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Effect of Raw Extract of *Hybanthus enneaspermus* (L.) F. Muell. on the Fertility Test in Female Mice (*Mus musculus* L)

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ABSTRACT

Hybanthus ennespermus (L.) (Violaceae) has no toxicity asper the earlier reports of fertility traditional medicine used by the tribal people of Grizzled Giant Squirrel Wildlife Sanctuary, Srivilliputur, Southern India. In the present study, fertility effect of *Hybanthus enneaspermus* (Violaceae) whole part of plant evaluated in adult female mice (*Mus musculus*). Raw extract of *Hybanthus enneaspermus* (REHE) was administered orally at the doses of 100 mg/kg body weight for a duration of 30 days and normal salinw (0.9%) to control groups. There were no significance changes in the body weight ($P<0.05$). The Possibility of marked increase in serum estradiol level ($P<0.05$) significantly. Administered of REHE in mice did not show any variation in the hematological and biochemical parameters. It is raw extract of *Hybanthus enneaspermus* (REHE) showed on above result indicated that this alcoholic free extract of *Hybanthus ennaespermus* confirm the fertility effect of above study.

***Keywords*:** *Hybanthus enneaspermus*, estrogenic effect, Biochemical and Hematological profiles

1. INTRODUCTION

Herbal medicinal plants are widely used by people in developing countries. The family Violaceae, *Hybanthus enneaspermus* whole plant is well known Ayurvedic and Siddha medicinal effect. This is a common plant of wide region of India and sub continents (James, 1999). The whole plant has been prescribed for its traditional use as fertility agent among tribal villagers. The oral fertility pill, injectable hormone therapy, GnRH, FSH and LH are having more side effect such as hot flashes, blurred vision, nausea, bloating and headach etc. (Klein and Rowland 1988; Elbetieha and Al-Hanood, 1997). Some of the reproductive potential of alcoholic extract of herbs is toxic side effect of physical parameters (World Health Organization, 1983; El-Ashmawy, 2007). Hence, the search for a suitable product from medicinal plants has been tested for their fertility effect in mice (Adaay and Mosa, 2012). So the present study was designed for evaluation of the dose dependent fertility property of alcoholic free raw extract of *Hybanthus enneaspermus* (REHE) in adult female mice.

Fig. 1. *Hybanthus enneaspermus*

Fig. 2. *Hybanthus enneaspermus* (flower)

2. MATERIALS AND METHODS

2. 1. Plant materials

Hybanthus enneaspermus (Violaceae) were collected from Delta region of Tamil Nadu, south India. It was dried in shade, powdered and raw extracted with clean water.

2. 2. Animal used

Healthy mature cyclic female swiss mice, *Mus musculus*, with the body weight of 26-27 g were procured from the Department of Zoology, Animal House, A.V.C. College, Mannampandal, Mayiladuthurai, India. They were maintained at 270 with food and water adlibitum. All the experiments were carried out with the approved of institutional animal ethical committee.

2. 3. Toxicity study

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

2. 4. Animal experiments

The female mice were divided in to two groups of 8 each. Normal slain 0.9%, 0.5 ml/kg per mouse per day was administered orally by an intragasteric catheter in group 1 for duration of 30 days. REHE at the doses of 100 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. Biochemical estimation

2. 5. 1. Protein content

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

2. 5. 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. Hormone assay

2. 6. 1. Estradiol

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

2. 6. 2. Progesterone

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

2. 6. 3. Hematological parameters

Estimation of RBC and WBC count (Arthur, 1980).

