International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.6, No.2, pp 783-793, April-June 2014

Evaluation of Potential Antifertility activity of Total Flavonoids, Isolated from *Portulaca oleracea* L on female albino rats

HanumanatappaBherigi Nayaka[1], Ramesh L Londonkar[1]* and Umesh M K[1]

[1]Department of Post Graduate Studies and Research in Biotechnology, Gulbarga University Gulbarga-585106, Karnataka, India

***Corres.author: londonkarramesh53@gmail.com**

Abstract: The present study isto evaluate anti-fertility activity of total flavonoids isolated from*P. oleracea L* in female rats.Air-dried aerial part of *P. oleracea* L was used for extraction. Flavonoids were administered orally for 1-7 and 7-14 days for evaluation of anti-implantation and Abortifecient activities respectively. After completion of last dose in both studies, animals were sacrificed under light anesthesia with ether. The number of implantation sites and aborted embryoswere recorded respectively. Biochemical changes were analyzed for determination of total protein and cholesterol content in uterus. The Qualitative and Quantitative tests for total flavonoids were also carried out. When flavonoids administered orally, a strong anti-implantation activity (3.22±0.02), and Abortifecient activity (05.00) was shown at high dose (500mg/kg of b w). Biochemical changes in uterus for total protein (131.66±2.88) and cholesterol (301.15±1.62) was observed at the tested dose levels (500 mg/kg of b w). The flavonoids shows further significant ($p>0.05$) increase in uterus weight(74.13 ±3.78)at the same dose indicating that hormonal changes in body. The qualitative tests for flavonoids were performed and alltests were positive for flavonoids. These observations suggest that,flavonoids of *P. oleracea* L have anti-fertility effect in female rats.

Keywords: *Portulacaoleracea* L, anti-fertility, Flavonoids, Phytomedicine.

Introduction

Global search for anti-fertility agents is continued to tackle the problem of population explosion that may lead to economic and health impact on the family in particular and the society in general especially in developing countries like china and India where the population growth is very high (Ministry of Health, 2003). Although contraceptives containing estrogen and progesterone are effective and popular, the risks associated to the drugs have triggered the need to develop alternative methods from medicinal plants. Hence, there is a need for a suitable product search from indigenous medicinal plants that could effectively be used in the place of pills [1]. A number of investigations have been carried out on traditionally claimed anti-fertility plants to validate the claim. Recent literature review revealed that 48 out of 72 traditionally employed medical plants for fertility control had anti-fertility potential [2]. The plant *P. oleracea* L was proved to show the muscle relaxant activity [3], anti-inflammatory effect [4], in some middle east countries, it is considered as beneficial for small tumors and inflammation, urinary disorders, liver obstruction, and ulcer of mouth and stomach. Several researchers have shown that *P. oleracea* L is having anti-hyperglycocemic activity, anti-tumor activity, and antiulcer

activity [5]. This plant has also proved for gastric antiulcer activity [6]. The plant *P. oleracea*L (Porsulane) is commonly known as porsulane an herbaceous weed. This plant is an annual succulent prostrate herb; stem is about 15.30cm long, reddish, swollen at the nodes, quite glabrous. Leaves are freshly, sub-sessile, 6.25 mm long alternate or sub-opposite. Flower few together, in sessile terminal heads. Microscopic analysis of the leaf powder invariably shows spherical mineral crystals, sieve plants, tracheas with spiral, annular and scalariform thickening and vessels with bordered pits [7]. An aqueous extract of *P. oleracea* was shown to have skeletal muscle relaxant effects both *in vitro* and *in vivo*; other studies include: antibacterial and antifungal; wound healing; anti-inflammatory; uterine stimulant and diuretic in rabbits. The aim of the present study to evaluate anti-implantation and Abortifecient activities, estrogenic/anti-estrogenic activity, effect on genital organs weight and body weight of total flavonoids isolated from *P. oleracea* L in female albino rats.

Materials and Methods

Plant material

The aerial part of *P. oleracea* L was collected around Gulbarga University campus in June 2012. The plant was identified by a taxonomist and a voucher sample representing, Herbarium No. HGUG-5013 was deposited in the Herbarium of Medicinal Plants of the Department of Botany Gulbarga University Gulbarga, Karnataka.

