ANTI-STRESS ACTIVITY OF *EUPHORBIA THYMIFOLIA* L. AQUEOUS ROOT EXTRACT 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 anti-stress activity of aqueous 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. Aqueous 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 estrous cycle and organs weight. The results were analyzed by using one-way ANOVA followed by Dunnett's test. *Euphorbia thymifolia* root extract showed a significant protective effect which is evident by decrease in the duration of proestrous and increase in duration of estrous, metestrous, and diestrous phases. Whereas the weight of adrenal glands noticeably decreased in aqueous extract treated group confirming the anti-stress activity which was found to be dose dependent. The anti-stress activity 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, Estrous cycle, Organ weight.

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 anti-stress activity of aqueous extract of *Euphorbia thymifolia* root in rat models against experimentally induced stress models.

MATERIALS AND METHODS:

Plant materials collection and extraction:

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.

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

Experimental 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.

Experimental model and animal grouping for anti-stress study

Forced swimming stress (FSS) model: Animals with regular estrous cycle were selected and divided into four groups, six animals in each group. The stress was induced to all the rats through forced swimming 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.[9]

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: Rats were treated with aqueous extract of *Euphorbia thymifolia* root (100mg/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 administration of the extract.

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

Every day immediately after the stress session, vaginal smears were examined in all the groups for estimation of estrous cycle. After the last stress session on 28th day all animals were sacrificed by cervical dislocation. Liver, ovaries, uteri and adrenal glands were isolated and weighed.

Restraint stress (RS) model:

Animals with regular estrous cycle were selected & divided into four groups, six animals in each group. The stress was induced to all the rats trough restraint stress by placing them individually inside plastic cylindrical restrainers (21cm in length x 6cm in diameter) with ventilated sliding doors at ambient temperature.[9,10,11]

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: Rats were treated with aqueous extract of *Euphorbia thymifolia* root (100mg/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.

Group IV: Rats were treated with aqueous extract of *Euphorbia thymifolia* root (200mg/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.

Every day immediately after the stress session, vaginal smears were examined in all the groups for estimation of estrous cycle. After the last stress session on 28th day all animals were sacrificed by cervical dislocation. Liver, ovaries, uteri and adrenal glands were isolated and weighed.

Dose selection based on acute oral toxicity study: Two doses of aqueous 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 the Office of Prevention, Pesticides and Toxic Substances (OPPTS) guidelines following Up and Down procedure.[12] The aqueous extract of *Euphorbia thymifolia* L. root found safe up to 5000 mg/kg body weight.

RESULTS

Effect of aqueous extract of *Euphorbia thymifolia* root on estrous cycle (28 Days) in forced swimming stress model in female rats

Groups	Proestrous (Days)	Estrous (Days)	Metestrous (Days)	Diestrous (Days)
Vehicle control	4.5±0.34	6.66±0.33	7.0±0.44	9.83±0.30
FSS	16.67±0.42^{***a}	2.00±0.25^{***a}	2.16±0.30^{***a}	7.0±0.25^{**a}
FSS+AEET (100 mg/kg)	9.16±0.30^{***b}	3.66±0.21^{**b}	4.5±0.22^{***b}	10.67±0.49^{***b}
FSS+AEET (200 mg/kg)	6.83±0.30^{***b}	5.5±0.42^{***b}	5.8±0.16^{***b}	9.83±0.79^{**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; **P<0.01.

Effect of aqueous extract of *Euphorbia thymifolia* root on different organ weights in forced swimming stress model in female rats

Groups	Ovaries (g)	Uterus (g)	Adrenal glands (g)	Liver (g)
Vehicle control	0.031±0.0008	0.19±0.0005	0.01±0.0004	3.378±0.04
FSS	0.023±0.0006^{***a}	0.103±0.001^{***a}	0.021±0.0004^{***a}	2.362±0.02^{***a}
FSS+AEET (100 mg/kg)	0.026±0.0003^{**b}	0.145±0.002^{***b}	0.016±0.0004^{***b}	2.605±0.01^{***b}
FSS+AEET (200 mg/kg)	0.029±0.0001^{***b}	0.17±0.002^{***b}	0.013±0.0002^{***b}	3.04±0.05^{***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; **P<0.01.