2. 7. Statistical analysis

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

3. RESULT

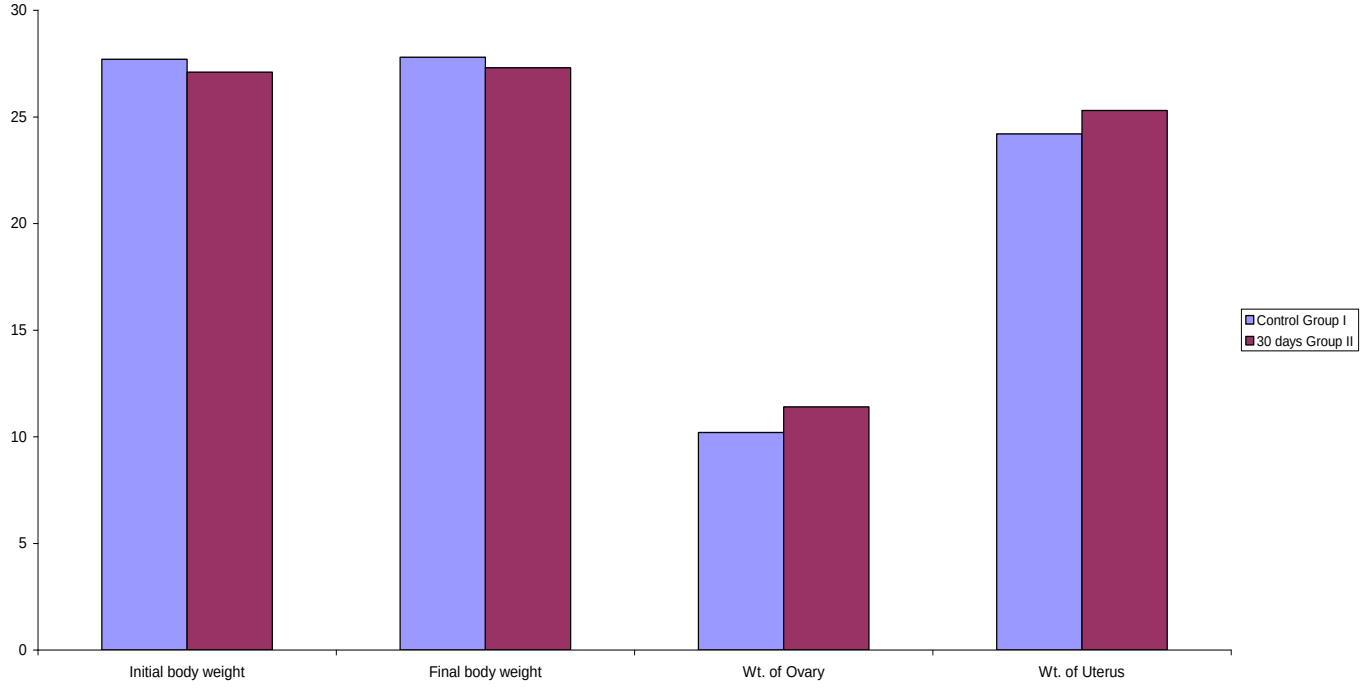
3. 1. Effect of REHE on estrous cycle, wet weight of ovaries and body weight

REHE no altered in estrous cycle, but little long estrus phase was observed during the 30 days study period of extract treated group (Table 1). The extract treated group there was no significant changes in the wet weight of ovaries and body weight (Table 1).

3. 2 .Effect of on serum estrogen level

Administration of REHE extract to the group 2 in the dose of 100 mg/kg showed significant ($P < 0.05$) enhance in the serum estradiol level as compared to the control group (Fig. 1, Table 1)

Figure 1. Effect of REHE treatment on the body and ovarian weight of female mice.