Chemicals

Methanol, ethanol, ethyl acetate, Petroleum ether, Diethyl ether, H_2SO_4, Chloroform, HCl, KOH, hexane, silica Gel 60-120 mesh, Tween 80 Phosphate buffer saline, FCR Reagent, all the chemical, solvents and reagents used were analytical grade obtained from Hi media.

Extraction of Total Flavonoids by soxhlet extraction method

Before extraction, *P. oleracea* L was crushed into powder by versatile plant pulverizer. The powder of the sample was degreased by soxhlet extractor with petroleum ether until the color of elute become colorless. The same powder sample was accurately weighed and placed in soxhlet extractor by adding with a ratio of 80 ml of ethanol: water (70:30) solvent, followed by the extraction for 5 hrs. and then extracted solution was concentrated. The extract was centrifuged at 11000 rpm for 30 min; supernatant was taken for further use [8].

Qualitative test for flavoinoids

The presence of flavonoids were further conformed by specific test for flavonoids like shinodtest, lead acetate test, sodium hydroxide test, Sulphuric acid test, aqueous test. These are the specific test, for detection of flavonoids [9].

Quantitative Determination of total flavonoid by UV-Spectra-photometric method

Firstly, 2 mL of the sample solution was accurately removed in a volumetric flask (10 mL) by adding 0.6 mL of NaNO2 (5%) solution, shaken up and then standing for 6 min. Secondly, 0.5 mL of the Al(NO3)3 (10%) solution was added to the volumetric flask, shaken, and was left to stand for 6 min. Finally, 3.0 mL of the NaOH (4.3%) solution was added to the volumetric flask, followed by addition of water to the scale, shaken, and left to stand for 15 min before determination. Using the sample solution without coloration as reference solution and 500 nm as determination wavelength, the coloration method was used to determine the content of flavonoids in the sample by ultraviolet–visible detector. Thestandard solutions and standard rutin curve was prepared as described [8].

Antifertility activity

Experimental animals

Experiment was performed on virgin female albino rats aged about seven weeks (100g) obtained from Lukman pharmacy college Gulbarga. The animals were acclimatized for 1-2 weeks before being used for the experiment. Fed with Standard palliated diet (Amrut laboratory animal feed diet Pune, Maharashtra India), and water were given ad libitum. They were housed under standard condition of temperature (24 ^{o}C), humidity (65%) light and

dark cycle (14:10 L), respectively. The initial body weight of each animal was recorded. The Vaginal smear of the each rat was studied microscopically for estrous cycle every morning between 8-9am. Only female rats with normal estrous cycle were selected for the anti-ovulatory activity evaluation. All experimental procedure were carried out in strict accordance with the guidelines prescribed by the committee for the purpose of control and supervisor on experimentation on animals (CPCSEA Registration No- 34800/2001) and were approved by the institutional animal ethical committee.

Test material administration

Administration of the extract was done with intra-gastric tube on the basis of the animal's body weight. The dose 250 and 500 mg/kg body weight for each animal was calculated considering the human dose (dry weight equivalent approximately 4 g/kg aqueous macerate employed as vaginal douche in divided doses) based on ethno medical use of the plant part [10].

Acute toxicity study

Toxicity studies were carried out in rat according to OECD guidelines. Flavonoids extract at different doses up to 1000 kg b w was administered and animals were observed for behavioral changes, any toxicity and mortality up to 48 hrs. There was no toxicity reaction or mortality was observed which found to be safe. Based on the acute toxicity results we have selected the dose 500mg/kg b w and 250mg/kg body weight as high and low dose respectively for evaluation of anti-ovulatory activity[11].

Anti-implantation activity

Anti-implantation activity was determined by following the method of [12]. Female rats of estrus phase were kept with male rats of proven fertility in the ratio of 2:1. The females were examined for vaginal sperms the animals which showed thick clumps of spermatozoa in vaginal smears were segregated from the male partner and divided into 3 groups each group containing 6 animals (n=6), first group serves as control by receiving 1% Tween 80, where in group 2 and 3 receive An effective doses of 250mg / kg body weight as a low dose and 500mg / kg of body weight as a high dose of total flavonoids extracts dissolved in 1% Tween 80, were orally administrated daily through catheter tubefor 7 days starting on day 1 of pregnancy to 7^{th} day .On day 10 of pregnancy, all the animal were laparotomies under light ether anesthesia and sterile conditions. Both uterine horns were examined for number of implants which were recorded.

