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Effect of Raw Extract of *Clitoria ternatea* L. on Sexual Stimulate Test of Female Genital Tract in Rat

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ABSTRACT

Clitoria ternatea have no toxicity as per the earlier reports of sexual stimulate effect of traditional medicine used by the tribal and fishery village women's of Point Calimere Wildlife Sanctuary, Nagapattinam district, Southern India. In the present study, reproductive stimuli and fertility effect of raw extract of *Clitoria ternatea* (RECT) was administered orally at the doses of 250 mg/kg body weight of female rat for a period of 30 days and normal clean water (Normal slain 0.9%, 0.5 ml/kg per day) to control groups. There were no significance changes in the body weight of RECT treated group when compared to control group. RECT treated female rat group vigorously mated with male when allowed on estrus phase of female rat observed in vaginal smears. The serum estradiol level was increased ($P < 0.05$) significantly. Administered the RECT showed no significant change in the biochemical parameters and hematological parameters of RBC, WBC count when compared to control group. It is concluded that the test of RECT could be due to its reproductive effect in female rat.

Keywords: *Clitoria ternatea*, Female rat, Estrogenic activity, Reproductive stimuli, Hematological and Biochemical profile

1. INTRODUCTION

The women sexual function is important for eliminating stress and infertility as a result happiest family healthy life will be going smoothly. The couples who do not have child to their face major problem of social life (Deir *et al*, 2003; Gnoth *et al*, 2005). The developing country women's particularly in Indian women hesitate to never talk about the sexual problem of her life; as a result married couples are isolated from the family life. Globally one fourth of the women population are face loss of sexual function and infertility. In India one third of young women population are health wise face problem of inhibit activity of sexual functions are caused by variety of factors such as weight loss or excessive weight gain, smoking, consume of alcoholic drink, stress, imbalanced reproductive hormonal function, loss of fantasy, loss of libido, loss of orgasm, obesity, polycystic ovarian syndrome (PCOS), diabetes mellitus. The causes of PCOS for young women are not harmful but it leads to hormonal imbalance (Barbieri, 2001). Women's infertility to be at greater risk factor for sexual dysfunction and that lower sex-life satisfaction scores often resulted in fertility related stress (Millheiser *et al*, 2010). The reproductive effect of methanolic extract of *Ficus asperifoli* administrated in female rat mated with male are resulted sperm positive in female rat vagina and in addition to tested administrated in normal immature rat showed marked level of estrogen synthesis (Watcho *et al*, 2007). A few good number of alcoholic extract of reproductive potential herbs are toxic side effect of physical parameters (1983; El-Ashmawy *et al*, 2007). The present study was designed for evaluation of the dose dependent reproductive stimuli and fertility effect of alcoholic free raw extract of *Clitoria ternatea* (RECT) in adult female rat.

2. MATERIALS AND METHODS

2. 1. Plant materials



Fig. 1. *Clitoria ternatea* L.

The *Clitoria ternatea* (root, flower, seed) equal portion were collected and dried in shade. Twenty five grams of the dried plant powder were suspended in 100 ml cold distilled water in a closed vessel, shaking with magnetic stirrer for 24 hours, centrifuged at 3000 rpm for 15 minutes and the supernatant was filtered using Whatman No.1 filter paper and the filtrate was then evaporated nearly to dryness (gummy residue). The yield was found to be 10%.. The gummy residue was dissolved in appropriate volume of distilled water and stored in a labeled sterile screw capped bottle at (-20 °C) until we use (Trease *et al*, 2002).

2. 2. Animal used

Healthy mature cyclic female albino rat, with the body weight of 100-120 gram were procured from the Department of Zoology, Animal House, A.V.C. College, Mannampandal, Mayiladuthurai, India. They were maintained at animal room temperature at (27±2 °C) with food and water ad libitum. The rat were closed by grid and placed in the department of animal house for acclimatization (Behringer, 1973). All the experiments were carried out with the approved of institutional animal ethical committee.