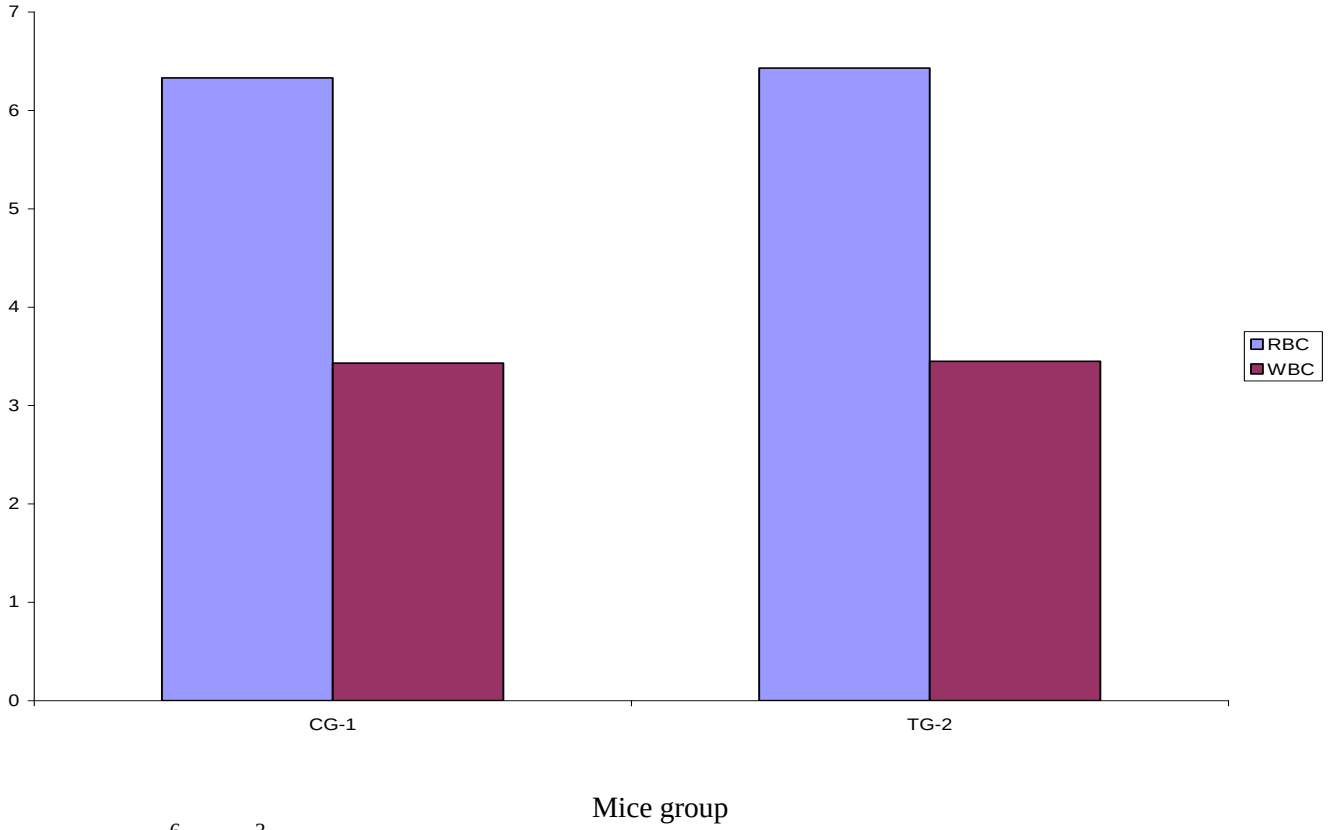
Wt. of Ovary (mg)
Wt. of Uterus (mg)
Data represents the mean + SEM (n = 6 for each group) Statistical $P < 0.05$, when compared to control group.

Table 1. Effect of REHE treatment on the body, ovarian and uterine weight of female mice.

Group	Treatment	Dose / mg / kg body wt.	Initial body weight (gm)	Final body weight (gm)	Wt. of ovary (mg)	Wt. of Uterus (mg)
I	Control (0.9 % saline)	0.5 ml	27.7 ± 1.0 ^{NS}	27.8 ± 0.9 ^{NS}	10.2 ± 0.8 ^{NS}	24.2 ± 1.8 ^{NS}
II	REHE	100	27.1 ± 1.7 ^{NS}	27.3 ± 0.8 ^{NS}	11.4 ± 0.6 ^{NS}	25.3 ± 1.6 ^{NS}

Each values represents Mean ± SEM of six animals
Statistical significance $P < 0.05$
NS = Not significant

Figure 2. Effect of REHE treatment on RBC and WBC in female mice.



