

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Evaluation of Antifertility Activity of *Melothria heterophylla* (Lour.) Cogn.

Harisha B^{1*}, Vikram Kumar Shetty², and Vrushabendra Swamy BM¹.

¹Department of Pharmacology, Gautham College of Pharmacy, RT Nagar, Bangalore-560032, Karnataka, India.

²Consultant Clinical Pharmacologist, Purva Panorama, Bannerghatta Road, Bangalore – 560076, Karnataka, India.

ABSTRACT

Several plants are traditionally used as birth control agents by the rural people in India. *Melothria heterophylla* is one of the folk medicinal plants commonly used as antifertility agent in some places in India. The present work was carried out to evaluate the claimed antifertility effect of the plant on male and female by carrying out pharmacological studies with the whole plant extract. Air dried plant of *Melothria heterophylla* (Lour.) Cogn was extracted using hydro alcohol. Extract was administered orally to Male wistar rats for 45 days. Effect of extract on reproductive organs, sperm count, serum testosterone, testicular cholesterol and alkaline phosphates levels were evaluated and changes in testicular histology was compared with the control rats. Progestogenic activity assay was performed by pregnancy maintenance test in female Sprague dawley rats. Clauberg Assay (endometrial proliferation) was performed on female imatured rabbits. pregnancy maintenance and clauberg assay was performed to investigate the probable progestational or anti-progestational mechanism of antifertility in immature female rabbits. *Melothria heterophylla* extract, when administered orally at a dose of 200, 400, 600 mg/kg body weight, it showed decrease in the weight of testis, seminal vesicles, epididymis, sperm count, and serum testosterone. Decrease in testicular cholesterol and alkaline phosphates level, when compared to the control. The histological examination of testis revealed that distorted seminiferous tubules with disorganised population of spermatogenic and supporting cells, severe hypercellularity of Leydig cells. Pregnancy maintenance was assessed and it is progestogenic. Clauberg assay in immature it shown progestational and not a anti-progestational activity.

Keywords: *Melothria heterophylla*, antifertility, Anti-progestational and Progestational.

*Corresponding author

INTRODUCTION

The future of life on the planet is under the pressure of the population explosion the world's population estimate, for mid-year 2011, is estimated at 6,928,198,253 [1] and continues to grow by 83 million people per year. During the last half-century, the world's population more than doubled. Contraceptive methods can be used to prevent unwanted pregnancies either temporarily or permanently. An ideal contraceptive should be safe, effective, acceptable, inexpensive, simple to administer and requiring little or no medical supervision. However, there are many contraceptive methods to control the fertility. The most widely used is the use of oral contraceptive. Even though they are useful, they are potentially dangerous. The high incidences of side-effects associated with the use of these drugs are given below cardiovascular effects, cancer, effect on reproductive tract, metabolic & endocrine effects [2,3].

Hence, there is an urgent need to substitute oral contraceptives by safe and effective agent. One such alternative are herbal contraceptive eg *Hibiscus rosa sinensis* for antiestrogenic activity and antispermatogenic activity, *Embelia ribes* for anti-implantation activity and *Montanoa frutescens* for anti-implantation activity [4], neem oil for spermicidal activity, *Malvaviscus conzattii* for antiandrogenic and antispermatogenic activity. *Melothria heterophylla* (Lour.) Cogn. Belongs to Cucurbitaceae family. Distributed throughout India, Srilanka, Myamnar, Afghanistan, Taiwan, Malaysia, Australia and some parts of China. A scandent dioecious perennial with several tuberous roots and with slender branched furrowed stems bearing simple tendrils. The plant is used as "Kapha" and "Vata" in Ayurvedic medicine [5]. The plant is used as "jangli-kakri" in Himachal Pradesh as a folk medicine [6]. The seeds of the plant are used for their purgative action. The leaves are used in allergic inflammations. In Konkan, the juice of the roots is mixed with cumin and sugar is given in cold milk as a remedy for spermatorrhoea [7]. The plant is also used in the treatment of diabetes.

The present study is under taken to evaluate the mechanism of antifertility activity of ethanolic extract of *Melothria heterophylla* (Lour.) Cogn. in male and females rats, investigate the role of progesterone and antiprogestational in the mechanism of antifertility.