Abortifecient activity

Abortifecient activity was determined by following the method of [12]. Female rats of estrus phase were kept with male rats of proven fertility in the ratio of 2:1. The females were examined for vaginal sperms the animals which showed thick clumps of spermatozoa in vaginal smears were segregated from the male partner and divided into3 groups each group containing 6 animals (n=6), first group serves as control by receiving 1% Tween 80, where in group 2 and 3 receive An effective doses of 250mg / kg body weight as a low dose and 500mg / kg of body weight as a high dose of total flavonoids extracts dissolved in 1% Tween 80, were orally administrated daily through catheter tube for 7 days starting on day 7^{th} of pregnancy to 14^{th} day .On day 15^{th} of pregnancy, all the animal were laparotomies under light ether anesthesia and sterile conditions. Both uterine horns were examined for number of Abortifecient embryos which were recorded.

Effect of the total flavonoids on the weight of genital organ and body weight

Fifteen matured female colony breed Wister strain albino rats were employed. The animals were divided in three groups (five each). The first and second groups received the extract 250 and 500 mg/kg of body weight for 10 days by intra-gastric route respectively. The second group received the extract 500 mg/kg of body weight. The third group receives vehicle (1% Tween 80) for the same number of days by the same route. On the 11th day, all the animals were weighed and sacrificed under diethyl ether anesthesia. The ovaries and uteri were dissected out, freed from surrounding tissues, blotted on filter paper and weighed quickly on a balance sensitive to 0.0001 g. The ovary and uterine ratios were then calculated by dividing the ovary and uterine weight in milligrams by body weight in grams as described by [13].

Estrogenic/anti-estrogenic activity

The total flavonoid extract of *P.olerace* L was found to be most active; hence, it was subjected for detailed investigation for potential estrogenic/anti-estrogenic activity. Bilaterally ovariectomised immature female rats (Wistar strain) of 25-30 days old, weighing between 30-40g were divided into 3 groups, each consisting of 6 animals (n=6). The Group I received vehicle (1% Tween 80) only and served as control. Group II received total flavonoid extract of 250mg/kg of body weight (Low dose) and Group III received total flavonoid extract at the doses of 500 mg/kg body weight (High dose) respectively. All the above treatments were given for 7 days. On the 8^{th} day of the experiment all the animals were sacrificed by decapitation and uteri were dissected, cleared from surrounding tissues, blotted on fitter paper, weighed quickly on a sensitive balance and fixed in Bouin's fluid for 24 hours. The tissues were dehydrated and embedded in paraffin. The paraffin sections were cut at 5µm and stained with Haematoxylin –eosin [14] for histological observations of uterus by the methods described by Deb et al, [15].

Biochemical analysis

The biochemical analysis in adrenal gland and uterus of the treated rats were carried out to know the effect of flavonoid extract on the total protein content, and total cholesterol content of both organs. The total protein and cholesterol content of both organs were estimated by the method as described by Lowry et al, good et al[16, 17], respectively.

Histopathology

The uterus of one side of each animal was fixed in bouins fluid dehydrated by using various grade of alcohol, cleared in toluene or xylene. Embedded in paraffin wax and sectioned at 5µm thickness. The paraffin sections were stained with haematoxyline and eosin for histopathological study [18].

Statistical analysis

Results are expressed as Mean ± SEM. The statistical analysis was carried out using one way ANOVA analysis. The p-value of 0.05 or less was considered significant for all experiment.

Results

The qualitative Determination of flavonoids

The qualitative test for flavonoids were performed and all the test were given positive by formation yellow colored precipitation and shinoda test has given positive by formation of pink color. The results of the entire test were shown in the Table 1.

Table 1: The results of qualitative test for flavonoids

Tests	Observations	Inference
Shinoda test	Formation of Pink Color	Presence of flavonoids
Lead acetate test	Yellow colored Precipitation was formed	Flavonoids are present
Sodium hydroxide test	Yellow color formation, after adding acid yellow color is disappeared	Presence of flavonoids
Sulphuric acid test	Yellow color formation	Flavonoids are present
Aqueous test	Formation of Yellow color	Presence of flavonoids

Quantitative Determination of total flavonoid

The total flavonoids determination result showed that the *P. oleracea* L contained more (339.21µg/ml) flavonoids. The basic structure of flavonoids was presented in Fig.1a, and most of the flavonoids in *P. oleraceaL*, have 3′, 4′-dihydroxy-substituted structure (as shown in Fig. 1b). The color reaction of flavonoids and chromogenic system and standard curve for estimation of total flavonoids were represented inFigure 1 and 2 respectively. Using the concentration of rutin standard solution as abscissa and the absorbency (Optical

density 0.640) as y-coordinate, the linear chart was constructed and the standard curve is shown in Figure 2, the regression equation was y=−0.0019x-0.0045 (R^2=0.9939).