2. 3. Toxicity study

LD50 of RECT was found to be 3g per kilogram body weight, in rat by oral administration.

2. 4. Animal experiments

The female rat was divided in to two groups of 8 each. Normal slain 0.9%, 0.5 ml/kg per rat per day was administered orally by an intra gasteric catheter in group 1 and RECL at the doses of 250 mg/kg per day are given to group 2 for duration of 30 days. Body weight was noted and estrus cycle was observed every day by microscopic examination of vaginal smear (Watcho *et al.*, 2007).

2. 5. Hematological parameters

Estimation of RBC and WBC count (Arthur, 1980)

2. 6. Biochemical estimation

2. 6. 1. Protein content

Total protein in the ovarian tissue homogenate was estimated by the method of Lowry *et al.*, (1951)

2. 6. 2. Cholesterol content

Ovaries were homogenized in appropriate ice cold buffer using glass homogenizer with Teflon pestle. The cholesterol was estimated by the method of Zlatkis *et al.* (1953)

2. 6. 3. Estimation of glucose

Total glucose in the tissue extract was determined by method of Hassid and Abraham (1957).

2. 6. 4. Hormone assay

2. 6. 4. 1. Estradiol

Serum estradiol was estimated using RIA kit obtained from diagnostic products corporation (DPC), USA.

2. 6. 4. 2. Progesterone

Progesterone concentration in serum was estimated by solid-phase RIA' procedure using kits obtained from diagnostic system laboratory (DSL) USA.

2. 7. Statistical analysis

Results are expressed as mean \pm S.E.M. Statistical analysis was done by student's t-test and the difference was considered statistically significant at $P \leq 0.05$.

3. RESULTS

3. 1. Effect of RECT on estrus cycle, wet weight of ovaries and uterus and body weight

RECT no altered in estrous cycle, but little long estrus phase was observed during the 30 days study period of 250 mg/kg body weight of extract treated group 2 (Table 1, Fig. 2). The extract treat group 250 mg/kg body weight there was significantly ($P < 0.05$) changes in the wet weight of ovaries and uterus. There was no significant change in their body weight in control and treated groups.

3. 2. Effect of RECT on hematological parameters

The dose of (250 mg/kg b wt.) RECT treatment group 2 was no any significant changes in hematological parameters of RBC and WBC (Table 2, Fig. 3) when compared to control group of female rat.

3. 3. Effect of RECT on biochemical parameters

The dose of (250 mg/kg b wt.) RECT treatment group 2 was no any significant changes in biochemical parameters of Protein (Table 3, Fig. 4), Glucose (Table 4, Fig. 5), Cholesterol (Table 5, Fig. 6) in compared to control group of female rat.

3. 4. Effect of RECT on serum estrogen level

Administration of RECT extract to the group 2 in the dose of 250 mg/kg showed significant ($P \leq 0.05$) enhance in the serum estradiol level as compared to the control group (Table 6, Fig. 7)

3. 5. Effect of RECT on serum progesterone level

The mice RECT treated with 250 mg/kg of body weight of extract showed no significant changes in the serum progesterone when compared with control (0.9% saline) group 1 (Table 6, Fig. 7).

4. DISCUSSION AND CONCLUSION

In the present study, no significant change in the body weight was observed in *Clitoria ternatea* raw extract treated rat when compared to the control (Table 1, Fig. 2). Similar finding has been recorded by (Adaay and Mosa, (2012). The plant raw extract of *Clitoria ternatea* treated female rat groups for 30 days duration had no altered in estrous cycle, but little long estrus phase was observed during the RECT treated female rat group. Administration of raw extract of RECT showed no significant changes in the haematological parameter of WBC and RBC count when compared to control group (Table 2, Figure 3).

The present study unaltered pattern of the biochemical parameter of protein, cholesterol, glucose, in ovary and uterus showed by the raw extract of *Clitoria ternatea* extract treated in female rat group. (Table 3, 4, 5 Figure 4, 5 & 6).

