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Research Article

Protective Role of Seed Extract of *Tephrosia purpurea* in Letrozole Induced Polycystic Ovary Syndrome in Wistar Rats

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Abstract

Background and Objective: Polycystic Ovary Syndrome (PCOS) is very common heterogeneous endocrinological and metabolic disorder in women of reproductive age which leads to infertility/subfertility. The medications available are associated with so many side effects. Therefore; the current study aimed to elucidate the therapeutic efficacy of seed extract of *Tephrosia purpurea* (TP) in letrozole induced polycystic ovary syndrome (PCOS) in female rat model. **Materials and Methods:** Letrozole was administered orally (1 mg kg⁻¹ body weight) for 21 days to induce PCOS condition in female rat model (*Rattus norvegicus*). Three different doses of ethanolic seed extract were administered to PCOS induced rats orally (100, 200 and 300 mg kg⁻¹) for 28 days. On the completion of experimental period, different parameters were studied viz. ovarian weight, ovarian tissue biochemistry (lipid peroxidation, reduced glutathione and superoxide dismutase), hormone assays (testosterone, estrogen, progesterone, FSH, LH, leptin) and histopathology. **Results:** Ovarian PCOS-induced female rats revealed significant increase in ovarian weight lipid peroxidation and blood plasma testosterone, luteinizing hormone and follicle stimulating hormone when compared to control group, whereas, there was a reduction in the level of blood plasma estradiol, progesterone leptin and ovarian tissue reduced glutathione and superoxide dismutase activities. The ethanolic seed extract restored the anomalies in biochemical, hormonal and histological parameters suggesting it might be used in the management of PCOS. **Conclusion:** Therefore, it might be suggested that ethanolic seed extract of *Tephrosia purpurea* might be used in the management of PCOS.

Key words: Polycystic ovary syndrome, *Tephrosia purpurea*, metabolic disorder, reproductive age, letrozole, testosterone, lipid peroxidation

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Polycystic ovary syndrome (PCOS) is one of the most common reproductive and endocrine disorders affecting ovarian follicular development and consequently ovulation which occurs due to imbalance in steroidal hormones. So far it is being recognized that PCOS results from combination of biochemical, genetic as well as environmental factors including hyperandrogenism¹⁻⁴. This disorder affects not only reproductive physiology leading to an anovulatory condition but also the endocrine and metabolic systems⁵. Elevated levels of androgens are considered a characteristic sign of this syndrome, caused by an excess of ovarian androgen production from multiple small follicles, anovulation and insulin resistance in the reproductive life span of female⁶. Therefore, higher androgen level is the main marker of PCOS patients⁷.

The PCOS can be treated and cured in a number of ways. Current available modes of treatment of PCO are still a matter of investigation and controversial among the researchers. Many of the proposed drugs are reported to have various side effects following prolonged usage^{8,9}. These medications are used to regulate the reproductive cycle and to stimulate ovulation. So far effective treatment to manage PCOS is a challenge because various drugs used in its treatment cater to different symptoms.

Generally herbs can be defined as a plant part or extract of its part (leaves, root, bark, seed) used for various medicinal purposes including the fragrance of flowers. Since ancient times many naturally available traditionally herbal medicine generally with few or no side effects are used for the treatment of various health issues. Gradually traditional herbal medicine has received a significant attention in global health debate because of their promotive, preventive, curative and rehabilitative role¹⁰.

The search for alternative therapies for PCOS management is receiving increased attention by the researchers if it remains untreated may cause infertility problems^{11,12}. Plants are known to possess a multitude of bioactive compounds which may be helpful in the remedy of various diseases including PCOS. *Tephrosia purpurea* is well known for its richness in various bioactive components¹³. *Tephrosia purpurea* is a species of flowering plant in the family, Fabaceae, a highly branched, sub-erect, herbaceous perennial herb, which grows in poor soils. It has about 400 species distributed throughout the world among which 24 species were recorded in India¹⁴. It is commonly known as 'Sharapunkha' which means that it has the property of healing