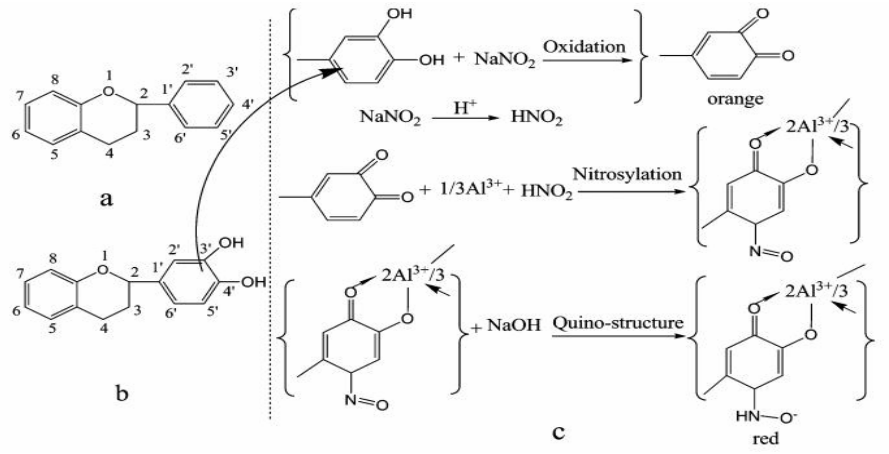

Figure 1: The color reaction of flavonoids and chromogenic system

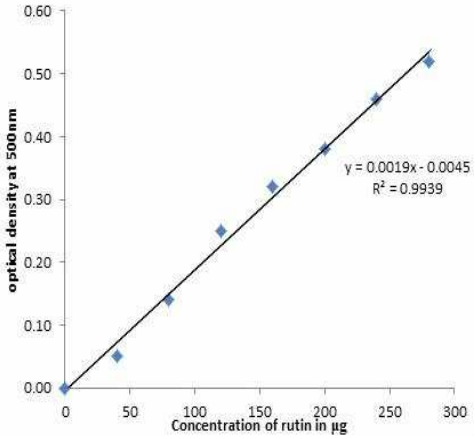

Figure 2: The standard curve of the Flavonoid Rutin

Antifertility activity

Acute toxicity.

Over the study duration of 2-3 days, there were no deaths recorded in the experimental group of animals while giving the dose ranging from 100 to 1000mg/kg of b w of flavonoid extract of *Portulecaolerace L*.The animals did not show any change in general behavior, skin effection, defecation, loss of hairs or other physiological activities. Hence, 250 and 500mg/kg of b w were fixed as low and high doses respectively to evaluate the anti-ovulation activity of flavonoid extract of *Portulecaolerace L*

Anti-implantation activity

The evaluated total flavonoids of *P. oleracea* L for anti-implantation activity in albino rats have shown the 50% (4.11±0.01) inhibition of implantation at low dose (250mg/kg b w), where in case of high dose (500mg/kg b w) it has more significant ($p<0.05$) inhibition by 70% (3.22±0.02) when compared with control group (8.12±0.44). The result of anti-implantation activity was observed after 24 hrs. of last dose as shown in the Figure 3. The numbers of implantation sites wererecorded, statisticallyanalyzed and represented as Mean± SD as given in Table 2.

Figure 3: Anti-implantation activity of total flavonoids of *P. oleracea* L in albino rats: C-Control, FLD-Flavonoid Low Dose, and FHD-Flavonoid High Dose.