The above mentioned fact was supported by earlier study in which the toxicity against *Hybanthus enneaspermus* extract treated animal model showed improve normal position of bio chemical parameters (Haseena bhanu *et al.*, 2011). In the present study, there were a no significant changes in the ovary and uterus cholesterol content recorded in *Clitoria ternatea* extract treated female rat group when compared with control group. The without changes in ovary and uterine cholesterol content are: RECT might be helps to maintained normal position of cholesterol synthesis in the ovary and uterus.

The estrogen syntheses are stimulated by gonads secretion of FHS. In the present study RECT extract treated group estradiol level in the blood was significantly increased with control one. Since the precursor of estradiol synthesis is might be hypothalamus furnish to stimulate factor of sex hormones. The possibility of marked increase in estradiol level recorded following the *Clitoria ternatea* raw extract treatment are: (a) regulation of steroid genesis (b) little enhance stimulation of FHS. The above mentioned fact was supported by earlier study in which the treatment of raw extract of *Hybanthus enneaspermus* extract treated mice resulted enhance serum estradiol (Chandru *et al.*, 2017).

In this regard in addition to suggested in reproductive hormones stimulation is due to might be phytoestrogen content of the plant as studies their treatment of *Tribulus terrestris* extract rising of FHS, LH effective on the reproductive parameters of female mice (Adaay and Mosa, 2012). In the dose depended RECT treated rat there was no significant changes in progesterone was might be due to regular synthesis of reproductive key enzyme 3β and 17β Hydroxy steroid dehydrogenase (HSD) as convert pregnenolone to progesterone was normal synthesis of RECT treated from female rat. Similar finding has been reported (Moza far *et al.*, 2011; Chandru *et al.*, 2017; Malabadi *et al.*, 2005; Parimaladevi *et al.* 2004).

5. CONCLUSION

The present study resulted alcoholic free RECT did not have any toxic effects was evidence from the unaltered hematologic profile and biochemical parameters from female rat. It is concluded that the reproductive effect of preliminary test of RECL could be stimulate its safe oestrogen synthesis.

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Table 1. Effect RECT on the Body Weight of Control and Treated Female Rat.

Group	Treatment	Dose /mg/kg Body wt.	Initial body weight (gm)	Final body weight (gm)
1	Control (saline (0.9%	0.5 ml	153 ± 6.1 ^{NS}	153 ± 4.1 ^{NS}
2	RECL	250	153 ± 7.2 ^{NS}	153 ± 5.1 ^{NS}

Each values are represents mean ± S.E.M of six rat
 Statistical significant at *P<0.05, when compared to control group