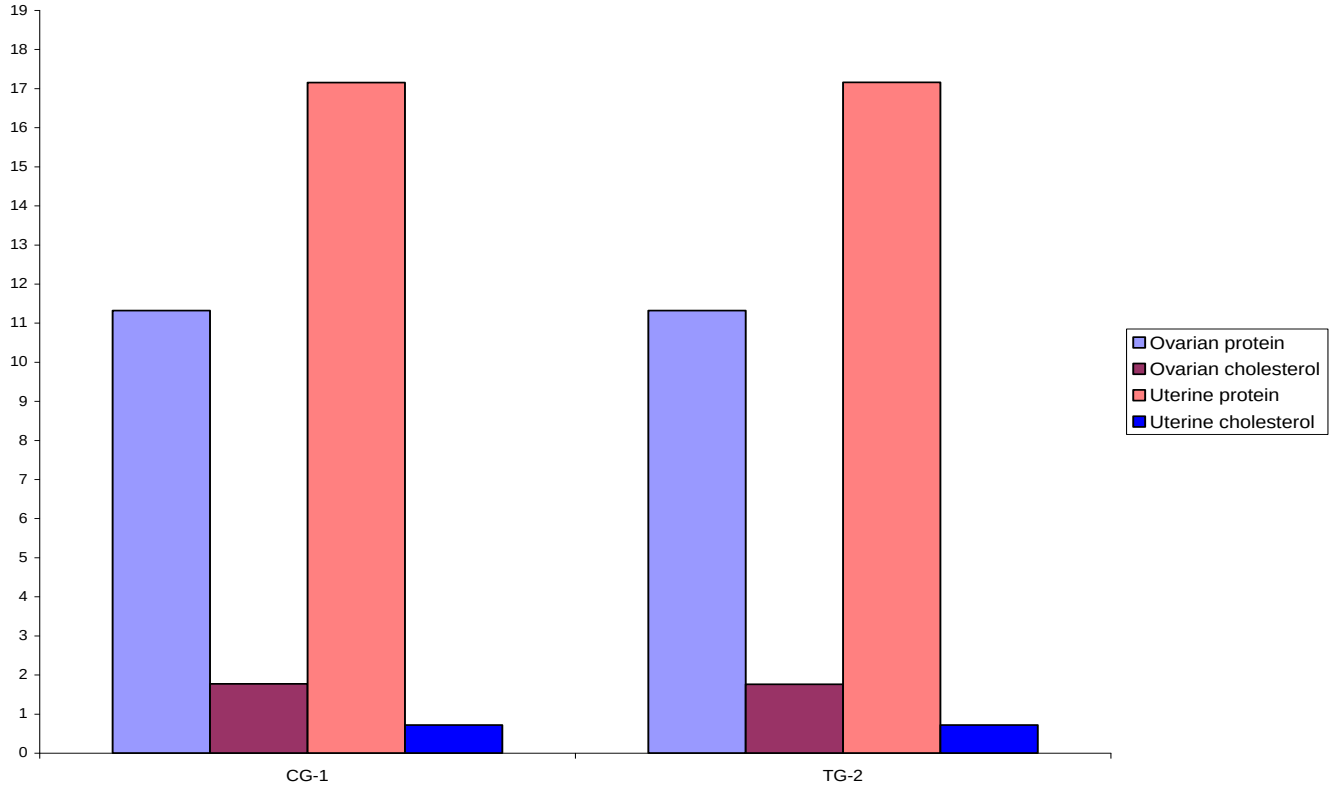
RBC (10^6 / mm^3)
WBC (10^6 / mm^3)
Data represents the mean + SEM (n=6 for each group) Statistical $P < 0.05$, when compared to control group.

Table 2. Effect of REHE treatment on RBC and WBC in female mice.

