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FERTILITY INDUCING EFFECT OF *CADABA FRUTICOSA* (L.) DRUCE IN FEMALE ALBINO RATS

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ABSTRACT

The parts of plant *Cadaba fruticosa* are used for female reproductive disorders. Hence the present research was carried out to assess the fertility inducing effect of aerial parts of *Cadaba fruticosa* in wistar albino female rats. The effect of ethanolic extract of *Cadaba fruticosa* on ethinyl estradiol induced anovulatory infertility model was examined. The extract at two different doses of 125 mg/kg and 250 mg/kg were administered orally as low and high dose respectively. The effect of extract on estrous cycle, serum estrogen level, serum FSH level, and fertility index were also evaluated to study the fertility inducing activity. The high dose of ethanolic extract of *Cadaba fruticosa* exhibited statistically a high significant ($P<0.001$) level in regularization of estrous cycle, statistically a high significant increase ($P<0.001$) in serum estrogen level and statistically a significant increase ($P<0.01$) in serum FSH level were observed when compared to negative control group. The high dose of ethanolic extract produced statistically a high significant ($P<0.001$) increase in number of newborns when compared to negative control group. The ethanolic extract from aerial parts of *Cadaba fruticosa* induces fertility in ethinyl estradiol induced anovulatory female rats.

Keywords: Clomiphene citrate, Ethinyl estradiol, ELISA, Estrous cycle, Fertility, Infertility

INTRODUCTION

Infertility, although not life threatening, instigates extreme mental distress and disturbance that can only be best portrayed by infertile couples themselves. According to 2010 report, 48.5 million couples were infertile worldwide. Levels of primary infertility altered a little between 1990 and 2010, in most world regions [1]. Poly cystic ovarian syndrome (PCOS) is the most common female endocrine disorder with a prevalence of approximately 5–10% in women of reproductive age [2]. Patients with PCOS normally exist with a history of infertility due to anovulation. Anovulation may result in the imbalance of hormones. During ovulation process, more numbers of hormones are involved and their interactions must be balanced, and any disturbances in the hormonal balance can obstruct ovulation that leads to anovulation [3]. To treat infertility, traditionally an immense number of plants have been applied, for example; *Asparagus racemosus, Carmona retusa, Dioscorea villosa, Leea microphylla, Lepidium meyenii, Ocimum americanum, Withania somnifera,* and *Ziziphus jujube etc.* In India, *Cadaba fruticosa* commonly known as Indian Cadaba; is used traditionally to treat various female reproductive health problems such as amenorrhea, dysmenorrheal and uterine obstructions [4] without scientific core. *C. fruticosa* has many active phytoconstituents such as non-tannin phenolics, kaempferol, new spermidine alkaloid cadabicine, L-stachydrine and 3-hydroxystachydrine, three novel spermidine alkaloids one capparisine and an aromatic acid, ά,β-dihydroferulic acid, novel sesquiterpenoid cadabicine methyl ether and cadabicine diacetate, besides a sesquiterpene and cadibicilone. It also contains 3(4-formylphenoxy) -4- methoxybenzaldehyde, methyl cinnamate, methyl ferulate ether, ether of p-cinnamic acid-m- ferulic acid, thiazolidine compound. It also shows the presence of quercetin, isoorientin, hydroxybenzoic acid, syringic acid, vanillic acid and 2-hydroxy- 4-methoxy benzoic acid [4, 5]. Previous studies on the plant exposed that it possesses antibacterial and antifungal [6], antipyretic [7], antidiabetic [8], hepatoprotective [9], antioxidant [10], cytotoxic and anticancer [11]. Hence an attempt has been made to investigate the plant for its fertility inducing activity.