all types of wounds¹⁵. It grows on hard and stony grounds¹⁶. Phytochemical investigations suggested that *Tephrosia purpurea* is known for the presence of glycosides such as rutin, quercetin and osyritin; retinoids-deguelin, elliptone, rotenone and tephrosin; flavonoids like lanceolatin A, B and C, purpurin, purpurenone, purpuritenin and sterols such as β -sitosterol¹⁷. The HPTLC estimations showed the presence of quercetin¹⁸. It provides many important components of some preparations such as Tephroli and Yakrifit which are used for various liver disorders¹⁹. In Ayurvedic systems of medicine, various parts of this plant are used as remedy for impotency, asthma, gonorrhoea, rheumatism, diarrhoea, ulcer and urinary disorders²⁰. A current literature survey suggests *Tephrosia purpurea* as a valuable herbal therapy because of its anti-oxidant²¹, anti-bacterial^{17,22}, anti-inflammatory²³, hepatoprotective²⁴, anti-diabetic activity²⁵, wound healing²⁶ properties. A number of other plants like *Glycyrrhiza glabra*²⁷, *Panax ginseng*²⁸, *Mentha spicata*⁷, *Linum usitatissimum*²⁹, *Aloe barbedensis*³⁰, *Cinnamomum zeylanicum*³¹, *Matricaria Chamomilla*³², *Silybum marianum*³³, *Labisia pumila*³⁴ have been utilized to treat the various complications which are associated with endocrine dysfunction like PCOS³⁵.

Considering the beneficial effects reported for *Tephrosia purpurea*, the present study tested the possibility that seed extracts from this plant could be used as a protective herbal therapy to treat PCOS in the female rat.

MATERIALS AND METHODS

Animals and chemicals: Female albino rats of Wistar strain (170 \pm 10 g b.wt.) of same age were used in this study. Animals were housed under standard husbandry conditions (25 \pm 2°C temp, 60-70% relative humidity and 12 h photoperiod) and had access to standard rat feed and drinking water *ad libitum*. The animals were treated and cared in accordance with the guidelines recommended by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Government of India. The study was approved by Institutional Animal Ethics Committee, Guru Ghasidas Vishwavidyalaya, Bilaspur (994/GO/ERe/S/06/CPCSEA).

Chemicals were procured from Sigma-Aldrich Company, USA and Himedia Laboratories Ltd. Mumbai, India. Letrozole was procured from Novartis India Ltd. All the diagnostic kits for hormone assay were procured from Sigma-Aldrich, USA, Monobind Inc., Costa Mesa, USA, DRG International, GmbH, USA.

Collection of plant material (*Tephrosia purpurea*) and extract preparation:

Plant material *Tephrosia purpurea* was collected from the premises of Guru Ghasidas Vishwavidyalaya, Koni, Bilaspur Chhattisgarh, India. The plant identity was authenticated by the taxonomist from Department of Botany, Guru Ghasidas Vishwavidyalaya and a specimen was kept in herbarium of the Department of Botany, Guru Ghasidas Vishwavidyalaya. The plants were shade dried and seeds were separated from the pods. Seed were crushed and the ethanolic (100% ethanol) extract was prepared by using an accelerated solvent extraction unit (DIONEX). Extract was concentrated using rotator vacuum evaporatory (Concentrator plus, Effendorf) revolving at 4000 rpm till sample extract becomes dry and was kept under refrigeration (4°C) till further use.

Preparation of letrozole and TP doses: Letrozole was dissolved in 1% CMC (carboxymethyl cellulose) as a vehicle solvent and the desired volume was added to double distilled water to obtain the concentration of 1 mg kg⁻¹ b.w., *Tephrosia purpurea* extract was prepared by dissolving in distilled water to make final concentrations.

PCOS induction: All the groups except control and *Tephrosia purpurea* (*per se*) were administered letrozole orally daily at a dose of 1 mg kg⁻¹ dissolved in 0.5% CMC for 21 days to induce PCOS condition in female rat model (*Rattus norvegicus*). Three different doses of ethanolic seed extract were administered to PCOS induced rats orally (100, 200 and 300 mg kg⁻¹) for 28 days.

Experimental design: After an acclimatization period of two weeks, rats were randomly divided into following experimental groups:

- Group I** : Normal control (1% CMC, p.o.)
- Group II** : PCOS control (letrozole -1 mg kg⁻¹ p.o.)
- Group III** : *Tephrosia purpurea* (*per se*) (300 mg kg⁻¹ p.o.)
- Group IV** : PCOS (1 mg kg⁻¹ p.o.)+ *Tephrosia purpurea* (100 mg kg⁻¹ p.o.)
- Group V** : PCOS (1 mg kg⁻¹ p.o.)+ *Tephrosia purpurea* (200 mg kg⁻¹ p.o.)
- Group VI** : PCOS (1 mg kg⁻¹ p.o.)+ *Tephrosia purpurea* (300 mg kg⁻¹ p.o.)