Group	Treatment	Dose / mg / kg body wt.	RBC 10^6 / mm^3	WBC 10^3 / mm^3
I	Control (saline 0.9 %)	0.5 ml	6.33 ± 0.46^{NS}	3.43 ± 0.86^{NS}
II	REHE	100	6.43 ± 0.33^{NS}	3.45 ± 0.65^{NS}

Each values represents Mean ± SEM of six animals
Statistical significance $P < 0.05$
NS = Not significant

Figure 3. Effect of REHE treatment on the biochemical parameters of Control and Treated female mice



Mice group

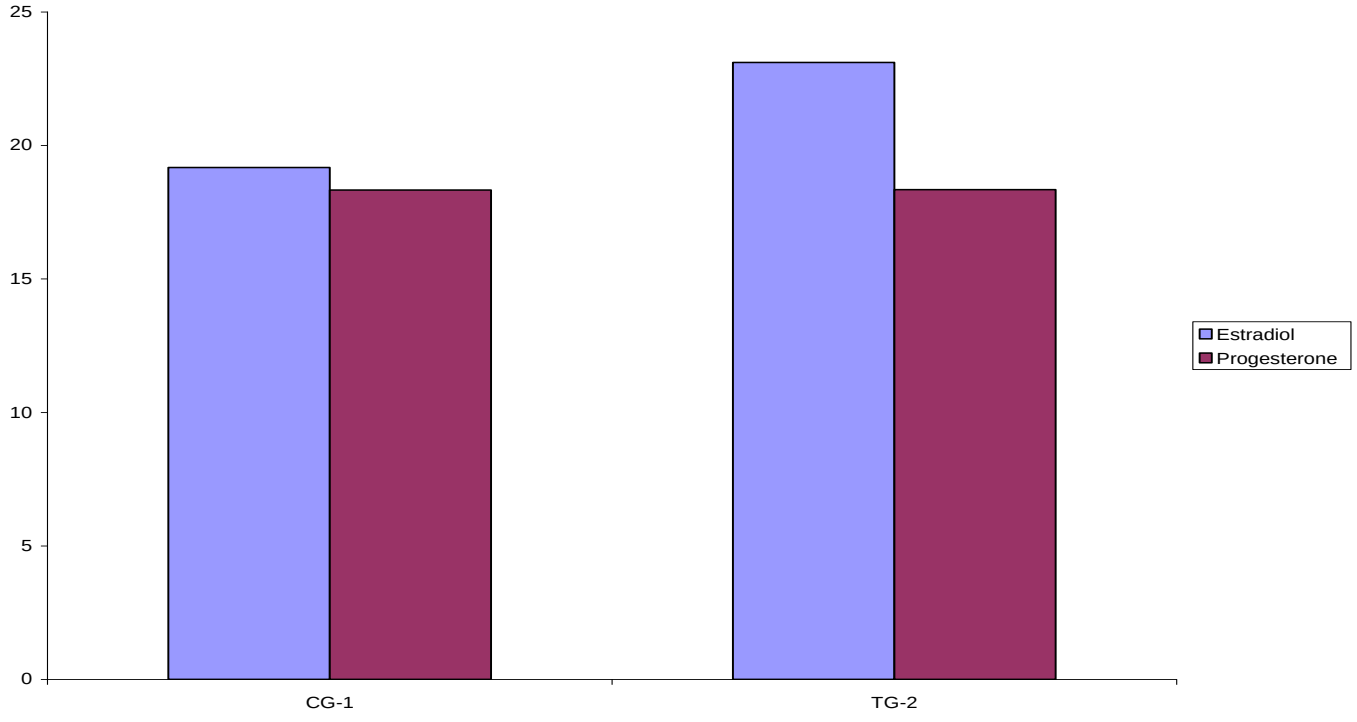
Ovarian Protein (µg/100 mg fresh tissue) ovarian cholesterol (mg/100 mg/fresh tissue)
Uterine protein (µg/100 mg fresh tissue) uterine cholesterol (mg/100 mg/fresh tissue)
Data represents the mean + SEM (n = 6 for each group) Statistical $P < 0.05$, when compared to control group.

Table 3. Effect of REHE treatment on the biochemical parameters.