MATERIALS AND METHODS

Plant substance:

The fresh aerial parts of plant *C. fruticosa* (L.) Druce belongs to the family Capparidaceae were collected after flowering

season from natural habitats of Viruthunagar District of Tamil Nadu, India. Taxonomically identified and authenticated by botanist Dr. V. Chelladurai, Research Officer- Botany (Retd.), Central Council for Research in Ayurveda and Siddha, Government of India. The voucher specimen number P2401 of *C. fruticosa* have been deposited at the herbarium in Department of Botany, Presidency College, Chennai, India. The samples were washed thoroughly in running tap water to remove soil particles and adhered debris and finally washed with sterile distilled water. The aerial parts of plant were shade dried and ground into fine powder. The powdered material was extracted with ethanol for 24 h using soxhlet apparatus. The yield of the ethanolic extract of *C. fruticosa* was 14% w/w.

Experimental animals:

Swiss albino female mice weighing between 25 and 30 g and female wistar albino rats weighing between 150 and 180 g were used. The experimental protocol was approved (Approval No. 1046/ 29.11.2013) by the Institutional Animal Ethical Committee of Rajah Muthiah Medical College and Hospital (RMMC&H) (Reg. No. 160/ 1999/ CPCSEA), Annamalai University, Annamalai Nagar, Cuddalore District of Tamil Nadu, India. Acclimatization, housing and feeding conditions were followed as per Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines.

Inducing and Standard drug:

A known oral contraceptive drug ethinyl estradiol (Pubchem CID 5991) was used to induce anovulation. A known fertility inducing agent clomiphene citrate (Pubchem CID 2800) was used as standard drug in ethinyl estradiol induced anovulatory infertility model. Ethinyl estradiol and clomiphene citrate were procured from Sigma- Aldrich, USA.

ELISA kits:

Rat serum estrogen and FSH ELISA kits were procured from Shanghai Yehua biological ltd., Shanghai, China.

Acute oral toxicity study:

The experiment was conducted on adult female young swiss albino mice weighing between 25-30 g. The ethanolic extract of *C. fruticosa* was formulated as suspension in distilled water with 2% tween 80 as a suspending agent. For the acute toxicity studies freshly prepared suspension was used. Test was performed as per OECD 423 guideline for testing of chemicals [12]. The animals were treated orally with ethanolic extract at a dose of 2000 mg/kg bw. The zero percent mortality for ethanolic extract of *C.*

fruticosa was found at a dose of 2000mg/kg. The test was repeated with another group. There was no morbidity/mortality was found at the dose of 2000 mg/kg bw. The findings indicated that the ethanolic extract of *C. fruticosa* was safe when tested in mice at dose level of 2000 mg/kg bw. It comes under the GHS category 5. Hence the LD_{50} cut-off value of ethanolic extract of *C. fruticosa* was 2500 mg/kg bw. The $1/10^{th}$ dose (250 mg/kg bw) and $1/20^{th}$ dose (125 mg/kg bw) were taken as higher and lower doses from LD_{50} cut-off value for further study.

Inducing method for anovulation:

All groups other than group 1, with regular cycling female rats were treated orally twice daily for four consecutive days with ethinyl estradiol 0.03 mg/kg bw, starting on the day of estrus after confirmation through smear test, to inhibit ovulation. On day 5 after the final dose, ethinyl estradiol blocked the ovulation [13, 14].

Statistical analysis:

The data from biological evaluation were treated by the one way Analysis of Variance (ANOVA) test and presented as Mean±SEM (standard error on the mean). The Dunnett test was used for the multiple comparisons of means using prism graphpad version 5.0 and values of $P < 0.05$ were considered as statistically significant.

Experimental design for fertility inducing activity:

A total of 60 female wistar albino rats having normal estrous cycle grouped into 5 having twelve animals each was used for this study including three control groups and two test group. Group 1 served as normal control in which normal animals treated with vehicle (2% tween 80), group 2 served as negative control in which induced animals treated with vehicle and group 3 served as positive control in which induced animals treated with standard clomiphene citrate 0.1 mg/kg bw [15-17]. Group 4 and 5 served as test in which induced animals treated with low (125 mg/kg bw) and high (250 mg/kg bw) dose of ethanolic extract of *C. fruticosa* respectively. The details of grouping of animals are given below in Table 1. The treatment was given for 30 consecutive days. During the treatment period all the animals were examined for their length of the estrous cycle. At the end of treatment period (after 30 days), 6 animals in each experimental groups were selected and anesthetized using diethyl ether. Blood was collected by penetrating the retro orbital plexus. Serum estrogen and FSH levels were evaluated using ELISA kits. The remaining animals in each group used for the fertility test [18].