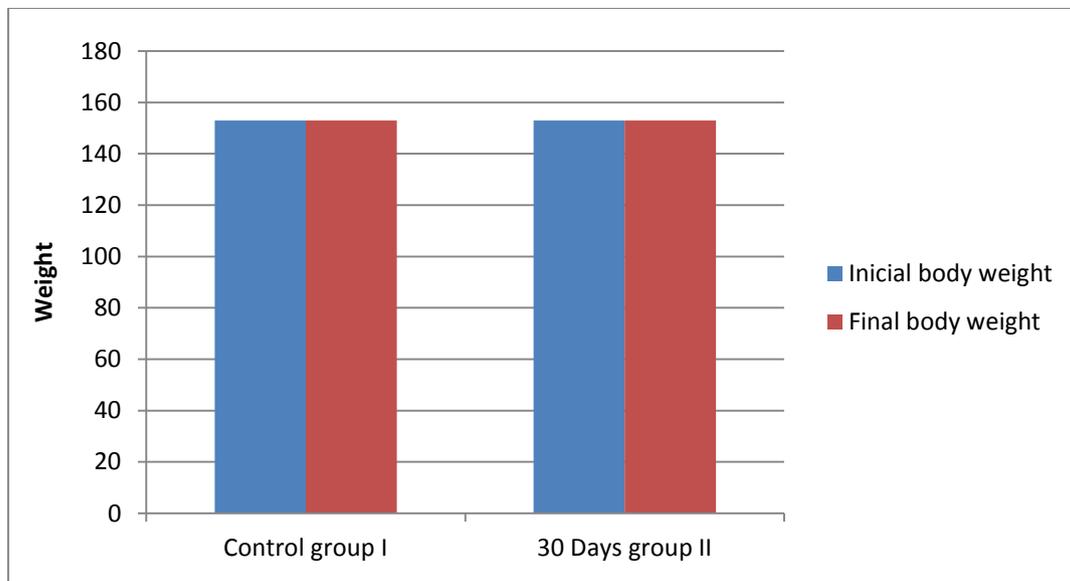


Fig. 2. Effect RECT on the Body Weight of Control and Treated Female Rat.

Table 2. Effect of RECT on the RBC 10⁶/mm³ and WBC 10³/mm³ count of Control and Treated Female Rat.

Group	Treatment	Dose/mg/kg Body wt.	RBC 10 ⁶ /mm ³	WBC 10 ³ /mm ³
1	Control (0.9% saline)	0.5 ml	8.2± 0.13 ^{NS}	4.2 ± 0.13 ^{NS}
2	RECL	250	4.2 ± 0.13 ^{NS}	4.2 ± 0.16 ^{NS}

Each values are represents mean ± S.E.M of six rat
 Statistical significant at *P<0.05, when compared to control group

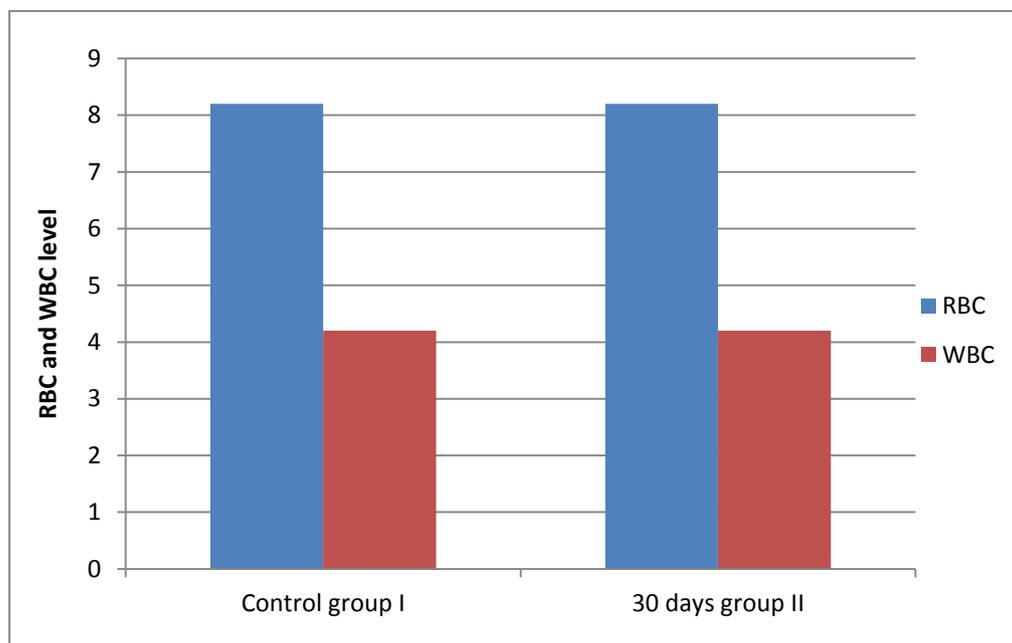


Fig. 3. Effect of RECT on the RBC 10⁶/mm³ and WBC 10³/mm³ Count of Control and Treated Female Rat.