Table 2: Effect of total flavonoids of *P. oleracea L* on implantation activity in albino rats

Group No.	Treatment	Dose (Mg/kg of b w)	Dosing days	Body weight (g)	No. of Implantations (Mean ± SD)*
I	Control*	0.5 ml	1-7	120	8.12±0.44
II	FLD*	250	1-7	120	4.11±0.01
III	FHD*	500	1-7	120	3.22±0.02**

*Control: 1% Tween 80**,** FLD: Flavonoid Low Dose and FHD: Flavonoid High Dose,(Mean ± SD): Mean ± Standard Deviation.
** indicates significant at $p<0.05$.

Abortifecient activity

The evaluated total flavonoids of *P. oleracea* L for Abortifecient activity in albino rats have shown the 30% (03.00) aborted embryo at low dose (250mg/kg b w), where in case of high dose (500mg/kg b w) it has most significant ($p<0.05$) aborted embryos by 50% (05.00) when compared with control group (0.00). The result of Abortifecient activity was observed after 24 h. of last dose as shown in the Figure 4. The numbers of implantation sites were recorded, statistically analyzed and represented as Mean value as given in Table 3.

Figure 4: Abortifecient activity of total flavonoids of *P. oleracea* L in albino rats: C-Control, FLD-Flavonoid Low Dose, and FHD-Flavonoid High Dose.

Table 3: Effect of total flavonoids of *P. oleracea L* on Abortifecient activity in albino rats

Group No.	Treatment	Dose (Mg/kg of b w)	Dosing days	Body weight (g)	No. of Aborted Embryos (Mean ± SD)*
I	Control*	0.5 ml	7-14	120	0.00±0.00
II	FLD*	250	7-14	120	03.00±0.11
III	FHD*	500	7-14	120	05.00±0.02**

*Control: 1% Tween 80**,** FLD: Flavonoid Low Dose and FHD: Flavonoid High Dose,(Mean ± SD): Mean ± Standard Deviation.
** indicates significant at $p<0.05$.

Effect of flavonoids of *P. oleracea* L on weight of genital organ and body weight

The weights of the ovary and uterus of both low and high doses increased significantly when compared to control group as given in Table 4. In the present study the increase in the wet weight of the ovary in the extract treated animals compared to the control animals may indicate inhibition of ovulation through suppression of follicular stimulating hormone.

Table 4: Effect of total flavonoids of *P. oleracea L* on Body weight and Genital weight

Group No.	Treatment	Dose (Mg/kg of b w)	Dosing days	Body weight (g)		Genital organs weight (g)				
				Initial	Final	Ovary	Uterus	Adrenal	Kidney	Liver
I	Control*	0.5 ml	1-10	100	105	0.10	0.25	0.13	1.30	6.42
II	FLD*	250	1-10	100	110	0.16	0.31	0.11	1.25	6.94
III	FHD*	500	1-10	100	125	0.20	0.15	0.08	1.33	6.70

*Control: 1% Tween 80**,** FLD: Flavonoid Low Dose and FHD: Flavonoid High Dose.

Estrogenic /anti-estrogenic activity

The oral administration of the total flavonoids extract of *P.olerace* L at 250mg and 500mg/kg body weight caused a significant decrease in the uterine weight (92.66±2.51, 74.33±3.78) in immature rats when compared to control group (172.33±2.30),The treatment of flavonoids also altered the estrous cycle significantly characterized by a prolongation of the diestrous phase as represented in Table 5.the estrous cycle of the experimental animal were checked daily morning with in 10pm to 11pm and the different phages were recorded as represented in Figure 5.

Table 5: Effect of total flavonoids of *P. oleracea L* on Uterine weight, vaginal opening, Estrus cycle and Cornification of vaginal epithelial cells in bilaterally Ovariectomised immature rats

Group No.	Treatment	Dose (Mg/kg of b w)	Dosing days	Body weight (g)	Estrous cycle	Uterine weight (Mg/kg b w) (Mean ±SD)**	Vaginal opening & Cornification
I	Control*	0.5ml	1-7	40	Regular	172.33±2.30	0/0
II	FLD*	250	1-7	40	Irregular	92.66±2.51	6/6
III	FHD*	500	1-7	40	Irregular	74.13 ±3.78	6/6

*Control: 1% Tween 80**,** FLD: Flavonoid Low Dose and FHD: Flavonoid High Dose.**Values are Mean ± SD, number of animals in each group was six (n=6), *$p<0.05$