Table 1: Experimental grouping of animals

S. No.	Group	Treatment for 30 days
1	Group I [CONTROL]	Animals served as normal control, treated with vehicle 2% v/v tween 80 p.o
2	Group II [EE]	Animals served as negative control, ethinyl estradiol induced animals treated with 2% v/v tween 80 p.o
3	Group III [STD]	Animals served as positive control, induced animals treated with clomiphene citrate 0.1 mg/kg bw p.o
4	Group IV [EECF 125]	Animals served as test, induced animals treated with ethanolic extract of *C. fruticosa* 125 mg/kg bw p.o
5	Group V [EECF 250]	Animals served as test, induced animals treated with ethanolic extract of *C. fruticosa* 250 mg/kg bw p.o

Estrous cycle monitoring:

The stages of estrous cycle were determined by preparing the vaginal smears [19]. For this, first thin cotton bud was taken which was dipped into normal saline (0.9 % w/v). The rat was held loosely on left hand. While the vaginal margins were separated, the cotton bud rotated inside clockwise twice with an angle of 45°. The material obtained from the pledget was transferred to a clean grease free microscope slide. The slide was stained with methylene blue (0.05% w/v) for 7 min. The slide was gently washed using plain water to remove excess stain and the stained slide was left in a room temperature for 10 min. The slide was later examined for the various stages of estrous cycle under the microscope. The stages of estrous cycle were classified according to the cell type observed under microscope.

Evaluation of serum estrogen and FSH level:

The separated serum samples were analyzed for estrogen and FSH as per manufacturer operating instructions, using enzyme linked immunosorbant assay (ELISA) based on biotin double antibody sandwich technology. Samples, standard solution and reagents were prepared according to the manufacturer instruction and added in coated ELISA plate. The plate was covered with seal plate membrane and shaken gently. The covered plate was incubated at 37° C for 60 min. After incubation the plate was washed with washing solution for 5 times. The plate was blotted using tissue paper. After blotting 50 µL of chromogen A and chromogen B were added in all wells. The plate was incubated at 37° C for 10 min. Further 50 µL of stop solution was added to stop the reaction. The

absorbance was measured immediately at 450 nm by ELISA plate reader.

Evaluation of fertility index:

The remaining animals were allowed to mate on proestrus stage with a male rat of proven fertility. Mating was confirmed by vaginal smear examination. The day on which sperm was spotted in vaginal smear was designated as day 1 of pregnancy [17, 20]. After confirmation of pregnancy, the animals were separated and maintained in a new cage. From the 20th day of the pregnancy, all animals were noted for births. After birth the number of viable and death newborns (if any) were recorded. The newborns were weighed and inspected for 10 days after birth for any deformity.

RESULTS

Effect of extract on estrous cycle:

Initially irregular estrous cycle was observed in all treated groups, estrous cycle abnormalities arise as lengthened diestrus stage. Treatment with 250 mg/kg dose of ethanolic extract of *C. fruticosa* showed statistically a high significant (P<0.001) level on regularization estrous cycle length as that of standard treated group particularly shortened the metestrus and diestrus phase when compared to negative control group. The effect of ethanolic extract on the estrous cycle is depicted in fig. 1.

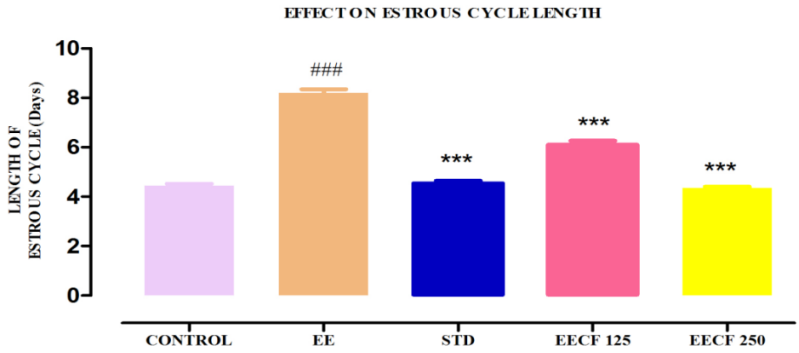

Fig. 1: Effect of ethanolic extract of *C. fruticosa* on estrous cycle length

All data are expressed as mean±SEM (n=12). ###P<0.001; when compared to control group, ***P<0.001; when compared to negative control group.