MATERIAL AND METHODS

Plant Material

The whole plant of *Melothria heterophylla* (Lour.) Cogn were collected from Tirupathi, identified and authenticated by Dr. K. Madhava Chetty, Dept.of Botany, Sri Venkateswara University, Tirupathi, and Andhra Pradesh.

Preparation of Extract

The powdered drug (300gm) was packed in Soxhlet apparatus and extracted with 70% Ethanol. The extract was concentrated, dried. The dried extract (HAMH) stored in refrigerator <10^o C and percentage of yield calculated.

Preliminary Qualitative Phytochemical Analysis [8]

Phytochemical analysis was carried out by using the standard procedures. Alkaloids, carbohydrates, flavonoids, glycosides, phytosterols/terpenes, proteins and saponins and lipids were qualitatively analyzed.

Experimental Animals

Male Wistar rats of either sex weighing between 150-200 g were procured from the Biogen, Bangalore, Karnataka. Sprague-Dawley of female and male sex was procured from In Vivo Biosciences, Bangalore and Rabbits are procured from Rabbit Farm the animals were acclimatized for seven days under laboratory conditions. The animals were fed with commercially available rat pelleted diet (Amruth) feeds & foods, Bangalore). Water was allowed ad libitum under strict hygienic conditions. Animals were housed six per cage at $27 \pm 2^\circ\text{C}$ with constant 55% humidity, on a 12-h light/dark cycle. The study protocols were duly approved by the Institutional Animal Ethics Committee (IAEC) (Approval no-IAEC/017/12/2010) of Gautham College of Pharmacy, Bangalore. Studies were performed in accordance with the CPCSEA guidelines.

Acute Toxicity Studies [9]

The test performed according OECD guidelines, the procedure was divided into two phases, Phase I (observation made on day one), and Phase II (observed the animals since next 14 days). Two set of healthy female rats (each set of 3 rats) were used for the experiment. First set animals were divided and fasted for 18 hours deprived from food, water withdrawn before 4 hours of the dosing, body weights were noted before and after dosing with HAMH (2000mg/kg) orally. Individually animals were observed for 4 hours to see any clinical symptoms, any change in behavior or mortality. 6 hours post dosing again body weights recorded. From the next day onwards, each day 1 hour the behavioral change, clinical symptoms or mortality was observed in the same animals for next 14 days and animal body weights were recorded on 8th and 14th day. The same procedure was repeated with another set of animals to nullify the errors.

Antifertility activity in Male Rats [10-12]

Male albino rats of wistar strain 120-150 gm were used for experiment animals were maintained under control standard laboratory condition, with free access to pellet diet and water ad libitum. The animal was divided into four groups of six animals each.

Group- I: Received only normal saline (p.o daily) for 45 days and served as control.

Group- II ,III, IV : Received HAMH at the dose 200 , 400, 600mg/kg body weight (p.o daily) for 45 days.

Sacrificiation Schedule

On the 45th day i.e. 24hr after last dose animals were weighed, blood was collected retro orbital, serum was separated and sacrificed using ether anesthesia. The testes, epididymis, seminal vesicle were dissected out and freed from the adhering tissue, blotted on a filter paper and weighed.

Sperm Count

The Cauda epididymis was chopped in 10ml normal saline, the aliquots of sperm suspension was Filled up to 0.5 mark in WBC pipette and diluted with saline up to 11 mark. Sperm count was done in Neubauer's chamber in WBC squares. Sperm count/ml was calculated as follows.

$$X * 20 * 10^4 / \text{cm}^2 / \text{ml} / \text{epididymis}.$$

Where 'X' is the average mean of spermatozoa of all 4 squares.

Testosterone Radio-immunno Assay

Blood samples were collected retro orbitally and allowed to clot at room temperature for about 1hr and the serum was separated by centrifuging at 3000-4000 rpm for 15 min. Serum levels of testosterone were assayed by using Testosterone coated-tube radioimmunoassay kit.

Tissue Biochemistry

The other testes (left) were kept at -20oc until assayed for cholesterol and ALP. The testes were homogenized with ice- cold distilled water in a pre-cooled mortar and pestle to contain 10 mg per ml .The homogenate was centrifuged at 3000 rpm for 15 min and the supernatant was used for estimation of cholesterol content and alkaline phosphates activity (ALP) using diagnostic kit.