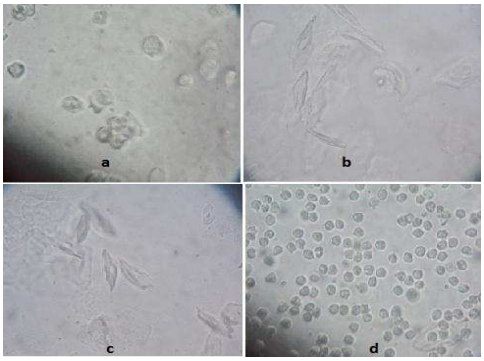

Figure 5: Different phases of estrous cycle: a-Proestrous, b-Estrous, c-Metaestrous, d-Diestrous

Biochemical analysis

Protein content of the uterus was reduced 50 % significantly 131.66±2.88 with flavonoids extract of both low and high doses respectively when compared to control group (239.33±0.57). Where in case of cholesterol content was reduced 30 % when compared to control group (301.15)as represented in Table 6.Cholesterol is the precursor for many of steroidalhormone due to this un-utilization of cholesterol there will be less synthesis of steroidalhormones which leads hormonal imbalance in the rat body resulting to antifertility.

Table 6: Effect of total flavonoids of *P. oleracea L* on Biochemical change in uterus

Group No.	Treatment	Dose (Mg/kg of b w)	Dosing days	Organ	Protein (Mg/100g) (Mean ± SD)**	Cholesterol (Mg/100g) (Mean ± SD)**
I	Control*	0.5ml	1-15	Uterus	239.33±0.57	504.66±4.16
II	FLD*	250	1-15	Uterus	131.66±2.88*	300.15±0.21*
III	FHD*	500	1-15	Uterus	131.66±2.88*	301.15±1.62*

*Control: 1% Tween 80, FLD: Flavonoids Low Dose and FHD: Flavonoids High Dose, b. w: body weight.

**Values are Mean ± SD, number of animals in each group was triplicate (n=3), *$p<0.05$.

Histopathology

The uterotropic changes such as the diameter of the uterus and thickness of the endometrium were significantly increased when compared with control rats. The epithelial layer of the endometrium consisted spindle shaped cells with basal nuclei. The lumen, perimetrium, myometrium and endometrium of the uterus were represented in the Figure 6.

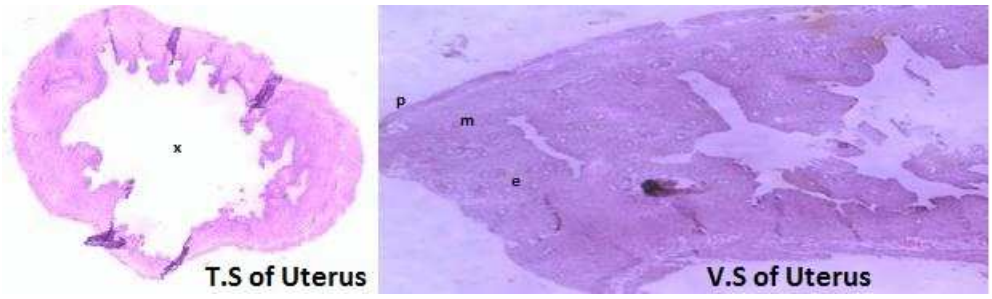

FIGURE 6: Histology of uterus (H&E, 5-µm sections) showing Trans Section and Vertical Section:X-Luminal Diameter of the Uterus, p-Perimetrium, m-Myometrium, e-Endometrium