Group	Treatment	Dose / mg / kg body wt.	Ovarian protein (µg / 100mg fresh tissue)	Ovarian cholesterol (mg / 100mg/fresh tissue)	Uterine protein (µg /100 mg fresh tissue)	Uterine cholesterol (mg / 100mg/fresh tissue)
I	Control (saline 0.9 %)	0.5 ml	11.32 ± 0.14 ^{NS}	1.77 ± 0.009 ^{NS}	17.15 ± 0.9 ^{NS}	0.72 ± 0.009 ^{NS}
II	REHE	100	11.32 ± 0.16 ^{NS}	1.76 ± 0.005 ^{NS}	17.16 ± 0.21 ^{NS}	0.72 ± 0.007 ^{NS}

Each values represents Mean ± SEM of six animals
Statistical significance $P < 0.05$
NS = Not significant

Figure 4. Effect of REHE treatment on the serum estradiol and pogesterone level of control and treated female mice



Estradial (Pg/ml)
Progesterone (ng /ml)
Data represents the mean ± SEM (n=6 for each group) Statistical $P < 0.05$, when compared to control group.
* Significant

Table 4. Effect of REHE treatment on the serum estradiol and pogesterone in female mice.

Group	Treatment	Dose / mg / kg body wt.	Estradiol (Pg / ml)	Progesterone (ng / ml)
I	Control (saline 0.9 %)	0.5 ml	19.17 ± 0.06 ^{NS}	18.33 ± 0.18 ^{NS}
II	REHE	100	23.11 ± 0.07 *	18.34 ± 0.17 ^{NS}

Each values represents Mean ± SEM of six animals
Statistical significance P < 0.05
NS = Not significant
Note: * significant

3. 3. Effect of REHE on biochemical parameters

In mice treated with REHE there were no significant changes in the protein, cholesterol and RBC, WBC when compared to control group (Table; 3; Fig. 3)

3. 4. Effect of serum progesterone level

The mice REHE treated with 100 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 4; Fig. 4).

4. DISCUSSION AND CONCLUSION

The REHE treated mice group had no altered in oestrous cycle, but little long estrus phase observed. In this record Adaay and Mosa (2012) reported aqueous extract of *Tribulus terrestris* on increase the reproductive parameter in female mice.

In the present study, there were a no significant changes in the ovary and uterian cholesterol content recorded in REHE extract treated group when compared with control group. The without changes in ovary and uterus cholesterol content are the *Hybanthus ennaespermus* extract not interfere with the cholesterol synthesis in ovary and uterus. The possibility of no hyperlipidemic effect of the extract could be stimulated its gonads and gonad releases or synthesis follicle stimulation of FSH. The above mentioned fact was supported by earlier study in which *Foeniculum vulgare* effect on folliculogenesis in female mice (Khazaei *et al* 2011).

In the extract REHE treated group marked increase in estradiol level in the serum was the precursor of estradiol sys\nthesis in ovarian cholesterol might be not enhanced. In addition the increased serum estradiol level in serum was could be due to the *Hybanthus enneaspermus* extract might be stimulate the FSH and regulation of steroid genesis in the blood. In this regard an earlier study designed to find the estrogenic effect of *Pueraria mirifica, Pfaffia paniculata* showed the increased estrogenic activity in female mice (Cherdshewasart *et al*,

2007; Oshima and Gu, 2003). The REHE treated mice there was no significant changes in blood serum progesterone was which are not blocking key enzyme of 3β and17β Hydroxy steroid dehydrogenase (HSD) as converted pregnenolone to progesterone. So that unaltered cholesterol level not interferes that regular synthesis of progesterone. In this study similar finding of the activity of LH are *Foeniculum vulgare* extract, treated mice showed by no changes in prolactin

The present study no significant changes in the body weight and wet weight of ovary and uterus of REHE treated female mice. REHE did not have any toxic effects which was evident from the unaltered hematologic profile RBC, WBC and biochemical parameters of protein, cholesterol in ovary and uterus of mice. The estrogenic effect of Raw Extract of *Hybanthus enneaspermus* might be due to its fertility effect in mice model. Since there is no toxic effect. It is concluded that the Raw Extract of *Hybanthus enneaspermus* in this above fertility test could be used to further study of examination of same animal model.

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