Testicular Histopathology

At the time dissection of rat one (right) of two testes of each animal was collected. A small punctures of the capsule was made with the tip of scalpel. Subsequently the testes were preserved in neutral formalin buffer 10% for 24h.

Pregnancy Maintenance Test [12-15]

Progestational activity was assessed by pregnancy maintenance test. Mature Sprague-Dawley female rats weighing 200-250g was used for the experiment. Animals were maintained under controlled standard laboratory conditions, with free access to pellet diet and water ad libitum. The female rats were inseminated by placing with male rats overnight in the ratio of (2:1). The day that the sperm were found in the vaginal smear was considered the day-1 of pregnancy. On the 8th day of the pregnancy the females were ovariectomized,

if found pregnant upon examination of the uterus. Then the drug was administered as follows.

Group I Received tween 80 of 0.5ml (p.o daily) and 0.1 μ g of Estradiol valerate (s.c) from 8th to 19th day, serves as the control

Group II Received Progesterone 3mg/rat/day, s.c, and 0.1 μ g of Estradiol valerate (s.c) from 8th to 19th day served as reference standard

Group III, IV, V Received HAMH at the 200, 400, 600 mg/kg body weight (p.o daily) and 0.1 μ g of Estradiol valerate (s.c) from 8th to 19th day. On the 20th day, the animals were autopsied, presence or absence of the implantation sites and the number of live embryos were recorded.

CLAUBERG ASSAY [12-15]

Progestational and antiprogestational activity was assessed in rabbits using Clauberg assay as described in Vogel. Immature rabbits weighing 550-650g were used for experiment. The animals were maintained under standard experimental conditions. The animals were grouped into five groups of 6 animals each. All animals were injected s.c, with estradiol valerate at the dose of 8.3 μ g/kg for a period of 6 days. After estrogen priming, they were treated with other drugs as follows.

Group- I: Received 0.5% normal saline (p.o daily) for 5 days.

Group- II, III, IV: Received HAMH at the 200,400 ,600mg/kg (p.o daily) for 5 days.

Group- V: Received norethisterone 0.75 mg/kg (p.o daily) for 5 days.

Group VI, VII, VIII : Received norethisterone 0.75 mg/kg and HAMH at the dose 200 ,400 ,600mg/kg (p.o daily) for 5 days.

The animals were sacrificed on the 15th day. The uterus was dissected out, adherent tissues were removed, blotted on a filter paper and was preserved in the Neutral formalin buffer 10% for 24 hr, then dehydrated in alcohol and embedded in paraffin wax. The sections of 5 μ m were cut and stained with haematoxylin-eosin and examined under digital microscope.

Statistical Analysis

The values are expressed as Mean \pm SEM. The data was analysed by using one way ANOVA followed by Dunnett's test using Graph pad prism software. Statistical significance was set at $P \leq 0.05$.

RESULTS

Extraction

Extraction of *Melotheria heterophylla* (Lour.) Cogn was carried out by using the Soxhlet apparatus with 70% v/v ethanol solvent the percentage yield of extract is given below

Table No. 1: Extractive Yield and Percentage Yield of *Melotheria heterophylla* (Lour.) Cogn

Weight of the Plant Used	792 gms
Yield	86
Percentage yield	10.86%

Investigation of Preliminary Qualitative Phytochemical Analysis

Table No 2: Qualitative Phytochemical Analysis

Sl no	Test	HAMH
I	Alkaloids	+
II	Carbohydrates	-
III	Flavonoids	+
IV	Glycosides	+
V	Phytosterols/Terpens	+
VI	Proteins	+
VII	Tannins	+
VIII	Saponins	-

- Absent + Indicates presence

Acute Toxicity Studies

In both phase I and Phase II procedures, none of the animals show any toxicity upon the single administration of HAMH (2000 mg/kg). None of the animals showed any toxicity. Thus 200, 400, and 600mg/kg body weight p.o doses were selected for the present study.

Antifertility Activity in Male Rats Effect of Extract on Total Sperm Count

Reduction in serum testosterone levels was observed in all treatment groups when compared with Group- I is shown in Table No 5.3. The respective levels in Groups- II, III and

IV were 34.83 ± 1.447 , 28.83 ± 1.014 and 27.50 ± 0.99 versus 44.33 ± 1.28 in Group- I. Oral administration of extract at the dose of 200mg/kg, 400mg/kg and 600mg/kg showed very significance ($P < 0.001$) reduction in total sperm count. Most significance reduction of total sperm count 27.50 ± 0.99 was observed at the dose of 600mg/kg p.o.

