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Ovulation Inducing Activity in PCOD Induced Female Wister Albino Rats and *In vitro* Studies of Free Radical Scavenging Activity of *Kadugurogini chooranam* (*Picrorhiza scrophulariiflora*)

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Abstract

Siddha system of medicine is oldest traditional system in south India. In these system herbals plays a vital role in prevention and curing the disease. Siddhar's who told the amazing solution for aging process. Causes of aging are due to increased level of free radicals that damage the major constituents of the cell. Herbals naturally have free radical scavenging activity because Presence of phyto-constiuents like polyphenols generally indicates the rejuvenating properties. The present study also to validate the Estrodial induced PCOS female wistar albino rats to treat with extract of Kadugurogini Chooranam (Picrorhiza scrophulariiflora) and the level of hormones were detected for the ovulation inducing activity and analysis the anti-radical properties from the decoction of Kadugurogini Chooranam (Picrorhiza scrophulariiflora) through the four parameters superoxide, nitricoxide, lipid peroxidation, ABTS radical activity with standard antioxidant and positive control is used as a ascorbic acid. Therefore, aqueous root extracts of Kadugurogini Chooranam have considerable antioxidant properties. In preliminary study, the Kadugurogini Chooranam posse's significant effects on hormonal level on PCOS induced female wister albino rats.

Keywords: Siddha, Phytochemicals, Antioxidant, Medicinal Herbs, PCOS, Kadugurogini Chooranam.

INTRODUCTION

The Siddhars, who founded our Siddha system, categorised a wide range of illnesses and provided great treatments for them. Additionally, soodhagavayu is one of the diseases that are classified as affecting women in Siddha literature. Stein Levanthal's condition is also called as PCOS in current terminology. The main problem of PCOS is infertility, which is caused by the ovaries' hyper androgenic dysfunction, which also causes hirsuitism, bilateral enlargement of the ovaries, and menstrual cycle irregularities [1]. With a prevalence of 2.2% to 26% among adult women aged 18 to 35, or 1 in 15 women worldwide, it is a significant economic health burden that is predicted to grow along with obesity.

Herbs and home-grown items have been utilized customarily to remedy nearly all afflictions of human as well as other creatures [2]. Herbals are the foremost widely utilized drugs within the world nowadays. A full eighty-five percent (85%) of the world's population utilizes herbs as their essential medications [3]. These herbs are referred to as "rejuvenating herbs" (Kayakalpam) since they are used to heal diseases in addition to scavenging free radicals.

Herbs' anti-oxidant properties aid in shielding cells from free radical damage. *Kadugurogini Chooranam* (Picrorhiza scrophulariiflora Pennell, Family: Scrophulariaceae), also known as kutki, is a little perennial medicinal herb of the alpine region that is threatened. The purpose of the current study is to validate the ovulation inducing action of this plant. The plant grows in the Himalayan region on both organic soils and damp, rocky slopes. It can be found in the Himalayan region (from Bhutan's Garhwal to the southeast of Tibet), north Burma, and west China. Generally speaking, it favors growing in rocky crevices [4].

It develops for the most part on messy and cliffy mountains. One of the dynamic metabolites of P. kurroa is flavonoid apocynin and it has been detailed to weaken Parkinson's, hypoxia and ischemia reperfusion by its inhibitory activity on NADH oxidase; communicated amid oxidative stress [5]. Aim of the present

study is to reveal the result of ovulation inducing activity and antioxidant property of *Kadugurogini Chooranam*.

MATERIALS AND METHODS

Plant Material

The crude root of Picrorhiza scrophulariiflora was collected from herbal raw material provider in Chennai. The confirmation was done by DR. S. Sankaranarayanan M.Sc., M.Phil., Ph.D, Department of Medicinal Botany, Government Siddha Medical College, Arumbakkam, and Chennai. A voucher example (GSMC/MB-128/2017) was kept within the department.

Preparation of Extracts

The root of Picrorhiza scrophulariiflora were air-dried beneath shade, pulverized by a mechanical processor, passed through work estimate 40 and after that put away in hermetically sealed holders. 20 gram of powdered root of Picrorhiza scrophulariiflora was kept in 200 ml cone shaped flask and included 100 ml of sterile water.

The mouth of the cone shaped flask was secured with aluminum foil and kept in a responding shaker for 24h for persistent disturbance at 150rev/min for careful blending conjointly complete explanation of dynamic materials to break up within the particular dissolvable. At that point, extricate was sifted by utilizing muslin cloth taken after by Whatman no 1 channel paper and at last sifted by utilizing vacuum and pressure pump.