Effect of extract on serum estrogen and FSH level:

The linear regression equation of the standard curves of estrogen and FSH were adopted to find out the concentration of the corresponding sample, using OD values of the samples. The effect of ethanolic extract of *C. fruticosa* on the serum estrogen level is illustrated in fig. 2. The negative control group showed statistically a high significant (P<0.001) decrease in serum estrogen level when compared to control group. Standard

group showed statistically a high significant (P<0.001) increase in the level of estrogen when compared to negative control group. *C. fruticosa* at 125 mg/kg dose level exhibited significant level of increase (P<0.01) in estrogen level when compared to negative control group. *C. fruticosa* at 250 mg/kg showed statistically a high significant (P<0.001) increase in level of estrogen when compared to negative control group.

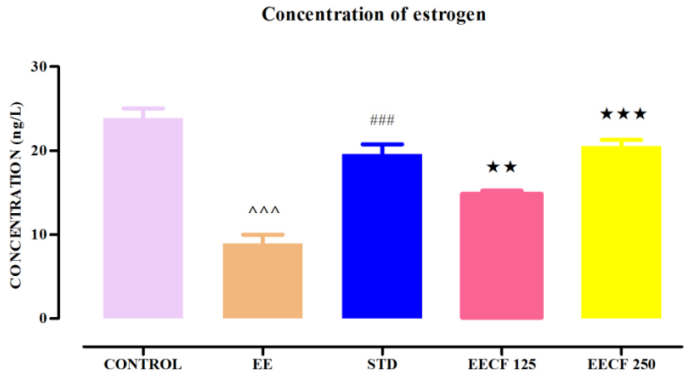

Fig. 2: Effect of ethanolic extract of *C. fruticosa* on estrogen level

All data are expressed as mean±SEM (n=6). ^^^P<0.001; when compared to control group, ###P<0.001; when compared to negative control group. **P<0.01, ***P<0.001; when compared to negative control group.

The effect of ethanolic extract of *C. fruticosa* on the serum FSH level is illustrated in fig. 3. The negative control group showed statistically a high significant (P<0.001) decrease in serum FSH level when compared to control group. Standard group showed statistically a significant (P<0.01) increase in the level of FSH when compared to negative control group. *C. fruticosa* at both dose levels exhibited a non significant level of FSH when compared to standard group. *C. fruticosa* at 250 mg/kg showed a statistically a high significant (P>0.001) increase in the level of FSH when compared to negative control group.

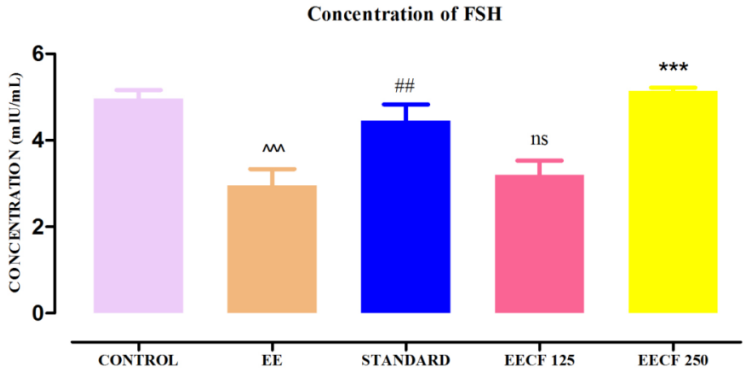

Fig. 3: Effect of ethanolic extract of *C. fruticosa* on FSH level

All data are expressed as mean±SEM (n=6). ^^^P<0.001; when compared to control group, ##P<0.01; when compared to negative control group, nsP>0.05, ***P<0.001; when compared to negative control group.