Table No 3: Effect of *Melothria heterophylla* (Lour.) Cogn Extract on Total Sperm Count of Male Rats.

Groups	Treatment	Epididymal Sperm count ($\times 10^5$)/ml
Group I	Saline	44.33 ± 1.28
Group II	HAMH 200mg/kg	$34.83 \pm 1.447^{***}$
Group III	HAMH 400mg/kg	$28.83 \pm 1.014^{***}$
Group IV	HAMH 600mg/kg	$27.50 \pm 0.99^{***}$

Values are Mean \pm SEM (n=6) one way ANOVA followed by Dunnett's test. Where, *** $P < 0.001$. All the values are compared with the Group I. HAMH: Hydro alcoholic extract of *Melothria heterophylla*

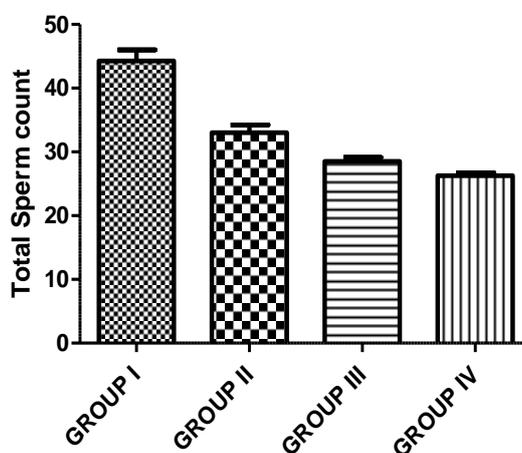


Figure No 5: Effect of *Melothria heterophylla* (Lour.) Cogn Extract on the Total Sperm Count in Male Rats.

Effect of Extract on Body and Reproductive Organ Weights

The final body weights of rats of all groups increased markedly when compared with their respective initial body weights and are shown in Table No- 4. A great decline in the weights of testis, epididymis and seminal vesicle (expressed in mg/100gms of body weight) were observed in all treatment groups when compared with Group- I animals and are shown in Table No- 4. Oral administration of extract at the dose of 200mg/kg, 400mg/kg and 600mg/kg p.o showed very significance ($P < 0.001$) decrease in epididymis. Most significance reduction of epididymis weights 0.256 ± 0.004 respectively, was observed at the dose of 600mg/kg p.o. Oral administration of extract at the dose of 200mg/kg showed significant ($P < 0.01$) decrease in seminal vesicle and testis weight. Oral administration of extract at the dose of 400mg/kg and 600mg/kg showed very significance ($P < 0.001$) decrease in testis and seminal vesicle weight. Most significance reduction of testis and seminal vesicle weight 1.258 ± 0.029 and 0.402 ± 0.025 respectively was observed at the dose of 600mg/kg p.o.

Table No 4: Effect of *Melothria heterophylla* (Lour.) Cogn Extract on Body and Reproductive Organ Weights of Male Rats.

Groups	Treatment	Body Weights (gms)		Reproductive Organ Weights (gms)		
		Initial	Final	Testis	Epididymis	Seminal Vesicles
Group I	Saline	171.3 ± 2.716	211.5± 3.89	1.638 ±0.023	0.387±0.015	0.676±0.009
Group II	HAMH 200mg/kg	171.8 ± 3.55	210.8 ±1.30	1.492±0.021 **	0.309±0.008***	0.583±0.014 **
Group III	HAMH 400mg/kg	169.8 ± 3.21	207.7± 2.53	1.392±0.040 ***	0.268±0.013***	0.480±0.009 ***
Group IV	HAMH 600mg/kg	170.7±3.52	210.2±1.74	1.258±0.029 ***	0.256±0.004***	0.402±0.025 ***

Values are Mean ± SEM (n=6) one way ANOVA followed by Dunnett’s test. Where, *** P<0.001 and ** P<0.01. All values are compared with Group I. HAMH: Hydro alcoholic extract of *Melothria heterophylla*

