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Protective Effect of *Euphorbia Thymifolia* L. Root on Reproductive Dysfunction in Female Rats

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ABSTRACT

Euphorbia thymifolia root is having the protective effect against female reproductive dysfunctions. This study is to evaluate the protective effect of ethanolic extract of *Euphorbia thymifolia* root in treating female reproductive dysfunction induced by stress. Forced swimming stress (15min/day for 28 days) and restraint stress (3h/day for 28 days) were the methods employed to induce female reproductive dysfunction in rats. Ethanolic extract of *Euphorbia thymifolia* root was given to rats in two doses, 100 mg/kg and 200 mg/kg for 28 days along with induction of stress and its effectiveness was assessed by observing changes in FSH, LH, Prolactin, Estradiol and Progesterone. The results were analysed by using one-way ANOVA followed by Dunnett's test. *Euphorbia thymifolia* root extract showed a significant protective effect which is evident by increase in the levels of FSH, LH, Progesterone and decrease in the levels of Prolactin and Estradiol which was found to be dose dependent. The protective effect may be due to the presence of various phytochemical constituents like alkaloids, flavonoids and other constituents present in the *Euphorbia thymifolia* root.

Keywords: *Euphorbia thymifolia* L. root, Forced swimming stress, Restraint stress.

INTRODUCTION

Infertility is defined as the inability to conceive after trying for at least one year. Infertility is a raising problem in today's society, influencing around 15% of couples globally. The event of infertility has moved ahead to expanding velocity and may impact 11% of couples of conceptive age.[1] As per World Health Organization, 2–10% and 10–25% of couples worldwide are unable to conceive due to primary and secondary infertility causes respectively. Among these couples, causative components are found in about 30-40% in females and 10–30% in males. In 15-30% of cases, both partners have detectable abnormalities[2] and in some cases without any cause.

There are so many confounding factors that can cause or continue to infertility. The major causes of female infertility are due to ovarian dysfunction, tubal obstruction, polycystic ovarian syndrome, endometriosis, stress and other unexplained factors.[3] Reproductive functions are suppressed under various stress conditions which includes infection, malnutrition, lifestyle factors, restraint, strenuous exercise, surgical trauma, heat, cold, noise exposures and environmental pollution.[4] Prolonged or chronic stress causes anovulation which results in infertility due to suppression of gonadotrophic hormones and oxidative stress.[5]

Normal aging reduces a woman's ability to become pregnant. With age, ovulation becomes slower and less effective, Aging begins to reduce fertility as early as age 30. Pregnancy rates are very low after age 44. Infertility, like any disease, is simply a sign that something is not right inside the body and must be fixed. The body can reverse infertility naturally if given the correct resources. Currently, female infertilities are treated by natural plants, drugs, surgical procedures in addition to dietary and life style changes.

Treatment of infertility with drugs and surgical procedures may lead to complications like multiple pregnancy, twins, ectopic pregnancy, stress, ovarian hyperstimulation syndrome, ovarian cancer, birth defects etc. Although significant advances have been made in treatment of reproductive disorders, there are serious limitations in existing therapies because of cost, utilization and toxicity. Medications from natural sources (medicinal plants) are attractive therapeutic alternatives and supplements to existing therapy and have not really been explored in depth.

Euphorbia thymifolia, also Known as *Chamaecyse thymifolia*, Dudhi, Dugdhikaa, Naagaarjuni and Swaaduparni.[6] This plant is reported to have antiviral,[7] antibacterial, antioxidant,[7] anti-inflammatory[8] and hepatoprotective[8] activities. Roots of *Euphorbia thymifolia* are known to show female fertility improving properties,[6] but not reported scientifically. So the current study was undertaken to evaluate the protective effect of ethanolic extract of *Euphorbia thymifolia* root in rat models against experimentally induced stress models.

MATERIALS AND METHODS

Plant: *Euphorbia thymifolia* fresh roots were collected from Tirupati, Andhra Pradesh, identified and authenticated by Dr. K. Madhava chetty, Asst. Professor, Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh, India.

Ethanolic extract: The roots of *Euphorbia thymifolia* L. was chopped and dried under shade at room temperature and submitted for extraction to Green Chem Herbal Extracts and Formulations, Bangalore, India. The ethanolic extract and COA were obtained from Dr. Rajendran, Green Chem Herbal Extracts and Formulations with Batch no: ETE/RD/01.