Effect of aqueous extract of *Euphorbia thymifolia* root on estrous cycle (28 Days) in restraint stress model in female rats

Groups	Proestrous (Day)	Estrous (Days)	Metestrous (Days)	Diestrous (Days)
Vehicle control	4.5±0.34	6.66±0.33	7.0±0.44	9.83±0.30
RS	14.67±0.33^{***a}	3.16±0.30^{***a}	3.6±0.49^{***a}	6.8±0.74^{***a}
RS+AEET (100 mg/kg)	8.6±0.33^{***b}	5±0.36^{**b}	5.5±0.22^{**b}	9.16±0.40^{**b}
RS+AEET (200 mg/kg)	7.0±0.25^{***b}	5.5±0.42^{***b}	5.8±0.30^{**b}	9.6±0.42^{***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.

Effect of aqueous extract of *Euphorbia thymifolia* root on different organ weights in restraint stress model in female rats

Groups	Ovaries (g)	Uterus (g)	Adrenal glands (g)	Liver (g)
Vehicle control	0.031±0.0008	0.19±0.0005	0.01±0.0004	3.378±0.04
RS	0.024±0.0002^{***a}	0.110±0.003^{***a}	0.019±0.0001^{***a}	2.50±0.01^{***a}
RS+AEET (100 mg/kg)	0.026±0.0002^{***b}	0.13±0.001^{**b}	0.015±0.0005^{***b}	2.67±0.02^{**b}
RS+AEET (200 mg/kg)	0.028±0.0002^{***b}	0.16±0.002^{***b}	0.012±0.0003^{***b}	2.93±0.04^{***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.

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.

Usually in rats the ovulation process occurs in two phases, those are named as pre-ovulatory phase and post-ovulatory phase. The pre-ovulatory phase consisting of proestrous phase (beginning of new cycle), estrous phase (sexual receptivity), similarly the post-ovulatory phase consisting of metestrous phase (shortly after ovulation) and diestrous phase. 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]

The stress induced rats showed significant increase in the mean days of proestrous phase and significant decrease in the mean days of estrous, metestrous and diestrous phases indicating the arrest of follicular development at the initial stages. This disruption in the growth and differentiation of preovulatory follicles may be due to the non-availability of steroidal hormones, which are essential for their maturation and differentiation.[14] *Euphorbia thymifolia* treated rats showed significant decrease in the mean days of proestrous phase which indicates the development of follicles. Significant increase in the mean days of estrous, metestrous and diestrous phases also indicates the further maturation of the follicles, formation of graffian follicles and corpus luteum.

Ovaries are considered to be an aggregate of three endocrine tissues, the stroma, the follicle and the corpus luteum. The weight of these tissues constitutes the net weight of ovaries. The stress induced rats showed significant decrease in the weight of ovaries. The decrease in weight of ovaries may be due to decrease in activity of stroma, follicle and corpus luteum in the ovary, non-availability of either gonadotrophic or steroidal hormones.[15] *Euphorbia thymifolia* treated groups showed significant increase in the weight of ovaries which may be due to the influence of gonadotrophic and steroidal hormones.

The stress treated rats showed significant decrease in the weight of uterus and liver. The decrease in weight of uterus may be due to the non-availability of the hormones required for the development of uterus and the decrease in weight of liver may be due to oxidative stress and depletion of stored contents.[16] *Euphorbia thymifolia* treated groups showed significant increase in the weight of uterus and liver which may be due to uterotrophic effect and hepatoprotective activity combined with antioxidant activity showing a synergistic effect to prevent the process of initiation and progress of hepatocellular damage respectively.[16]

The stress induced rats showed significant increase in the weight of adrenal glands. The increase in weight of adrenal glands may be due to the active involvement of the hypothalamic-pituitary-adrenal (HPA) axis & sympathetic activation, which is highly responsive to stress. The adrenal hypertrophy takes place in response to the secretion of adrenocorticotrophic hormone (ACTH) from the pituitary for increased corticosterone from cortical cells to combat stress.[17,18] *Euphorbia thymifolia* treated groups showed significant decrease in the weight of adrenal gland which may be due to the reversal of the stress-induced adrenomedullary response and decreased production of corticotropic hormone. Similar studies with different herbal sources were reported by Adkar Prafulla *et al*.,[19] and Santosh kumar gupta *et al*.[20]

CONCLUSION

The experimental studies carried out on aqueous extract of *Euphorbia thymifolia* root showed retardation and anti-stress effect towards the process leading towards 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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