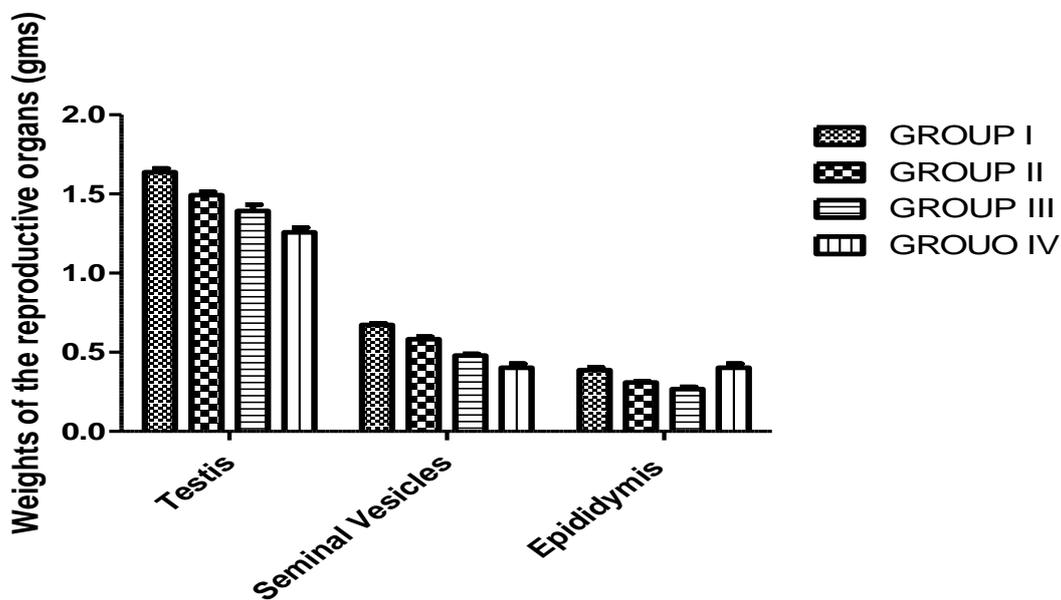


Figure No 2: Effect of *Melothria heterophylla* (Lour.) Cogn Extract on the Body and Reproductive Organ Weights in Male Rats.

Effect of Extract on Serum Testosterone Level in Male Rats

Reduction in serum testosterone levels was observed in all treatment groups when compared with Group- I is shown in Table No-5.5. The respective levels in Groups- II, III and IV were 4.872 ± 0.148 , 3.987 ± 0.112 and 3.067 ± 0.158 versus 11.6 ± 0.015 in Group- I. Oral administration of extract at the dose of 200mg/kg, 400mg/kg and 600mg/kg showed very significance (P<0.001) reduction in serum testosterone levels. Most significance reduction of serum testosterone level 3.067 ± 0.158 was observed at the dose of 600mg/kg p.o .

Table No 5: Effect of *Melothria heterophylla* (Lour.) Cogn Extract on Serum Testosterone Level of Male Rats.

Groups	Treatment	Testosterone (ng /mL)
Group I	Saline	11.6 ± 0.015
Group II	HAMH 200mg/kg	4.872 ± 0.148***
Group III	HAMH 400mg/kg	3.987 ± 0.112***
Group IV	HAMH 600mg/kg	3.067 ± 0.158***

Values are Mean ± SEM (n=6) one way ANOVA followed by Dunnett’s test. Where, *** P<0.001. All values are compared with Group I. HAMH: Hydro alcoholic extract of *Melothria heterophylla*

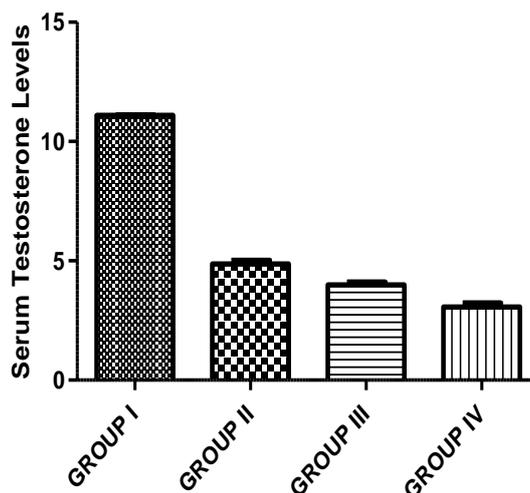


Figure No 3: Effect of *Melothria heterophylla* (Lour.) Cogn Extract on the Serum Testosterone Level in Male Rats.