Animals: Experimental study was carried out using adult female Wistar albino rats weighing between 175-200g. Animals were housed in a group of 6 in polyethylene cages under standard housing conditions of 12-12h light and dark cycle, temperature 22±2°C and humidity 50±10% with standard feed pellet and free access to water *ad libitum*. Standard hygiene conditions were maintained. Experiment was conducted with strict compliance to ethical principles and guidelines formulated by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and performed in accordance with the Institutional Animal Ethics Committee (IAEC/NCP/66/11) of Nargund college of Pharmacy, Bangalore.

Dose selection based on Acute oral toxicity study: Two doses of ethanolic extract 100 mg/kg and 200 mg/kg of *Euphorbia thymifolia* L. root were selected as per the acute oral toxicity study performed in accordance with Office of Prevention, Pesticides and Toxic Substances (OPPTS) guidelines following the Up and Down procedure.[9] The ethanolic extract of *Euphorbia thymifolia* L. root found safe up to 5000 mg/kg body weight.

Forced swimming stress (FSS) model: Animals with regular estrous cycle were selected and divided into four groups, six animals in each group. The forced swimming stress was induced to all the rats by placing them individually in acrylic plastic pool (60 cm in height x 30 cm in diameter) filled with water up to a depth of 50cm for 15 min/ day for 28 days at ambient room temperature.[10]

Group I: Vehicle control - distilled water, orally (5 mL/kg b.w) for 28 days.

Group II: Forced swimming stress (15 min/day) for 28 days.

Group III and IV: Rats were treated with ethanolic extract of *Euphorbia thymifolia* root (EEET) (100 mg/kg and 200 mg/kg b.w, per oral), continued for 28 days along with induction of stress. Animals were subjected to forced swimming stress for 15 min/day after half an hour of administration of the extract.

After the last stress session on 28th day blood was collected from all the groups by puncturing retro-orbital plexus. Serum was separated. Serum FSH, LH, Prolactin, Estradiol, Progesterone levels were estimated using fully automated chemiluminescence immunoassay (C.L.I.A), certified by Dr. Vamseedhar. A, Pathologist, Sanvi Diagnostic Centre.

Restraint stress (RS) model:

The animals with regular estrous cycle were selected & divided into four groups, six animals in each group. The restraint stress was induced to all the rats by placing them individually inside the plastic cylindrical restrainers (21cm in length x 6cm in diameter) with ventilated sliding doors at ambient temperature.[10, 11, 12]

Group I: Vehicle control - distilled water, orally (5 mL/kg b.w) for 28 days.

Group II: Restraint stress (3h/day) for 28days.

Group III and IV: Rats were treated with ethanolic extract of *Euphorbia thymifolia* root (EEET) (100 mg/kg and 200 mg/kg b.w, p.o), continued for 28 days along with induction of stress. Animals were subjected to restraint stress for 3h/day after half an hour of administration of the extract.

After the last stress session on 28th day blood was collected from all the groups by puncturing retro-orbital plexus. Serum was separated. Serum FSH, LH, Prolactin, Estradiol, Progesterone levels were estimated using fully automated chemiluminescence immunoassay (C.L.I.A), certified by Dr. Vamseedhar. A, Pathologist, Sanvi Diagnostic Centre.

RESULTS

Effect of ethanolic extract of *Euphorbia thymifolia* root on serum FSH, LH, Prolactin, Estradiol and Progesterone levels in forced swimming stress model in female rats

Groups	FSH mIU/mL	LH mIU/mL	Prolactin ng/mL	Estradiol ng/mL	Progesterone ng/mL
Vehicle control	6.253±0.19	17.26±0.30	3.830±0.17	54.51±1.24	39.49±0.86
FSS	2.275±0.19***a	4.50±0.17***a	16.40±0.71***a	98.78±1.62***a	11.26±0.19***a
FSS + EEET (100mg/kg)	4.188±0.12***b	9.07±0.22***b	9.04±0.16***b	69.25±1.14***b	21.28±0.41***b
FSS + EEET (200mg/kg)	5.22±0.30***b	11.03±0.20***b	7.08±0.15***b	67.13±0.85***b	26.19±0.75***b

Values are expressed as Mean ± SEM. Data were analyzed by one way ANOVA followed by Dunnett's *t* test. Number of animals in each group n = 6, [a] comparison made with vehicle control group, [b] comparison made with Forced swimming stress group. *** P<0.001.