Vaginal smear observation: The PCOS condition was confirmed by preparation of vaginal smear to observe reproductive cycle irregularities. Vaginal secretions were collected daily with a plastic pipette by inserting the tip in the vagina of female rats, filled with 15 µL of normal saline. One

drop of collected vaginal fluid was placed on glass slides. Vaginal fluid was fixed by placing the slides on a slide warming table and stained with methylene blue staining solution. After staining, slides were washed to remove the excess stain, dried and observed under light microscope to assess the changes in the estrus cycle. Three types of cells were recognized: Round and nucleated cells were epithelial cells; irregular cells without a nucleus were the cornified cells and the small round cells were the leucocytes, their mutual proportion was used for the determination of different phases of the estrus cycle³⁶.

Sample collection: After completion of experimental period, the rats of all the groups were sacrificed by euthanization in desiccator by using diethyl ether as an anesthesia. The ovaries were collected for weight analysis, lipid peroxidation assay, reduced Glutathione and Superoxide Dismutase assays. The blood samples were collected directly through cardiac puncture in heparinized tubes and centrifuged at 3000 g for 15 min to collect the plasma. The blood plasma was stored at -20°C for ELISA of various hormones. All assays were performed with freshly isolated samples.

Weight analysis: Ovaries from all experimental groups were carefully separated and weighed after the removal of adherent fat. They were weighed using micro-balance (MAB182, WENSAR, Chennai, India) with accuracy of 0.01 mg. The ovarian weight was expressed in grams and documented.

Tissue biochemistry: Excised ovaries were washed in ice cold normal saline and blotted dry for biochemical estimations.

Lipid peroxidation: Thiobarbituric acid reactive substances (TBARS) are produced during oxidative damage to cell membrane. Malondialdehyde (MDA), one of the major lipid breakdown product and commonly used parameter to assess lipid peroxidation. Ovaries were excised and weighed for the preparation of 10% tissue homogenates in 20 mM Tris Hydrochloride (HCl) buffer (pH-7.4). The homogenates were centrifuged at 3000 g for 15 min at 4°C and supernatant was subjected to thiobarbituric acid (TBA) assay by mixing it with 8.1% SDS, 20% acetic acid, 0.8% TBA and boiling for 1 h at 95°C. The reaction mixture was immediately cooled in running water and vigorously shaken with n-butanol and pyridine reagent (15:1) and centrifuged for 10 min at 1500 g. The absorbance (A) of the upper phase was measured at 534 nm. LPO was expressed as TBARS in nmol/g tissue weight by taking 1,1,3,3 tetraethoxypropane (TEP) as standard. The standard curve was calibrated using 10 nM concentration of TEP³⁷.

Reduced glutathione: The thiol content of ovarian tissues was measured by using the modified method of Sedlak and Lindsay³⁸. About 0.1 mL of tissue homogenate was added to 1.5 mL of 0.2M Tris buffer (pH 8.2) and 0.1 mL of 0.01 M dithio-bis (2-nitrobenzoic acid, DTNB), methanol was added to adjust total volume upto 10 mL. The sample mixture was incubated at 37 °C for 30 min. Following incubation period of 30 min the mixture was centrifuged at 3000 rpm for 15 min. The absorbance of the yellow colored supernatant was measured at 412 nm using Perkin Elmer Spectrophotometer (Lambda 25). The molar extinction coefficient of 13,100 was used to calculate GSH content.

Superoxide dismutase: Superoxide dismutase (SOD) level in ovarian tissues was assessed by using the modified method of Kakkar *et al.*³⁹. About 0.2 mL of tissue homogenate and to this whole reaction mixture containing (1.2 mL of sodium pyrophosphate, pH 7.0, 0.052 Mm, 0.1 mL of phenazine methosulphate (PMS), 185 µL and 0.3 mL of NBT (300 µM). The NADH (0.5 µM) was added to each tube at 30 °C. Reaction was stopped by the addition of 2 mL of glacial acetic acid. The reaction mixture was stirred and 4 mL of n-butanol and allowed to stand for 10 min. The mixture was centrifuged to separate butanol layer containing chromogen and absorbance was taken at 560 nm. The pure butanol was used as blank.

Histopathological studies: Ovaries of the different experimental groups were fixed immediately in Bouin's fixative, processed through different steps of hydration and dehydration to prepare blocks in paraffin wax and paraffin sections of 5 mm thickness were cut. Hematoxylin-eosin stained slides were observed under light microscope.