Ovulation inducing activity in female Wister albino rat model

Animals

Female Wistar albino rats 150–200 g, and immature female Wistar albino rats of 21–23 days old (40–60 g) were utilized in this consider. All tests were performed with the endorsement of IAEC of C.L. Baid Metha College of Pharmacy, Chennai, Tamil Nadu, India. The creatures were acclimatized for 10 days beneath research facility conditions. They were housed in polypropylene cages and kept up at 27°C ± 2°C, relative humidity 65 ± 10% beneath a 12-hour light/dark cycle. The creatures were nourished with rat pellet count calories and water advertisement libitum.

Aimal ethical clearance for performing the tests on animal was gotten from the Institutional Animal Ethical Committee (IAEC). Each test bunch had a partitioned set of creatures and care was taken to guarantee that creatures utilized for one reaction were not utilized somewhere else. Creatures were habituated to research facility conditions for 48 h earlier to exploratory convention which minimizes any nonspecific stress.

Method

Before starting treatment, the reproductive cycles of the rats were synchronized by the following method. 100µg estradiol dissolved in 2 ml olive oil was injected subcutaneously. After 24 hr period all rats are received intramuscular injections of 50 µg progesterone dissolved in olive oil. After few hours, vaginal smears were obtained by vagina lavage to monitor ovulation and oestrous cycle. Vaginal smears were prepared by washing vaginal opening with 0.9% w/v of sodium chloride with a glass dropper and placed in a clean glass slide and viewed under light microscope at 40X magnification. Examination of vaginal smears showed that all the animals were in the estrous stage.

All the animals were weighed daily after drug administration for 10 days. The suitable sensitive rats were divided into four groups of six each as follows;

Experimental design

- Group I - Normal Control animals 1ml/kg of CMC solution.
- Group II -rats were administered *Kadugurogini Chooranam* 100mg/kg for 10days,
- Group III- rats were administered *Kadugurogini Chooranam* 200mg/kg for 10 days
- Group IV- received Clomiphene 10mg/kg and served as standard. All the drugs were given orally.

After that 2ml of blood was collected by retro orbital puncture method. Blood samples were centrifuged for 15 minutes at 4000 rpm and the separated serum samples were frozen at -20°C and kept for later estimation of LH, FSH and Estradiol by ELISA method.

Hormonal Biochemical assay

The method employed was Microwell Enzyme Linked Immunosorbent Assay (ELISA) using analytical grade reagents.

Free radical scavenging ability was measured by following methods:

1. ABTS radical activity
2. Inhibition of lipid peroxidation activity
3. Superoxide scavenging assay activity
4. Nitric oxide radical scavenging activity

ABTS radical scavenging activity: [6]

ABTS solution (7 mM) was mixed with potassium persulfate (2.45 mM) solution and kept for overnight within the dull to abdicate a dull coloured solution containing ABTS radical cations. Before the study, the ABTS radical cation was diluted with 50% methanol for an starting absorbance of approximately 0.70±0.02 at 745 nm, with temperature control set at 30°C. Free radical movement was evaluated by blending 300 µl fluid root extricate of *Kadugurogini Chooranam* with 3.0 ml of ABTS working standard in a microcuvette. The decrease in absorbance was measured exactly one minute after mixing the solution, at that point up to 6 min.

Inhibition of lipid peroxidation: [7]

Lipid peroxidation restraint of aqueous root extract of *Kadugurogini Chooranam* was performed according to the previous method with minor modifications. In brief, one ml of each concentration (100, 250, 500, 750, and 1,000 mg/L [w/v]) of each Fluid root extract of *Kadugurogini Chooranam* was separately added to 50 Ml of phosphate buffered saline (PBS) at ae proportion of 1:4 (w/v), at that point 0.5 Ml of 24 mm ferrous sulfate and 0.5 Ml of PBS were added. The blend was shaken vigorously and incubated at 37°C for 15 min. Another, 0.5 Ml of 20% (w/v) trichloro acidic corrosive and 1 Ml of 0.8% (w/v) thiobarbituric corrosive were added to the mixture. After boiling at 95°C and cooling for 30 min, the blend was centrifuged at 2,200×g for 20 min at 25°C. The absorbance was measured at 532 nm by spectrophotometer (Shimadzu show UV-1601, Japan).