Discussion

In past year many studies have suggested that the use of plant extract for reproductive physiology of animals. However, much interest has shown in recent years to control fertility by using plants because they possess secondary metabolites they intern regulate the fertility. The total flavonoids isolated from *P. oleracea* L are also secondary metabolites, conformed by qualitative tests[19, 20]. Thetotal flavonoids were estimated on the basis of the principle that, Flavonoids with 3′, 4′-dihydroxy-substituted structure can show special color by reacting with the system of NaNO2– Al(NO3)3–NaOH. This method is based on the reaction of aluminum ion with flavonoid at alkaline medium forming red chelates. By measuring the absorption of such red chelates, it is possible to determine the flavonoids[8].In the present study the reports of the preliminary anti-implantation activity of total flavonoids extracts of *P. oleracea L* autopsy on day 10 revealed that all the control rats (treated with a vehicle of 1% Tween 80) were pregnant and had a normal number of implantations and normal duration of diestrus, on treatment with total flavonoids extract at both 250mg / kg and 500mg / kg body weight was found that the number of implantationsin uteri horns decreased 50% and 70% significantly at their respective doses. This loss of implantation caused by the extract (total flavonoids) may be due to anti zygotic, blastocyst toxic or anti implantation activity as described in previous studies[21]. The Abortifecient activity has shown by total flavonoids admiration for 7 days resulting that, 30% and 50% at both low (250mg/kg) and high (500mg/kg) dose level respectively. The Abortifecient activity of ethanol extract of *P. oleracea L* is mainly due to its estrogenic activity which imbalances the required progesterone and estrogen ratio. High dose of estrogen disproportionate to progesterone leads to re-sorption of features.The prolongation of diestrous phase may explain the remote chance of the rats to get pregnant. The observation that there was no significant change in the diestrous phase and estrous cycle after withdrawing the flavonoids from those of the control could explain the reversible nature of the anti-fertility effect of the extract which has also been observed from the preliminary studies as mentioned above. The present study is comparable with the studies described in previous studies [22, 23].It appears that the total flavonoids extract of *P. oleracea* L at both doses have strong estrogenicity, since various flavonoids have been reported to possess contraceptive property by regulating the estrogen level[24, 25]. Who had reported anti-fertility effect with similar observations in guinea pigs and rats on treatment with seed extract of *Ricinuscommunis*and root extract of *Rumexsteudelii* respectively, However, significant decrease in the duration of proestrus and metestrus stage in experimental group was recorded than those of control animals. These changes were found to revert back after withdrawal of the treatment except the proestrus stage in groups with higher dose of treatment. The prolongation of diestrous phase may lower the chance of pregnancy in animals.The absence of change in body weight after 7 days treatment with the extract revealed that there is no major negative impact on the general metabolic status of the animals. The biochemical study of present investigation revolved the role of cholesterol as an obligatory precursor in progestin biosynthesis in rat, rabbit and bovine luteal tissues have been reported earlier [26, 27].In the present study, the total flavonoids extract of *P. oleracea* L has proved to possess anti-implantation and abortificient activity, and estrogenic activity, the imbalance caused in progesterone and estrogen levels might be the reason for interruption of pregnancy.

Conclusion

The present study suggests that administrations of flavonoids extract of *P. olerace L* have shown promising anti-implantation and Abortifecient activities at high dose (500mg/kg of b w). The results of this study also revalued that, flavonoids effect on genital organs (ovary and uterus), alters Estrous cycle with a prolonged diestrous, and increases the uterine muscle weight and ovary weight leading to causes of anti-fertility. The results of Biochemical analysis and Histopathology studies strongly suggest that, total flavonoids of this plant have potential anti-fertility effect on female albino rats, thus present study support the Pharmacological basis of *P. oleracea*L Extract can be used for further development of contraceptive agent without side effect and cost effect.

Conflict of Interests

The authors declare that there is no conflict of interests.

Acknowledgements

The authors are grateful to University Grant Commission New Delhi (India) for providing financial support to carry out this research work under the scheme of Rajeev Gandhi National Fellowship (UGC-RGNF), The Authors also thankful to Gulbarga University Gulbarga, Karnataka (India), for providing lab facility to carry out this study.