Statistical analysis: The experimental data were expressed as Mean ± SE. Statistical analysis was performed using one-way ANOVA followed by Student's t-test with the SPSS 16.0 statistical software (SPSS, Chicago, IL, USA). A probability value $p < 0.05$ (*) or $p < 0.01$ (**) was considered to be statistically significant.

RESULTS

Effect on ovarian weight: Letrozole induced PCO rats showed significant increase in ovarian weight. However, alcoholic seed extract of *Tephrosia purpurea* was observed in ovarian letrozole induced PCO rats. About 300 mg kg⁻¹ dose of TP seed showed the significant reversal in ovarian weight (Fig. 1).

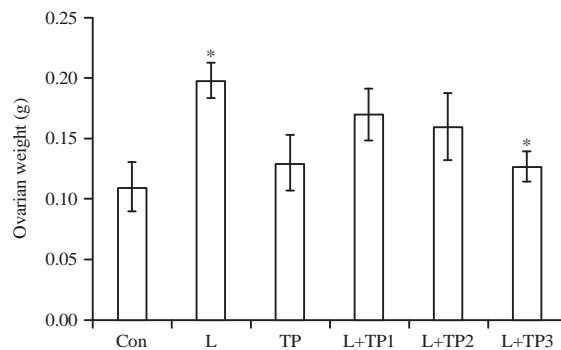


Fig. 1: Effect of alcoholic seed extract of *Tephrosia purpurea* on ovarian weight of letrozole induced (PCOS) rats. Histogram represents Mean+SE
Con: Control, L: Letrozole, TP: *Tephrosia purpurea*, n = 6, * $p < 0.05$, Con vs. L, L vs. L+TP1, L vs. L+TP2 and L vs. L+TP3

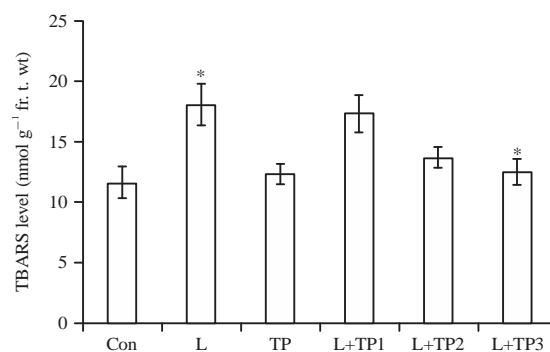


Fig. 2: Effect of alcoholic seed extract of *Tephrosia purpurea* on thiobarbituric reactive substance (TBARS) level of letrozole induced (PCOS) rats Data represents Mean+SE
Con: Control, L: Letrozole, TP: *Tephrosia purpurea*, n = 6, * $p < 0.05$, Con vs. L, L vs. L+TP1, L vs. L+TP2 and L vs. L+TP3

Tissue biochemistry observations

Oxidative stress and antioxidant status: Letrozole induced PCO rats revealed remarkable increase in lipid peroxidation expressed in terms of thiobarbituric acid (TBARS). The increment in lipid peroxidation (LPO) in letrozole induced polycystic rat ovaries suggests that free radicals generation and role of redox imbalance which is mainly responsible cause for PCO condition as a result of generation of reactive species. Administration of different doses of alcoholic seed extract *Tephrosia purpurea* resulted in significant decrease in its level because of the anti-oxidative property of plant extract (Fig. 2).

Letrozole induced PCOS female rats exhibited significant reduction in glutathione (GSH) level. Whereas, *Tephrosia purpurea* doses at three different concentrations 100, 200

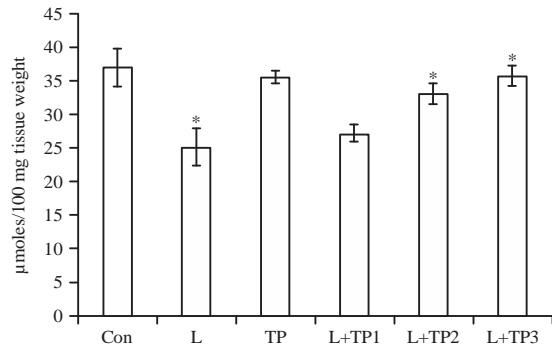


Fig. 3: Effect of alcoholic seed extract of *Tephrosia purpurea* on reduced glutathione (GSH) level of letrozole induced (PCOS) rats. Data represents Mean+SE

Con: Control, L: Letrozole, TP: *Tephrosia purpurea*, n = 6, *p<0.05, Con vs. L, L vs. L+TP1, L vs. L+TP2 and L vs. L+TP3

