fetuses obtained from exposed dams on gestation days 6 to 19. The result shows that both extracts had no significant ($P > 0.05$) effect on the fetal weights, number of live-born fetuses, total uterine implants, post-implantation loss, and birth index. A dose-dependent significant ($P < 0.05$) increase in the FCRL was observed only in the 250 and 500 mg/kg hydrophilic extract treated rats (Group 6 and 7, respectively) in comparison with control. The increase in FCRL in Group 7 was highly significant ($P < 0.01$). Other treated groups showed no significant effect on the FCRL, as shown in both tables.

No demonstrable sign implicated in maternal mortality was recorded in all the pregnant rats in the test groups. No anomaly was observed following gross morphological and internal organ assessment of living fetuses from the test groups when compared with the control (data not shown). Similarly, no abortion was recorded in all the pregnant rats in the test groups.

DISCUSSION

Plant extracts are made up of diverse kinds of phytochemicals, which are responsible for the biological activities associated with the extracts. These phytochemicals are extracted from plant materials using different solvents and extraction techniques. It has been reported that the type of solvent

Table 1: Effects of lipophilic leaf extracts of *Portulaca oleracea* on gestation/fetal indices

Gestation/fetal indices	Group 1 (Control)	Group 2 (125 mg/kg)	Group 3 (250 mg/kg)	Group 4 (500 mg/kg)
Fetal weight (g)	3.24±0.16	3.35±0.29	3.53±0.27	3.50±0.25
Fetal crown-rump length (cm)	3.44±0.12	3.51±0.08	3.55±0.04	3.51±0.10
Live-born fetuses (no)	8.40±0.89	7.60±1.14	7.60±1.14	9.00±1.41
Total uterine implants (no)	8.60±1.41	8.20±1.48	7.60±1.14	9.00±1.41
Post-implantation loss (%)	2.00±4.47	6.50±9.29	0.00±0.00	0.00±0.00
Birth index (%)	98.00±4.47	97.50±5.59	100.00±8.94	100.00±0.00

Values are given as mean±standard deviation for five rats in each group; experimental groups are compared with Group 1 (control). No significant difference at a 95% confidence interval ($P>0.05$)

Table 2: Effects of hydrophilic leaf extracts of *Portulaca oleracea* on gestation/fetal indices

Gestation/fetal indices	Group 1 (Control)	Group 5 (125 mg/kg)	Group 6 (250 mg/kg)	Group 7 (500 mg/kg)
Fetal weight (g)	3.24±0.16	3.43±0.25	3.50±0.36	3.69±0.30
Fetal crown-rump length (cm)	3.44±0.12	3.56±0.04	3.63±0.06[a]	3.71±0.10[a,b]
Live-born fetuses (no)	8.40±0.89	8.00±1.23	8.00±2.24	7.80±2.05
Total uterine implants (no)	8.60±1.41	8.00±1.23	8.00±2.24	7.80±2.05
Post-implantation loss (%)	2.00±4.47	0.00±0.00	0.00±0.00	0.00±0.00
Birth index (%)	98.00±4.47	100.00±0.00	100.00±0.00	100.00±0.00

Values are given as mean±standard deviation for five rats in each group; experimental groups are compared with Group 1 (control). [a, b]Indicate significant difference (at $P<0.05$ and $P<0.01$, respectively) compared to Group 1 (control)

used in the extraction process, to a large extent, determines the extract yield, the available biologically active compounds (phytochemicals) as well as the resulting pharmacological activities of the plant materials.[23] It is against this background that the solvents – chloroform and aqueous methanol were used in this study, to produce the lipophilic and hydrophilic extracts of *P. oleracea*, respectively.