Superoxide anion scavenging assay:[8]

The test for superoxide anion radical scavenging activity was supported by riboflavin-light-NBT system. Briefly, 1 ml of aqueous root extract of *Kadugurogini Chooranam* was taken at distinctive concentrations (25 to 500 µg/ml) and blended with 0.5 ml of phosphate buffer (50 mm, pH 7.6), 0.3 ml riboflavin (50 mm), 0.25 ml PMS (20 mm), and 0.1 ml NBT (0.5 mm). Response was begun by lighting up the response mixture employing a fluorescent light. After 20 min of incubation, the absorbance was measured at 560 nm. Ascorbic acid was utilized as standard.

Nitric oxide free radical scavenging activity:[9]

Saline Sodium nitroprusside (5mM) in phosphate buffered was mixed with diverse concentrations of fluid root extract of *Kadugurogini Chooranam* and incubated at 25℃ for 150 min. The test was mixed with griess reagent (1% sulfanilamide, 2% H3PO4 and 0.1% N-(1-napthyl) ethylenediamine dihydrochloride). The

absorbance of the chromophore formed during diazotization of nitrite with sulfanilamide and subsequent coupling with NED was perused at 546 nm employing a UV–VIS spectrophotometer. The restraint of nitric oxide formation was decided with regard to standard potassium nitrite within the same way with Griess reagent. The comes about have been communicated as ascorbic acid equivalent which has been utilized as a standard.

The percentage inhibition was calculated according to the formula:

Scavenging effect (%) = [(controlabsorbance−sampleabsorbance) /(controlasbsorbance)]×100

RESULT

Table 1: Effect of aqueous root extract of *Kadugurogini Chooranam* on PCOD induced female Wister albino rat:

S.NO	Group	Treatment and dose	Weight of Uterus/gm	Weight of ovary/gm
1	Normal	2ml/kg 2% CMC	0.421±1.20	0.123±1.10
2	Test group	*Kadugurogini Chooranam* – 5g/Kg	0.454±1.40	0.137±1.02
3	Test group	*Kadugurogini Chooranam* – 10g/Kg	0.457±2.11*	0.147±1.05*
4	STD	Clomiphene 10mg/Kg	0.488±0.87	0.160±1.01

Values are expressed as Mean±SEM (n= 6) one-way ANOVA followed by Dunnet's multiple comparison test. Where the values are ns P

Table 2: Effect of aqueous root extract of *Kadugurogini Chooranam* on Serum Concentration of reproductive hormones of female Wister albino rat

S. No	Group	Treatment dose	LH (IU/ml)	FSH (IU/ml)	Estradiol (pg/ml)	Progesterone (pg/ml)
1.	Normal	2ml/kg 2% CMC	0.28±0.12	0.33±0.24	54.10±3.5	9.03±1.65
2.	Test-I	100 mg /kg	0.30±0.07	0.36±0.10	38.78±1.2	6.30±2.32
3.	Test-II	200 mg /kg	0.36±0.02	0.55±0.16	33.41±1.1	6.68±0.41
4.	Standard	Clomiphene 10mg/kg	0.66±0.27	0.73±0.21	27.17±18	7.2±0.30

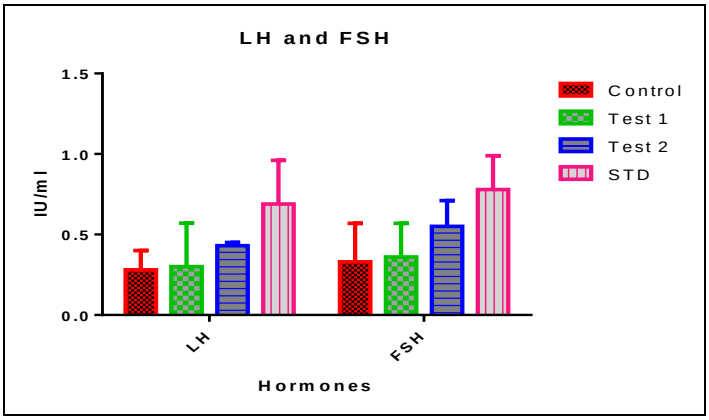

Figure 1: Effect of aqueous root extract of *Kadugurogini Chooranam* on Hormone level of PCOD induced female Wister albino rat