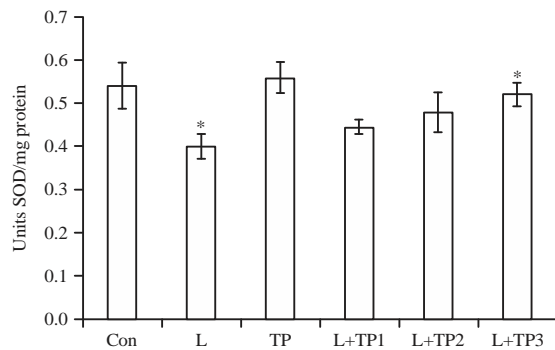


Fig. 4: Effect of alcoholic seed extract of *Tephrosia purpurea* on Superoxide dismutase (SOD) level of letrozole induced (PCOS) rats. Data represents Mean+SE

Con: Control, L: Letrozole, TP: *Tephrosia purpurea*, n = 6, *p<0.05, Con vs. L, L vs. L+TP1, L vs. L+TP2 and L vs. L+TP3

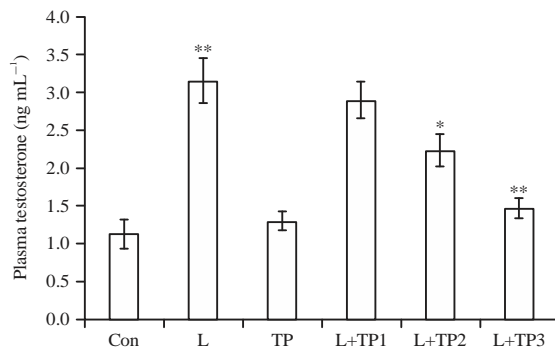


Fig. 5: Effect of alcoholic seed extract of *Tephrosia purpurea* on plasma testosterone level of letrozole induced (PCOS) rats. Data represents Mean+SE

Con: Control, L: Letrozole, TP: *Tephrosia purpurea*, n = 6, Con vs L. (**p<0.01), L vs. L+TP1, L vs. L+TP2 (*p<0.05) and L vs.+TP3 (**p<0.01)

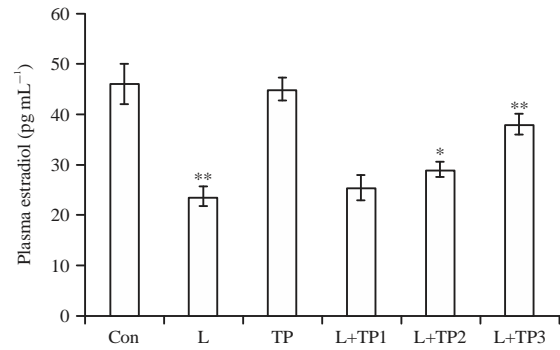


Fig. 6: Effect of alcoholic seed extract of *Tephrosia purpurea* on plasma Estradiol level of letrozole induced (PCOS) rats. Histogram represents Mean+SE

Con: Control, L: Letrozole; TP: *Tephrosia purpurea*, n = 6, Con vs. L (**p<0.01), L vs. L+TP1, L vs. L+TP2 (*p<0.05) and L vs. L+TP3 (**p<0.01)

and 300 mg kg⁻¹ showed recovery in antioxidant enzymes (Fig. 3). A significant decrease was observed in SOD activity of PCO rat. However, alcoholic seed extract were noted very effective in restoring the activity of SOD (Fig. 4).

Hormone analysis

Testosterone: The PCOS induced female rats showed significant increase in level of testosterone, as hyperandrogenism is one of the major etiology of PCOS. Administration of TP at the 300 mg kg⁻¹ were able to maintain the normal circulatory plasma testosterone level (Fig. 5).

Estradiol and progesterone: A significantly low circulatory plasma estradiol and progesterone level was found in letrozole induced PCO rats. The PCOS female rats followed by treatment of TP (300 mg kg⁻¹) increases the plasma estradiol level towards the control level (Fig. 6, 7).

Follicle stimulating hormone(FSH) and LH: Plasma level of FSH and LH revealed significant increase when compared to the control group. When the PCO females were treated with different doses of alcoholic seed extract of *Tephrosia purpurea* the LH and FSH circulatory plasma level was recovered significantly (Fig. 8, 9).

Leptin: Leptin level in plasma of PCO rats showed a significant increase as compared to control group. However, *Tephrosia purpurea* therapy administration showed recovery comparable to the control (Fig. 10).