Table 3. Effect of RECT on the Protein (mg /100 mg fresh tissue) Ovary and Uterus of Control and Treated Female Rat

Group	Treatment	Dose/mg/kg Body wt.	Ovarian protein (mg/ml)	Uterine protein (mg/ml)
1	Control + (0.9% saline)	0.5 ml	13.7 ± 0.6 ^{NS}	12.4 ± 0.1 ^{NS}
2	RECL	250	13.8 ± 0.1 ^{NS}	12.4 ± 0.4 ^{NS}

Each values are represents mean ± S.E.M of six rat
 Statistical significant at *P<0.05, when compared to control group

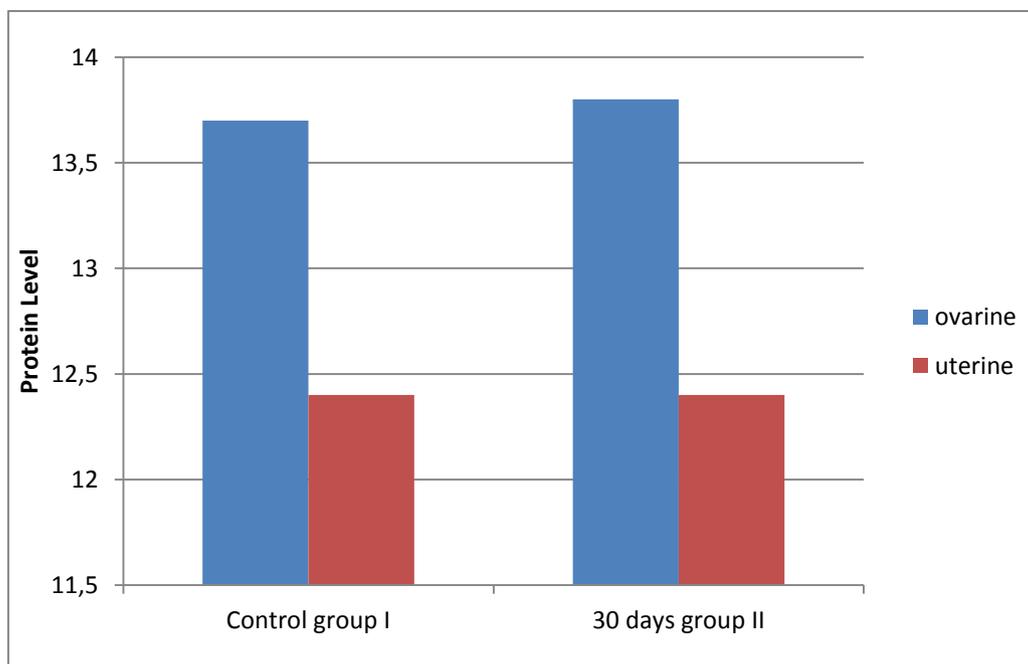


Fig. 4. Effect of RECT on the Protein (mg /100 mg fresh tissue) Ovary and Uterus of Control and Treated Female Rat

Table 4. Effect of RECT on the Glucose Level (mg /100 mg fresh tissue) Ovary and Uterus of Control and Treated Female Rat

Group	Treatment	Dose/mg/kg Body wt.	Estradiol (mg/ml)	Progesterone (mg/ml)
1	Control (0.9% saline)	0.5ml	20.11 ± 0.07 ^{NS}	19.39 ± 0.11
2	RECT	250	24.17 ± 0.08 ^{NS}	19.41 ± 0.13 ^{NS}

Each values are represents mean ± S.E.M of six rat
 Statistical significant at *P < 0.05, when compared to control group