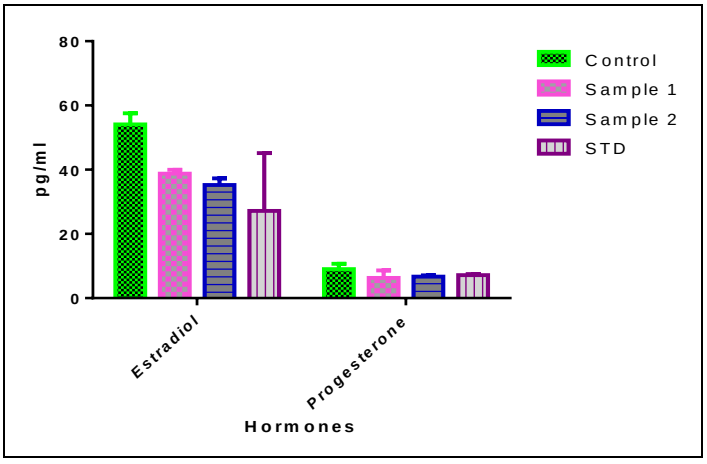

Figure 2: Effect of aqueous root extract of *Kadugurogini Chooranam* on Hormone level of PCOD induced female Wister albino rat

Discussion on ovulation inducing activity:

The two important fertility hormones plasma estrogen (B-estradiol) and progesterone, in females. The present study was to determine the effect of graded doses 100 and 200 mg/kg of aqueous root extracts of *Kadugurogini Chooranam*. In table 1 administration of aqueous root extracts of *Kadugurogini Chooranam* for test group at 5mg and 10 mg doses and results were noted, Clomiphene citrate used as a standard. Compared to the standard drug, in the dose of 10 mg aqueous root extracts of *Kadugurogini Chooranam* was significantly act on the weight of the uterus and ovary. The results showed that aqueous root extracts of *Kadugurogini Chooranam* significantly decreased plasma levels of estrogen and progesterone of the female Wistar rats in a dose dependent manner. Administration of aqueous root extract of *Kadugurogini Chooranam* reduced estrogen levels shown in Table-2 and Fig-1 and 2. The pituitary-gonadal axis is significant for the balancing of the reproductive system hence any distortion to this axis can be deleterious. Follicule stimulating hormone stimulates maturation of the Graafian follicle while leutinizing hormone causes it to synthesize testosterone which is then converted to estrogen by aromatase.

Proper equilibrium between estrogen and progesterone is essential for implantation, and any disturbance in the level of these hormones may affect the fertility. The phytochemical constituents such as isoflavones along with coumestans (also flavonoids) and lignans belong to a class of substances known as nonsteroidal phytoestrogens, and they produce infertility in animals. In addition, it has also been proved that several commonly occurring flavonoids mimic the biological effects of 17β-estradiol by virtue of their ability to bind and activate the nuclear estrogen receptors.

Free radical-scavenging ability using various methods by aqueous root extracts of *Kadugurogini Chooranam*

Tabel 3: EC_{50} value of root extracts of *Kadugurogini Chooranam*

S.NO	METHOD	Aqueous root extracts of *Kadugurogini Chooranam*	Standard Vitamin-C
1	ABTS radical activity	65.89	69.23
2	Inhibition of lipid peroxidation activity	62.16	68.25
3	Superoxide scavenging assay activity	68.49	73.59
4	Nitric oxide radical scavenging assay	61.49	66.16

The above results are shows EC_{50} value of the *Kadugurogini Chooranam.*

Discussion on free radical scavenging activity:

Compared to the standard the EC 50 values of aqueous root extracts of *Kadugurogini Chooranam* at different concentration have significant antioxidant property.

CONCLUSION

The present study has revealed that the aqueous root extracts of *Kadugurogini Chooranam* contains substantial amount of phenolic compounds and thus, can be inferred that these phenolics are responsible for its marked antioxidant activity as assayed through various *in vitro* models used in this study. This is consistent with several reports that have shown close relationship between total phenolic contents and ant oxidative activity of fruits, plants and vegetables. Therefore, aqueous root extracts of *Kadugurogini Chooranam* have considerable antioxidant properties and the consumption of this under-exploited plant may play a role in preventing human diseases in which free radicals are involved, such as cancer, cardiovascular disease, and premature aging. In preliminary study of *Kadugurogini Chooranam* possess a significant effect on hormonal level and weight of the uterus and ovary on PCOS induced female wister albino rats. However, further investigations on the *in vivo* studies of antioxidant activity of the different antioxidant mechanisms and ovulation inducing activity are warranted.

Conflict of Interest

None declared.

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None declared.

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