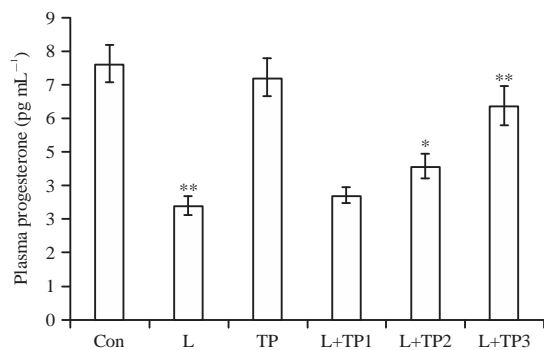


Fig. 7: Effect of alcoholic seed extract of *Tephrosia purpurea* on plasma progesterone level of letrozole induced (PCOS) rats. Histogram represents Mean+SE
 Con: Control, L: Letrozole, TP: *Tephrosia purpurea*, n = 6, Con vs. L (**p<0.01), L vs. L+TP1, L vs. L+TP2 (*p<0.05) and L vs. L+TP3 (**p<0.01)

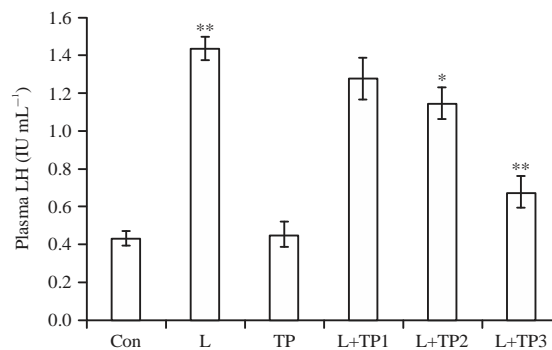


Fig. 9: Effect of alcoholic seed extract of *Tephrosia purpurea* on plasma LH level of letrozole induced (PCOS) rats. Histogram represents Mean+SE
 Con: Control, L: Letrozole, TP: *Tephrosia purpurea*, n = 6, Con vs. L (**p<0.01), L vs. L+TP1, L vs. L+TP2 (*p<0.05) and L vs. L+TP3 (**p<0.01)

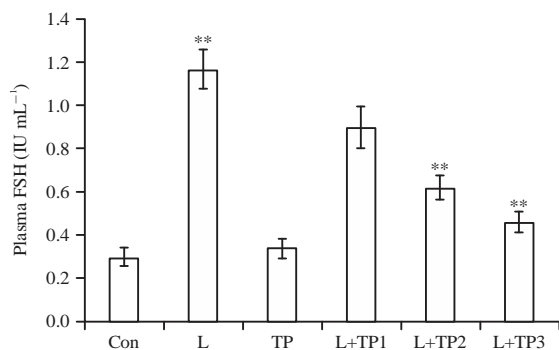


Fig. 8: Effect of alcoholic seed extract of *Tephrosia purpurea* on plasma FSH level of letrozole induced (PCOS) rats. Histogram represents Mean+SE
 Con: Control, L: Letrozole, TP: *Tephrosia purpurea*, n = 6, Con vs. L (**p<0.01), L vs. L+TP1, L vs. L+TP2 (**p<0.01) and L vs. L+TP3 (**p<0.01)

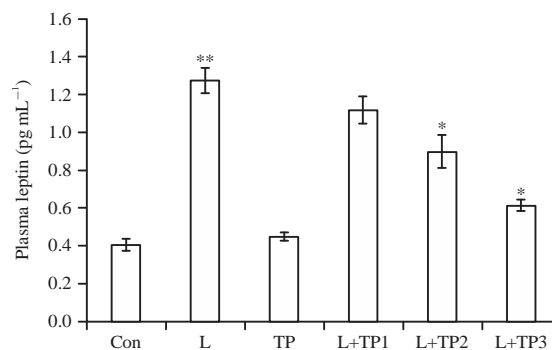


Fig. 10: Effect of alcoholic seed extract of *Tephrosia purpurea* on plasma leptin level of letrozole induced (PCOS) rats. Histogram represents Mean+SEM
 Con: Control; L: Letrozole; TP: *Tephrosia purpurea*, n = 6, Con vs. L (**p<0.01), L vs. L+TP1, L vs. L+TP2 (*p<0.05) and L vs. L+TP3 (*p<0.05)

Histopathology: Ovarian histomicrographs of the letrozole induced PCO female showed many immature cysts along with the marked atresia as well as withering of cells of granulosa layer. A significant number of atretic follicles were observed in the cortex of ovary which was not found in control ovarian histological architecture. Many cell debris were noted in the antrum of follicle. However, the different doses of alcoholic seed extracts of *Tephrosia purpurea* showed their efficacy very differently in according to the dose dependent manner. However, 300 mg kg⁻¹ dose of alcoholic seed extract of *Tephrosia purpurea* was noted to be most effective which reversed letrozole polycystic induced cellular architecture almost equivalent to control group of ovary (Fig. 11).