The preliminary phytochemical screening of the extracts which has been reported in our previous studies shows that the lipophilic extract, which could not dissolve in water due to its highly lipophilic nature and thus allowed for very few tests, indicated only the presence of triterpenoid/steroids,[24] while the hydrophilic extract showed the presence of saponins, cardiac glycosides, phlobatannins, alkaloids, carbohydrates, and steroids.[16]

Studies have shown that a wide range of indigenous plants used as herbal remedies may be harmful to developing conceptuses, leading to abortion, teratogenesis, and/or fetal death.[22,25,26]

In the present study, lipophilic and hydrophilic leaf extracts of *P. oleracea* did not cause any obvious abortion in the pregnant rats; rather, a dose-dependent increase in the FCRL was recorded in only the hydrophilic extract treated groups. No external and visceral anomalies were observed in the live-born fetuses from both lipophilic and hydrophilic extracts treated rats.

The non-significant decrease in the number of live-born fetuses from the treated dams which corresponded with the increased fetal weight could be due to some factors such as age, fecundity of dams, and accommodating ability of the uterus such that the more the number of live-born fetuses, the less the fetal weight, and vice versa. This finding is in agreement with the report by Obinna and Agu[21] who demonstrated that the higher the litter size from cypermethrin exposed dams, the less each pup weighed. This increase in FCRL of pups in all the treatment groups, especially in the hydrophilic extract group, excludes retarded growth in the fetuses. This finding with the absence of morphological and visceral anomaly in the exposed fetuses equally excludes teratogenesis. The non-significant post-implantation loss observed in the test Group 2 (125 mg/kg lipophilic extract group) may likely not have been caused by the extract since the control group also recorded the loss. This may justify the optimal birth index (100%) recorded in the other treatment groups.

From these findings, it can be inferred that extracts of *P. oleracea* as used in this study had no negative effect on the parameters measured both in the fetus and the gestating dam. This result is comparable with the work of Auharek *et al*.[27] which demonstrated that aqueous *Campomanesia xanthocarpa* (Berg.) administered to Wistar rats at a dose of 26.3 mg/kg daily from gestation day 1–20 neither disrupted the pregnancy nor impaired the fetal development; instead, it decreased the resorption sites, a factor considered to be beneficial to gestation. The study by Okoye *et al*. [28] on the effect of methanol extract of *Telfairia occidentalis* (fluted pumpkin) leaves on albino rats treated at 200 and 800 mg/kg doses from gestation day 1 to 21 also reported the absence of significant harmful effect on gestation generally and on the fetal indices evaluated. This finding contrasts with the study by Kagbo and Obinna[22] who worked on *Costus lucanuscianus* and reported a dose-dependent significant decrease in foetal crown-rump length and increase in percentage post-implantation death in gestating rats administered with100, 200, and 300 mg/kg doses of methanol leaf extract of Costus lucanuscianus (monkey sugarcane), from gestation day 6 to 19.

Given the findings of the fetal/gestational indices, the hydrophilic extract was shown to have marked effect than the lipophilic extract. This was obvious in the dose-dependent significant increase in FCRL of pups and 100% birth index in the hydrophilic extract test groups. Hydrophilic solvents such as methanol have been connected with the effective extraction of more active biological substances,[29] especially the hydrophilic compounds. The presence of more polar bioactive compounds in the hydrophilic extract compared with the lipophilic extract may have contributed to the variance in the physiological effect demonstrated in this study.

CONCLUSION

This study has demonstrated that leaf extracts of *P. oleracea* as used in this study is safe both to the fetus and the gestating dam; hence can be said to be gestation friendly. Secondly the hydrophilic leaf extracts of *P. oleracea* leaf have been shown to be more bioactive than the lipophilic extracts. Further studies are, however, recommended to elucidate the rationale behind the positive effect of this leaf extracts on gestation.

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study protocols were duly approved by the Research Ethics Committee of the Center for Research Management and Development, University of Port Harcourt, with the Ref. No: UPH/CEREMAD/REC/04. The rats for the study were humanely handled in accordance with the ethics and regulation guiding the use of research animals as approved by the University.

COMPETING INTERESTS

None was declared.

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