References

1. S. S. Pal, "Fertility control of female albino rats through Sesbanisesbian seeds," The *Journal of Research and Education in Indian Medicine*, 9, pp. 27–32, (1990).
2. R. Maurya, S. Srivastava, D. K. Kulshreshta, C. M. Gupta, "Traditional remedies for fertility regulation," *Current Medicinal Chemistry*, 11, pp.1431–1450, (2004).
3. F. Okwuasaba, C. Ejika, Parryo, "Effects of *Portulecaolerace* L on skllетal muscle in vitro," J. Ethanopharmacol, 27pp. 55-3, 1987.
4. K. Chan, M. W. Islam, M. Kamil, R. Radhakrishnan, N. M. Zakaria, M. Habibullah, and Attas, "The analgesic and anti-inflammatory effects of *Portulaca oleracea* L. subsp," Celak. *J. Ethnopharmacol*, 73, pp. 445-451, (2000).
5. A. Ivan, Ross, "*A Text book of medicinal plants of the world*," (2003).
6. Gholamrezakarmi, HosseinHosseinzadeh and NeginEttehad, "Evaluation of the gastric anti-ulsorogenic effect of *Portulecaolerace* L extracts," Phytother Res. 18 pp. 484-487, 2004.
7. G. Banerjee, A. Mukherjee, "Antibacterial activity of a common weed, *Portlecaolerace* L," *Gelobios* (Jodhpur), 30 (2-3) pp. 143-144, (2003).
8. Hongbin Zhu, Yuzhi Wang, Yuxuan Liu, Yalin Xia and Tian Tang, "Analysis of Flavonoids in *PortulacaoleraceaL*, by UV–Vis Spectrophotometry with Comparative Study on Different Extraction Technologies" *Food Anal. Methods*, 3, pp. 90–97, (2010).
9. C. K. Kokate, K. R. Khandelwal, A. P. Pawar and S. B. Gokhale,"*Practical Pharmacognocy*", NiraliPrakashan, New Delhi, India, pp.11-19, (1995).
10. B. Desta, "Ethiopian traditional herbal drugs. Part III: antifertility activity of 70 medicinal plants" *Journal of Ethnopharmacology*, 44, pp. 199–209, (1994).
11. R. A. Turner, "*Screening Methods in Pharmacology*," vol. II: Academic Press, New York, (1971).
12. U. Khanna, and R. R.Chaudhary, "Antiferlity screening of Plants. Part I. Investigation of Buteamonosperma (Lam) Kutze." *Indian Journal Medical research* 56, pp. 1575-1579, (1968).
13. H. G. Vogel, "Ovarian hormones," *In Pharmacological Assay*, second ed. Springer- Verlag, Berlin, Hidelberg, pp. 637–645, (1997).
14. W. G. Wiest, W. R. Kidwell and K. Balogh, "Progesterone catabolism in the rat ovary: A regulatory mechanism for progestational potency during pregnancy" *Endocrinol*, 82, pp. 844-849, (1968).
15. E. Gurr, "*Staining Animal Tissues: Practical and Theoretical*" Leonard Hill Limited, London, 233, (1962).
16. C. Good, H. Kramer and M. Somogyi, "The determination of glycogen,"*J. Biol. Chem*,100, pp. 485-491, (1933).
17. D. H. Lowry, N. J. Rosenbrough, A. L. Farr, and R. J. Randoll, R. J, *J. Biol. Chem*,265.193, (1951).
18. E. S. Hafez,"Reproduction and breeding,"*Techniques for laboratory animals*: Lea and febriger, Phildelphia, PA. (1970).
19. S. R. Chowdhury, C. M. Gupta and V.P. Kamboj, "*Current status in fertility regulation:Indigenous and modern a pproaches*" Central Drug Research Institute, Lucknow, India, (2001).
20. R. Kamal, R. S. Gupta and N. K. Lohiya, "Plants for male fertility regulation," *Phytother, Res*., 17: pp., 579-590, (2003).
21. S. K. Dhar, M. C. Nigam, K. K. Anand and V. Kaul, "Anti-implantation studies of some essential oil and t-Anethole", *Ind. Drugs*,27, 551-555, (1990).

22. E. Makonnen, L. Zerihun, G. Assefa, A. H. Rostom, "Antifertility activity of *Ricinus communis*seed in female guinea pigs," *East African Medical Journal*, 76, pp. 37–39, (1999).
23. E. Gebrie, E. Makonnen, A. Debella, L. Zerihun,"Phytochemical screening and pharmacological evaluations for the antifertility effect of the methanolic root extractof *Rumexsteudeli,"Journal of Ethnopharmacology*,96, pp.139–143, (2005).
24. L. L. Anderson, K. S. Moghissi and E. S. E. Hafez, "Biology of Mammalian Fertilization and Implantation," *Thomas, Springfield*, pp. 379, (1972).
25. S. Hariharan,"Laboratory animal's information service center news," ICMR, Hyderabad, India, (1980).
26. J. W. Wilks, G. B. Fuller, W. Hanse,"The role of cholesterol as a progestin precursor inrat, rabbit and bovine luteal tissue, "*Endocrinology*,87 pp. 581-7, (1970).
27. N. R. Mason, K. Savard, "Conversion of cholesterol to progesterone by corpus lutenum slices, "*Endocrinology*,75, pp. 215-21, (1964).

*****