DISCUSSION

Herbal medicines are valuable and freely available resource for primary health care. The heterogeneity of PCOS is reflected by the existence of several animal models, it is a challenge to create a single animal model that expresses all the characteristics of PCOS. The female rats mimics all women characters of PCO. Three doses of *Tephrosia purpurea* (100, 200 and 300 mg kg⁻¹ b.wt.) were given to the letrozole induced PCOS rats. Finding showed a dose dependent effect of the seed extract where the effective restoration was noted with the dose of 300 mg kg⁻¹ b.wt. This may be explained that this increase is because of the thickening of ovarian capsule

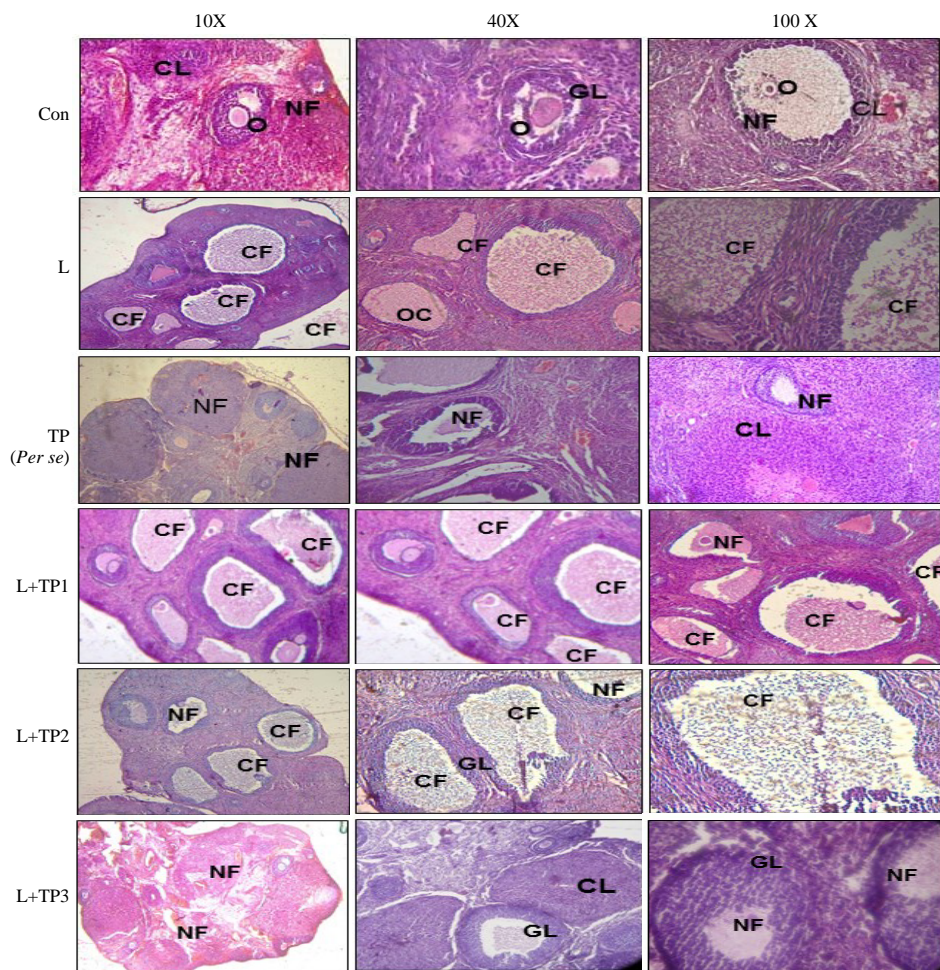


Fig. 11: Histomicrograph showing cellular architecture of ovarian tissue of rat in different experimental groups. Con: Control, L: Letrozole, TP: *Tephrosia purpurea* (10X left panel, 40X middle panel and 100X right panel)
CF: Cystic Follicle, GL: Granulosa layer, O: Oocyte, NF: Normal follicle, CL: Corpus luteum

and the hyperplasia of theca interna cells in the ovary. However, Administration of seed extract of TP brought decrease in the ovarian weight comparable to control.