Forced swimming group showed significant decrease in the level of serum FSH, LH, Progesterone and significant increase in the Prolactin and Estradiol levels when compared with vehicle control group. Groups treated with ethanolic extract of *Euphorbia thymifolia* root 100mg/kg body weight and 200mg/kg body weight along with the induction of forced swimming stress showed significant increase in the levels of serum FSH, LH, Progesterone and significant decrease in the Prolactin and Estradiol levels when compared with forced swimming stress group.

Effect of ethanolic extract of *Euphorbia thymifolia* root on serum FSH, LH, Prolactin, Estradiol and Progesterone levels in restraint stress model in female rats

Groups	FSH mIU/mL	LH mIU/mL	Prolactin ng/mL	Estradiol ng/mL	Progesterone ng/mL
Vehicle control	6.253±0.19	17.26±0.30	3.830±0.17	54.51±1.24	39.49±0.86
RS	2.72±0.11***a	6.38±0.19***a	14.06±0.43***a	88.85±1.384***a	14.25±0.32***a
RS + EEET (100mg/kg)	4.20±0.23***b	10.11±0.35***b	7.09±0.25***b	62.42±1.02***b	28.30±0.82***b
RS + EEET (200mg/kg)	5.3±0.23***b	13.41±0.39***b	5.04±0.15***b	57.81±1.075***b	32.34±0.84***b

Values are expressed as Mean ± SEM. Data were analyzed by one way ANOVA followed by Dunnett's *t* test. Number of animals in each group n = 6, [a] comparison made with vehicle control group, [b] comparison made with Restraint stress group. *** P<0.001, **P<0.01.

Restraint stress group showed significant decrease in the level of serum FSH, LH, Progesterone and significant increase in the Prolactin and Estradiol levels when compared with vehicle control. Groups that received with ethanolic extract of *Euphorbia thymifolia* root 100mg/kg body weight and 200mg/kg body weight along with the restraint stress showed significant increase in the levels of serum FSH, LH, Progesterone and significant decrease in the Prolactin and Estradiol levels when compared with restraint stress group.

DISCUSSION

Euphorbia thymifolia is well known in folk medicine and well recognized to have different activities towards health improvement. The plant consisting of different active ingredients and notably roots are known to have phytosterols, beta-sitosterol, brassicasterol, alkaloids and terpenes which are known to have protective effect in infertility.

Forced swimming stress (a moderate physical or metabolic stress) and Restraint stress (physical and psychological stress) are the stressor's which were known to induce female reproductive dysfunctions. These two methods were chosen to induce stress in rats.[11]

The estrous cycle in rats involves many histological, physiological, and morphological and biochemical changes within the ovary. During the estrous cycle the maturation and ovulation of preovulatory follicles takes place under the combined and balanced influence of ovarian and extra ovarian hormones. Imbalance in these hormones leads to irregularity in ovarian function and changes in the duration of estrous cycle.[13]

At the endocrine level, stress-induced release of β-endorphan has inhibitory effects upon gonadotropin-releasing hormone (GnRH) release, such that there is a decline of GnRH concentrations in the hypophysial-pituitary portal system within seconds. In addition, there is decreased sensitivity of pituitary gonadotropes to stimulatory effects of GnRH, thus greatly reducing the amount of luteinizing hormone (LH) secreted. A number of studies suggested that stress-induced secretion of both prolactin and glucocorticosteroids mediate this. Finally, glucocorticosteroids act at the level of the ovaries to decrease responsiveness to LH. This has been reported in a variety of species, and is likely due, at least in part to glucocorticosteroids induced decrease in LH receptor number. The result is an extended follicular stage, making overall reproductive cycle length longer and more irregular.[14]

Stress induced groups showed a significant effect on the levels of female reproductive hormones-FSH, LH, prolactin, estradiol and progesterone. Changes in the levels of these hormones was significantly less in animals treated with ethanolic extract of *Euphorbia thymifolia* when compared with the animals which did not receive *Euphorbia thymifolia* extract treatment, suggesting the ability of plant in preserving the female reproductive hormonal homeostasis in stress conditions.

CONCLUSION

The experimental studies carried out on ethanolic extract of *Euphorbia thymifolia* root showed protective effect in treating stress induced female reproductive dysfunction. Further work regarding isolation of bioactive compounds responsible for this potent activity will provide more insight about the role of plant.

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