Effect of extract on fertility index:

After birth, the number of delivered newborns was recorded. The negative control group was compared with control group. The standard group was compared with negative control group. The treatment groups such as ethanolic extract of *C. fruticosa* at 125 and 250 mg/kg were compared with standard group. The negative control group showed statistically a high significant (P<0.001) decrease in number of newborns when compared to control group. The standard group showed statistically a high significant (P<0.001) increase in number of newborns when compared to negative control group. Ethanolic extract of *C. fruticosa* at 125 mg/kg dose level showed a non significant (P>0.05) level of increase in newborns when compared to standard group and statistically a high significant (P<0.001) when compared to negative control group. Ethanolic extract of *C. fruticosa* at 250 mg/kg dose level exhibited statistically a high significant (P<0.001) level of increase in number of newborns when compared to negative control and standard group. The graphical representation of the effect of *C. fruticosa* on fertility assay is illustrated in fig. 4.

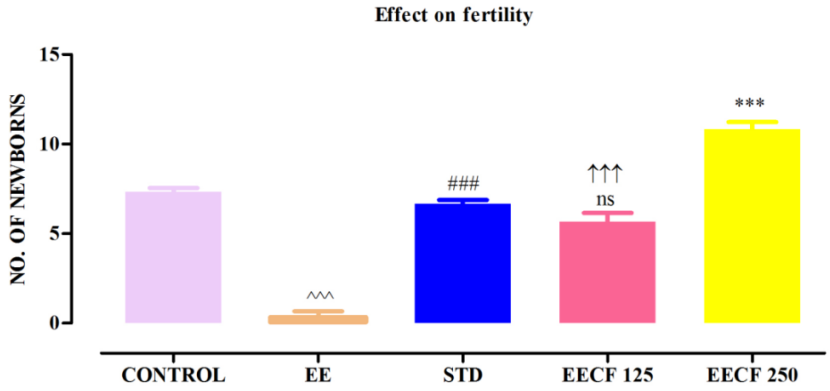

Fig. 4: Effect of ethanolic extract of *C. fruticosa* on fertility index
All data are expressed as mean±SEM (n=6). $^{\wedge\wedge\wedge}$P<0.001; when compared to control group, $^{\#\#\#}$P<0.001; when compared to negative control group, ^{ns}P>0.05; when compared to standard group, $^{\uparrow\uparrow\uparrow}$P<0.001; when compared to negative control group, ***P<0.01; when compared to negative control and standard group.

The negative control group showed statistically a high significant (P<0.001) decrease in weight of newborns when compared to control group. The standard group and ethanolic extract of *C. fruticosa* at both dose levels exhibited statistically a high significant (P<0.001) increase in weight of newborns when compared to negative control group. The graphical data are illustrated in fig. 5. There were no perceptible external deformities of the newborns at birth and daily up to the 10th day of birth during checking.

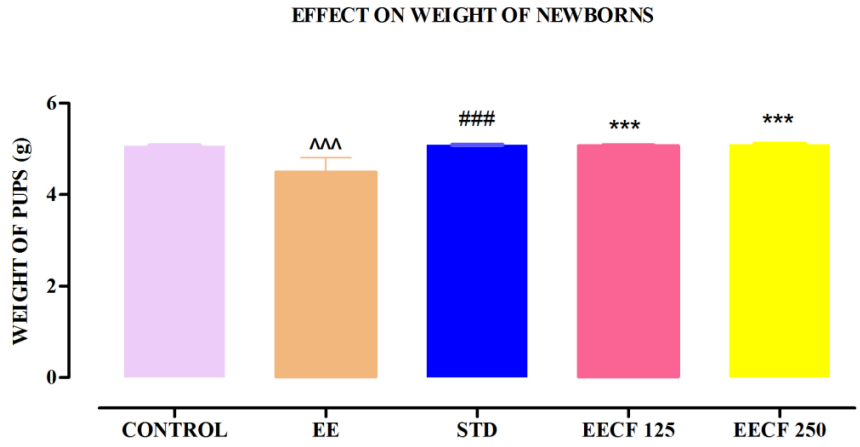

Fig. 5: Effect of ethanolic extract of *C. fruticosa* on weight of newborns
All data are expressed as mean±SEM (n=6). $^{\wedge\wedge\wedge}$P<0.001; when compared to control group, $^{\#\#\#}$P<0.001; when compared to negative control group, ***P<0.001; when compared to negative control group.