In the present study PCOS female rats showed irregularity in their estrous cycle. Vaginal smear of TP treated rats showed regularity in reproductive cycle which was not persistent with the estrous. This restoration in reproductive cycle may be considered as a sign of restoration towards normal condition. These findings are in line with the previous findings^{33,40}.

It was observed that the TP seed extract restored the ovarian weight towards the normal range which was noted significantly increased in letrozole induced PCO rats. Results of the present study is in agreement with the previous finding^{34,41}.

Significant decrease was found in the ovarian antioxidative enzymatic activities such as SOD activity and

GSH level of PCO rats, which confirms that during PCOS production of free radicals subsequently weakens the anti-oxidant system. Low level of anti-oxidative enzymes might lead to a situation of weak reproductive immunity and is the major cause behind increased free radical load. It has been previously studied that polyphenols and flavonoids are electron rich compounds hence they donate their electron to neutralize and eliminate the excessive generation of free and also reverse the oxidized state of the antioxidative enzymes⁴². The results are in agreement with earlier findings which reported that secondary bioactive compounds help in restraining the oxidative damages⁴². The safety profile of the seed extract was evaluated by administering the TP extract (*per se*) to separate experimental group, which does not show abnormal change in ovarian weight, LPO, GSH and SOD activity. It is reported that oxidative stress is one of the

pathological factor for PCOS^{43,44}. The concrete explanation of abnormally increment of oxidants might be altering the steroidogenesis in ovaries contributing to increased androgen production as noted in present study as well as by other studies^{43,45}.

The PCOS control group of female rats revealed remarkable reduction in circulatory of estradiol, progesterone and leptin level, but significantly increased testosterone level. Administration of alcoholic seed extract of TP were effectively reversed the abnormal decrease in circulatory plasma level of estradiol, progesterone and leptin. Further, earlier findings have reported that letrozole being a non-steroidal aromatase inhibitor blocks the conversion of testosterone to estradiol. This condition of results in the reduction in estradiol production^{46,47}. Letrozole being an aromatase inhibitor might have increased the circulatory androgen level^{48,49} of PCO rats. The TP treatment to PCOS rats normalized the testosterone level comparable to the normal in a dose dependent manner. Further, PCOS rats revealed increment in the plasma circulatory level of gonadotrophins (LH and FSH) indicating the disruption in coordination between their pulsatility and to that with the reproductive hormones (Testosterone, Estrogen, Progesterone, Leptin, LH and FSH). Seed extract of TP reverse the altered LH and FSH level near the control level, as plant herbal extracts shows modulation of pathogenicity induced by letrozole induced PCOS.

Histological observations showed number of atretic follicles, cystic formation having cell debris. Seed extracts of *Tephrosia purpurea* showed reduction in the size of cysts and less number of atretic follicles. In PCOS condition the corpora lutea is not formed or the numbers of corpora lutea are diminished indicating anovulation and the frequency of estrus cycle is almost nil in PCOS rats. Hyperandrogenism induced follicular atresia is thought to occur by entry of androgens into the granulosa layer of pre-antral follicles, where they bind to the cell receptors and cause the cell death. High circulatory level of androgens may cause deterioration of follicles by increasing the number of pyknotic granulosa cells and degenerating oocytes^{50,51}. However, the seed extract of *Tephrosia purpurea* treatment caused the disappearance of cysts in a dose dependent manner resulting in formation of a number of corpora lutea suggesting the restoration of ovulatory condition along with follicles at different developmental stages.

CONCLUSION

Tephrosia purpurea being excellent antioxidant because of the presence of many bioactive compounds among which

flavonoids is one of them. These bioactive compounds might have impacted directly at cellular level resulting in upregulation of the antioxidative enzymatic system (SOD, GSH), leading therefore in the rescue and restoration of the ovarian cellular damages noted during the induced PCOS condition. Hence, this herbal plant may be suggested as one of the potent traditional drug which can prevent ovarian dysfunction during PCO and in fertility management.

SIGNIFICANCE STATEMENT

The findings of study evidenced the efficacy of *Tephrosia purpurea* seed extract which can be considered as a potential traditional natural drug to treat PCOs. Therefore it might be projected the *Tephrosia purpurea* seed extract could be explored for its clinical use during PCO management in medical science.

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