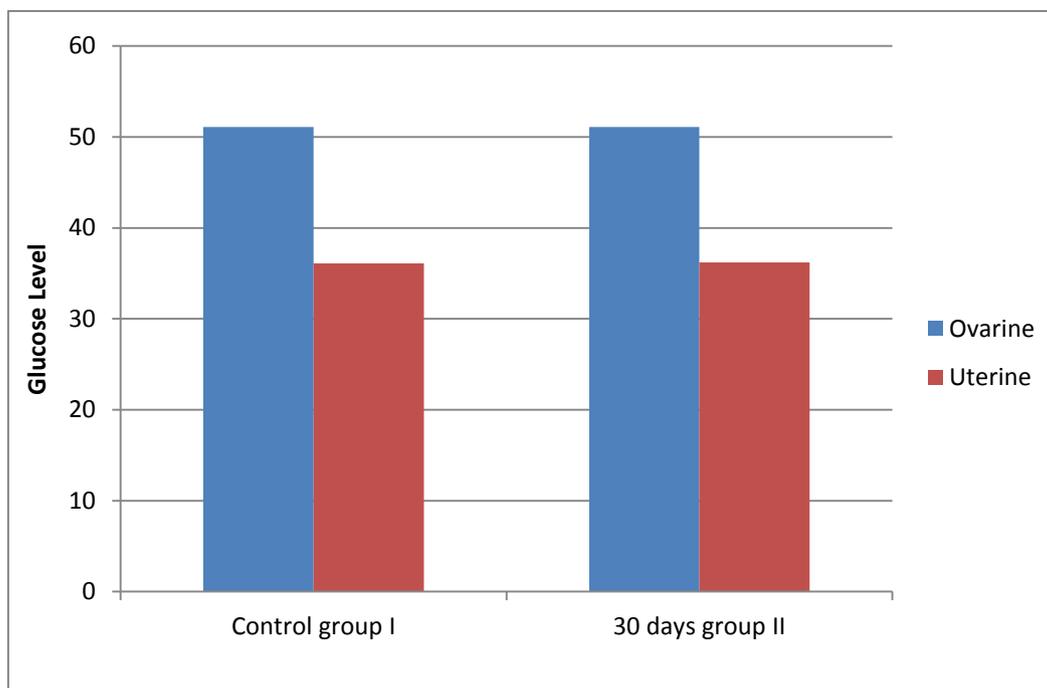


Fig. 5. Effect of RECT on the Glucose Level (mg /100 mg fresh tissue) Ovary and Uterus of Control and Treated Female Rat

Table 5. Effect of RECT on the Cholesterol Level (mg /100 mg fresh tissue) Ovary and uterus of Control and Treated Female Rat.

Group	Treatment	Dose/mg/kg Body wt.	Ovarian Cholesterol (mg/ml)	Uterine Cholesterol (mg/ml)
1	Control (0.9% saline)	0.5 ml	0.54 ± 0.33 ^{NS}	0.21 ± 0.01 ^{NS}
2	RECL	250	0.53 ± 0.06 ^{NS}	0.21 ± 0.02 ^{NS}

Each values are represents mean ± S.E.M of six rat
 Statistical significant at *P<0.05, when compared to control group