DISCUSSION

Many physiological, biochemical and histological alteration within the ovary takes place in females at the time of estrous cycle. The secretion of pituitary gonadotrophin and hypothalamic releasing hormones controls the synthesis of ovarian estrogen which is responsible for the different phases of estrous cycle. Under the balanced and combined influence of ovarian and extra ovarian hormones, the preovulatory follicles developed for the period of estrous cycle [21].

It was reported that the irregular estrous cycle was regularized in the mice with irregular cycle, by treating them with combined extract of *Turraeanthus africanus* and *Lepidium meyenii*. Author had posited that the regulation was may be due to the action of saponins, which have been used to treat sexual dysfunction, that is present in *L. meyenii* extract [20]. It was reported that the prolonged diestrus phase was also responsible for the antifertility activity which was proved in wistar rat by the administration of ethanolic extract of *Dalbergia saxatilis* [22]. Gonadotropin hormones are associated with the estrous cycle and also responsible for the growth and development of ovary and uterus [23]. It was reviewed that the oxidative stress is an contributory reason of irregular estrous cycle [24].

Normally rats have an estrous cycle of 4-5 days. The prolonged diestrus phase was observed in ethinyl estradiol induced group. It was quite possible that insufficient gonadotropin could have disturbed the estrous cycle. Treatment with ethanolic extract from aerial parts of *C. fruticosa* at high dose level might increases the level of gonadotropin hormone, and thus regularizing

the estrous cycle when compared to ethinyl estradiol induced group. It may be due to the presence of numerous antioxidant compounds in ethanolic extract from aerial parts of *C. fruticosa* which was analyzed by GC-MS [25].

Malfunction of gonads and cessation of reproductive cycles in females are generally observed as a result of decreased secretion of gonadotrophic hormones. It was reported that the fertility inducing effect of the aqueous extract of *Coccinia cordifolia* L. by significantly increasing the serum estrogen level in female wistar albino rats [16]. It was exposed that the increased FSH level in immature female Sprague–Dawley rats by treating them with *Symplocos racemosa* aqueous extract and revealed out the uses of plant in female reproductive disorder [26]. Since FSH is the primary hormone accountable for the follicle growth from primary up to the antral stage and the augmented production of estrogen.

It was investigated the effect of hydro alcoholic extract of saffron flowers on pituitary ovary axis in Sprague–Dawley rats and reported that the extract significantly increases the level of ovarian hormones such as estradiol, progesterone and also pituitary hormones like FSH, LH and thus enhances the fertility [27]. The level of estrogen and FSH were significantly normalized by the treatment with ethanolic extract of *C. fruticosa* at high dose level when compared with ethinyl estradiol group. According to the results of this study, the ethanolic extract of *C. fruticosa* may enhance the pituitary ovary axis activities which resulted in increasing the level of estrogen and FSH. This suggested that it has been used to treat infertility problems.

In many studies, the animals were sacrificed at middle of pregnancy to study the fertility activity. For example, fertility activity of aqueous extract of *Ficus platyphylla* was evaluated by sacrificing the animals at 10^{th} day of pregnancy to record the post implantation loss and litter size in normal female wistar rats [17]. In another study, fertility activity of *Turraeanthus africanus* and *Lepidium meyenii* was investigated by sacrificing all animals at 15^{th} day of pregnancy to count the number of fetuses in Balb/C strain mice model [20]. In one more study, the reproductive effect of aqueous and methanolic extract of *Ficus asperifolia* was studied by recording the implantation sites by laparoscopy at 10^{th} day of pregnancy under diazepam/ ketamine (10/50 mg/kg respectively) anesthesia [28].