Tissue Biochemistry

Effect of Extract on Testicular Cholesterol in Male Albino Rats.

Decrease in testicular cholesterol levels was observed in all treatment groups when compared with Group- I is shown in Table No- 6 The respective levels in Groups- II, III and IV were 9.99 ±1.49, 6.65 ±0.86 and 4.44 ±1.11 versus 16.66±1.21 in Group- I. Oral administration of extract at the dose of 200mg/kg, 400mg/kg and 600mg/kg p.o showed very significance (P<0.001) decrease in testicular cholesterol levels. Most significance decreasing of testicular cholesterol level 4.44 ±1.11 was observed at the dose of 600mg/kg p.o.

Effect of Extract on Alkaline Phosphates Levels (ALP) in Male Albino Rats.

Increasing in testicular alkaline phosphates levels was observed in all treatment groups when compared with Group- I is shown in Table No-6. The respective levels in Groups- II, III and IV were 669.5 ± 4.64, 752.0 ± 2.88 and 841.2 ± 4.28 versus 522.8±8.24 in Group- I. Oral administration of extract at the dose of 200mg/kg, 400mg/kg and 600mg/kg p.o showed very significance (P<0.001) increase in testicular ALP levels. Most significance increasing of testicular ALP level 841.2 ± 4.28 was observed at the dose of 600mg/kg p.o.

Table No 6: Effect of *Melothria heterophylla* (Lour.) Cogn Extract on Testicular Cholesterol and ALP Levels in Male Albino Rats.

Groups	Treatment	Cholesterol(mg/dl)	Alkaline phosphates (IU/L)
Group I	Saline	16.66±1.21	522.8±8.24
Group II	HAMH 200mg/kg	9.99 ±1.49***	669.5 ± 4.64***
Group III	HAMH 400mg/kg	6.65 ±0.86***	752.0 ± 2.88***
Group IV	HAMH 600mg/kg	4.44 ±1.11***	841.2 ± 4.28***

Values are Mean ± SEM (n=6) one way ANOVA followed by Dunnett’s test. Where, ***P<0.001. All values are compared with Group I. HAMH: Hydro alcoholic extract of *Melothria heterophylla*

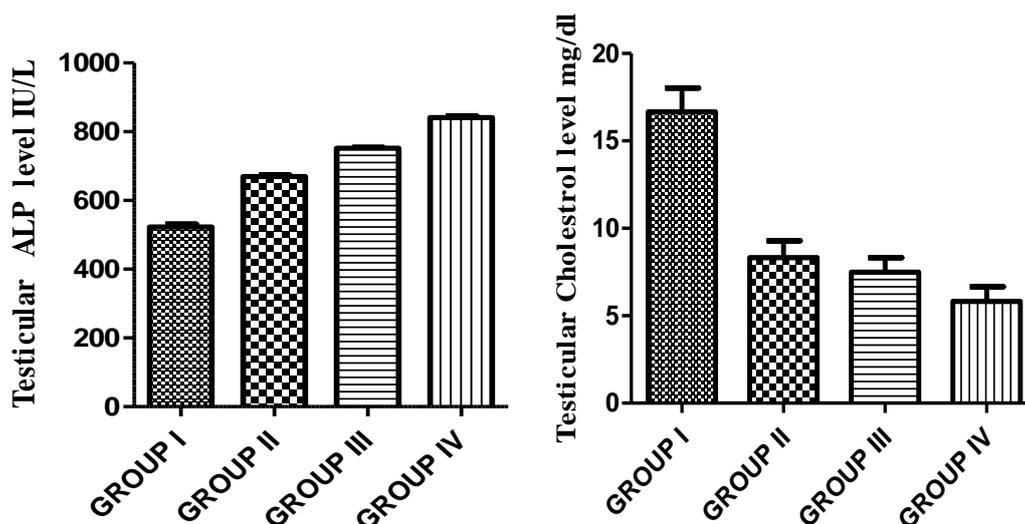


Fig 4: Effect of *Melothria heterophylla* (Lour.) Cogn Extract on Testicular Cholesterol and Alkaline Phosphates Levels (ALP) in Male Albino Rats

Histopathological Study of Testis of Rats

Group –I (Normal Control + Saline)

Section of testis of rat treated with Saline (control) showing normal spermatogenic activity in the seminiferous tubules, supporting cells, normal population of the leydig cell.