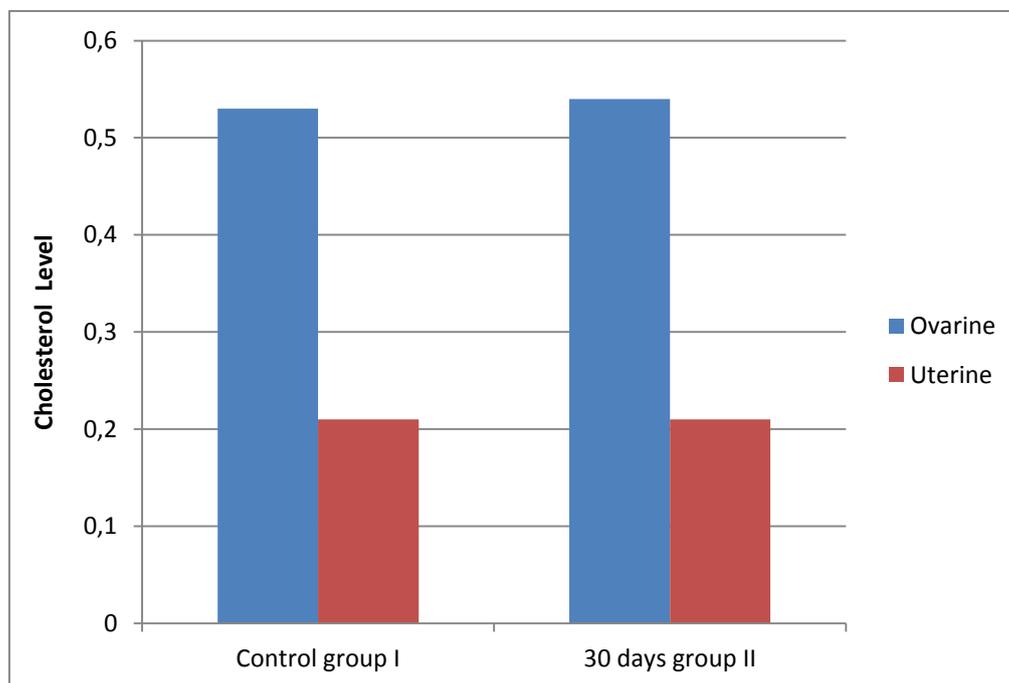


Fig. 6. Effect of RECT on the Cholesterol Level (mg /100 mg fresh tissue) Ovary and Uterus of Control and Treated Female Rat.

Table 6. Effect of RECT on the Serum Estradiol and Progesterone Level (mg /100 mg fresh tissue)

Group	Treatment	Dose/mg/kg Body wt.	Estrogen (pg/ml)	progesterone (ng/ml)
I	Control (0.9% saline)	0.5 ml	20.11 ± 0.07	19.39 ± 0.11 ^{NS}
II	RECT	250	24.17 ± 0.08*	19.41 ± 0.13 ^{NS}

Each values are represents mean ± S.E.M of six rat
 Statistical significant at *P<0.05, when compared to control group

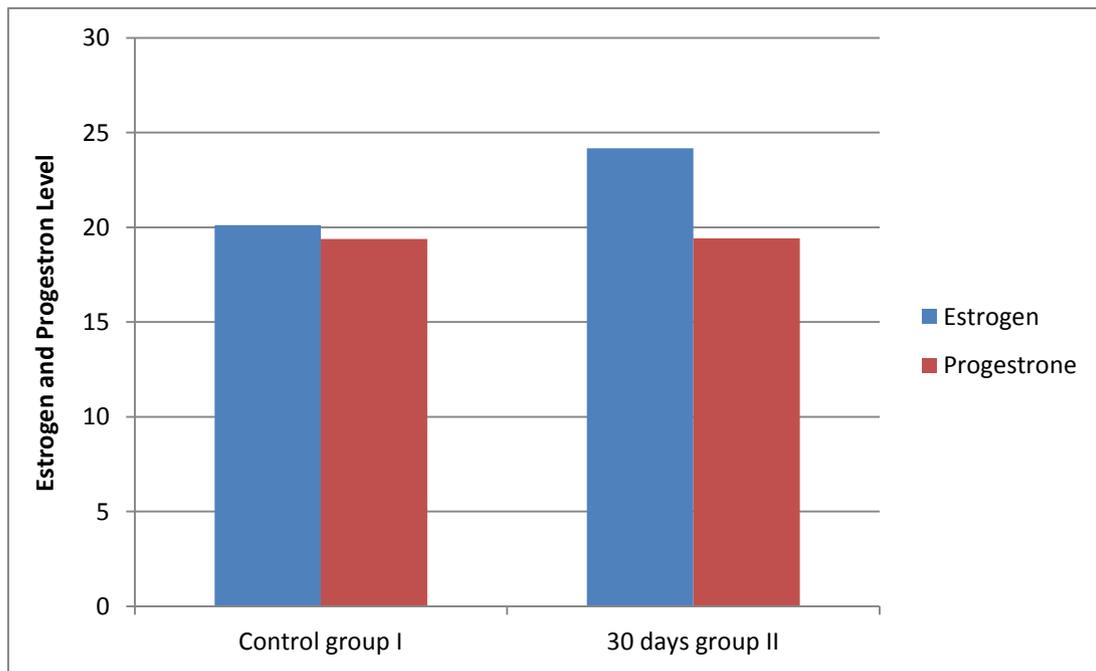


Fig. 7. Effect of RECT on the Serum Estradiol and Progesterone Level (mg /100 mg fresh tissue)