However in our study, the animals were allowed to give birth to their offspring. The

number of newborns and their weight were recorded to study the fertility activity of *Cadaba fruticosa*. Ethinyl estradiol group showed only 16.6% mating index and thus exhibited statistically a high significant level of decrease in number of newborns. Ethinyl estradiol mainly acts by bottle up the gonadotrophin and inhibits the ovulation.

From the results it was revealed that the ethanolic extract of *C. fruticosa* at high dose level exhibited statistically a high significant level of increase in number of newborns when compared to clomiphene citrate group. Literature review showed the presence of alkaloids, isoflavones, sterols and quercetin derivatives in *C. fruticosa*. The increased oxidative stress due to ethinyl estradiol might be minimized by the presence of antioxidants. Further there is increase in number of newborns as the dose is increased, indicating that there may be dose depenedent in the activity. The above said data strongly recommends the plant *C. fruticosa* for its fertility inducing activity.

CONCLUSION

On the basis of the findings, it was concluded that the ethanolic extract from aerial parts of *C. fruticosa* possesses fertility inducing activity. The fertility activity of *C. fruticosa* might be due to the synergistic effect of the compounds present in the extract. Further studies should be carried out to determine the constituents responsible for the activity by isolating the compounds.

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REFERENCES

1. Mascarenhas MN, Flaxman SR, Boerma T, Vanderpoel S, Stevens GA. National, Regional, and Global Trends in Infertility Prevalence Since 1990: A Systematic Analysis of 277 Health Surveys. PLOS Medicine. 2012; 9 (12): 1-12.
2. Kamel HH. Role of phyto-oestrogens in ovulation induction in women with polycystic ovarian syndrome. Eur J Obstet Gynecol Reprod Biol. 2013; 168: 60-63.
3. Guyton AC, Hall JE. Text Book of Medical Physiology. Unit XIV. Endocrinology and Reproduction 11th Ed. Elsevier Inc., Pennsylvania. 2006.
4. Khare CP. Indian Medicinal Plants- An illustrated medical dictionary. Springer. 2007; 107.
5. Ahmad VU, Amber A, Arif S, Chen MHM, Clardy J. Cadabicine, an alkaloid from *Cadaba farinosa*.

Phytochemistry. 1985; 24 (11): 2709-2711.

6. Selvamani P, Latha S. Studies on the antimicrobial activity of *Cadaba indica* lam. Indian J Pharm Sci. 2005; 67 (5): 637-638.
7. Mythreyi R, Sasikala E, Geetha A, Madhavan V. Antipyretic activity of leaves of *Cadaba fruticosa* (L.) Druce. Pharmacologyonline. 2008; 3: 136-142.
8. Arokiyaraj RS, Radha R, Martin S, Perinbam K. Phytochemical analysis and antidiabetic activity of *Cadaba fruticosa* R.Br. Indian J Sci Technol. 2008; 1 (6): 1-4.
9. Umesh BT, Anuj M, Vaibhav U, Avinash G, Hemalatha, Goswami DV. Hepatoprotective and Antioxidant activity of Root of *Cadaba farinosa*, Forsk against CCl_4 induced hepatotoxicity in rats. J Pharm Res. 2010; 3 (6): 1-5.
10. Al-Musayeib NM, Mohamed GA, Ibrahim SRM, Ross SA. Lupeol-3-O-decanoate, a new triterpene ester from *Cadaba farinosa* Forssk. growing in Saudi Arabia. Med Chem Res. 2013; 22 (11): 5297-5302.
11. Vadivel SD, Gopinath P, Santhosh JD, Balakumaran P. Anti-Cancerous Activity of Leaves of *Cadaba fruticosa* Druce using Human Lung Cancerous Cell Line A549 by *In vitro* Methods. International Journal of Pharma Research & Review. 2013; 2 (11): 6-17.
12. OECD guideline for testing of chemicals 423 adopted 17th December 2001. Available at: www.oecd.org/dataoecd/17/50/1948370.pdf
13. Bennink HJTC, Skouby S, Bouchard P, Holinka CF. Ovulation inhibition by estetrol in an *in vivo* model. Contraception. 2008; 77 (3): 186-190.
14. Rahman MA. Phytochemical and experimental study of Aparmaga (*Achyranthus aspera* Linn.) WSR to female anti-infertility activity. Presented for M.D (Ayurveda), Bangalore, Rajiv Gandhi University of Health Sciences, 2009.
15. Jadhav M, Menon S, Shailajan S. *In vivo* evaluation of M*imosa pudica* Linn. in the management of polycystic ovary using rat model. Int J Appl Biol Pharm.2012; 4 (1): 285-292.
16. Jha U, Asad M, Asdaq SMB, Das AK, Prasad VS. Fertility inducing effect of aerial parts of *Coccinia*