Group- II (HAMH 200mg/kg)

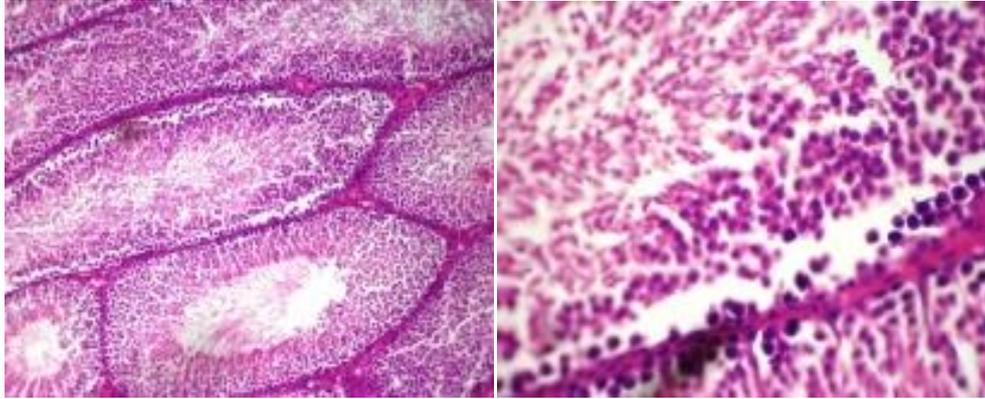
Section studied shows distorted seminiferous tubules with disorganised population of spermatogenic and supporting cells. The spermatocytes within the lumen are few with evidence of reduction spermatogenesis. Showing mild hypercellularity of leydig cells.

Group- III (HAMH 400mg/kg)

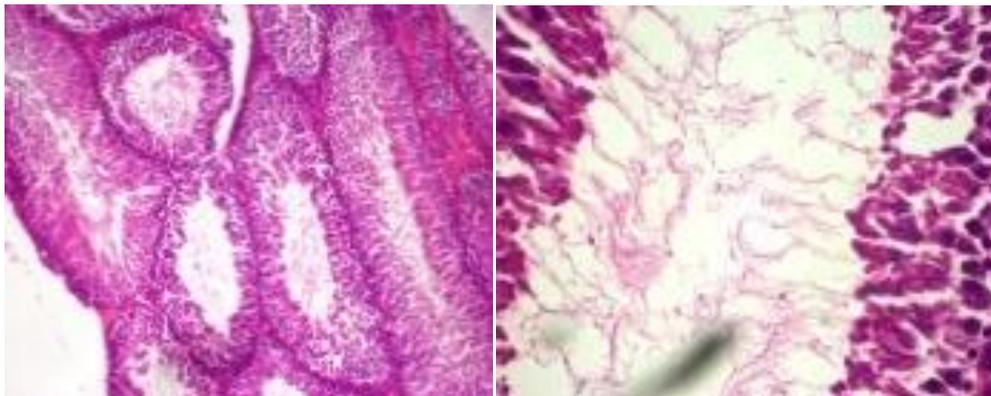
Section studied shows that distorted seminiferous tubules with disorganised population of spermatogenic and supporting cells. The spermatocytes within the lumen are very few with evidence of reduction spermatogenesis, showing severe hypercellularity of leydig cells.

Group- IV (HAMH 600mg/kg)

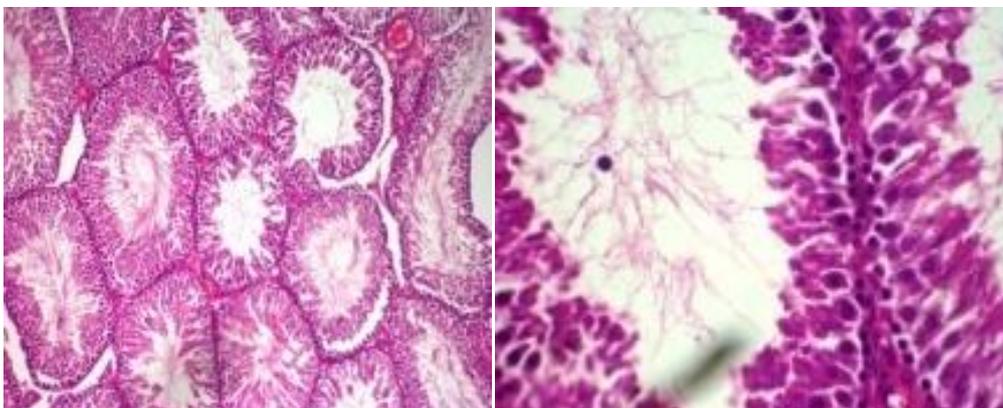
Section studied shows that distorted seminiferous tubules with disorganised population of spermatogenic and supporting cells. The spermatocytes within the lumen are very few with evidence of reduction spermatogenesis, showing severe hypercellularity of leydig cells