cordifolia L. in female rats. J Ethnopharmacol. 2010; 127: 561-564.

17. Ugwah-Oguejiofor CJ, Bello SO, Okolo RU, Etuk EU, Ugwah MO, Igbokwe VU. *Ficus platyphylla* promotes fertility in female *Rattus norvegicus* Wistar strain: a preliminary study. Reprod Biol Endocrinol. 2011; 9 (145): 1-6.
18. Lienou LL, Telefo BP, Bale B, Yemele D, Tagne RS, Goka SC, Lemfack CM, Mouokeu C, Moundipa PF. Effect of the aqueous extract of *Senecio biafrae* (Oliv. & Hiern) J. Moore on sexual maturation of immature female rat. BMC Complement Altern Med. 2012; 12 (36): 1-9.
19. Mary F, Eswaramohan T, Surendran SN, Murugananthan A, Romakeswaran P. Mukkuddu Maathrai (Triple Tablet), An Indigenous Medicine Elevates Serum Prolactin Level of Female Rats (*Rattus norvegicus*). Journal of Pharmacy. 2013; 3 (1): 38-42.
20. Lembe DM, Gasco M, Gonzales GF. Fertility and estrogenic activity of *Turraeanthus africanus* in combination with *Lepidium meyenii* (Black maca) in female mice. Eur J Integr Med. 2012; 4: 345-351.
21. Shivalingappa H, Satyanarayan ND, Purohit MG, Sharanabasappa A, Patil SB. Effect of ethanol extract of *Rivea hypocrateriformis* on the estrous cycle of the rat. J Ethnopharmacol. 2002; 82: 11-17.
22. Uchendu CN, Kamalu TN, Asuzu IU. A preliminary evaluation of antifertility activity of a triterpenoid glycoside from *Dalbergia saxatilis* in female Wistar rats. Pharmacol Res. 2000; 41(5): 521-525.
23. Sarwar AHMD, Sharma S, Arif M, Thakur SS, Athar F, Khillare B, Thakur SC. *Piper longum* hexane fraction induces infertility by modulation of inflammatory mediators and gonadotropin insufficiency in female rats. Int J Pharm Pharm Sci. 2014; 6 (2): 416-420.
24. Rong-Zhen Z, Dao-Wei Z. Oxidative stress and role of natural plant derived antioxidants in animal reproduction. J Integr Agric. 2013; 12 (10): 1826-1838.
25. Amudha M., Rani S. Assessing the bioactive constituents of *Cadaba fruticosa* (L.) Druce through GC-MS.

Int J Pharm Pharm Sci. 2014; 6 (2): 383-385.

26. Bhutani KK, Jadhav AN, Kalia V. Effect of *Symplocos racemosa* Roxb. on gonadotropin release in immature female rats and ovarian histology. J Ethnopharmacol. 2004; 94: 197-200.
27. Mokhtari M, Sharifi E, Daneshi A. Effects of hydro-alcoholic extract of red dried stigmas of *Crocus sativus* L. flowers (saffron) on the levels of pituitary-ovary hormones and folliculogenesis in rats. Int J Fertil Steril. 2010; 3 (4): 185-190.
28. Watcho P, Ngadjui S, Alango NP, Benoît NT, Kamanyi A. Reproductive effects of *Ficus asperifolia* (Moraceae) in female rats. Afr Health Sci. 2009; 9 (1): 49-53.