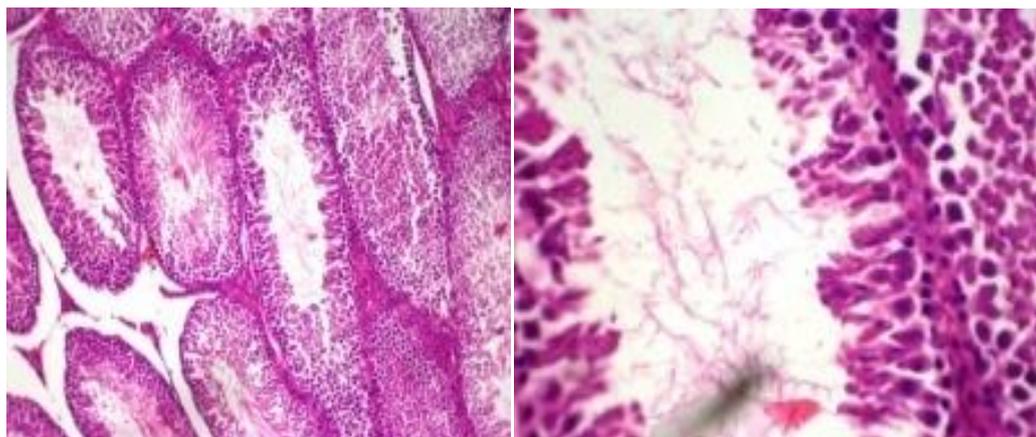
Group- I: Saline



Group- II: HAMH 200mg/kg



Group- III: HAMH 400mg/kg



Group IV: HAMH 600mg/kg

Figure No 5: Histopathology of Testes

Progestational and Antiprogestational Activity

Pregnancy Maintenance Test

Table No 7: Effect of Extract on Maintenance of Pregnancy in the Rat's Ovaricetomized on the 8th Day of Pregnancy.

Groups	Treatment	Mean viable Fetus	Net Success Index (%)
Group I	Saline + Estradiol 0.1µ g/rat /day (control)	0	0
Group II	Estradiol 0.1µ g/rat /day + progesterone 3mg/rat/day s.c. (reference standard)	7 ± 1.46	63.56 ± 13.27
Group III	Estradiol 0.1µ g/rat /day + HAMH 200mg/kg	4.16 ± 1.90	37.84 ± 17.15
Group IV	Estradiol 0.1µ g/rat /day + HAMH 400mg/kg	5.83 ± 1.88	53.03 ± 53.03
Group V	Estradiol 0.1µ g/rat /day + HAMH 600mg/kg	7.16 ± 1.47	66.50 ± 13.60

HAMH: Hydro alcoholic extract of *Melothria heterophylla*

Administration of estradiol 0.1µg/rat/day s.c. and progesterone 3mg/rat/day s.c for 13 days to the rats (on the 8th day of pregnancy) maintained pregnancy after ovariectomizing. The number of viable fetus seen at the time of autopsy (on the 20th day) and the net success index of pregnancy maintenance is calculated, shown in (Table 5. 7). In the control group the animals had complete abortion and the pregnancy was not maintained. Administration of estradiol 0.1µg/rat/day and HAMH at the dose of 200mg/kg/day, 400 mg

/kg and 600 mg/kg p.o maintain pregnancy. The effect of the drug extract was similar to that of the reference standard group. i.e HAMH shows progestational activity.

Clauber Assay

Effect of Extract on Histopathological Changes in the Uterus of Immature Rabbits after the Treatment.

Group- I

Section of immature rabbits uterus treated with normal saline (control) showing grade 0 type of proliferative changes in the endometrium. i.e. ramification of the uterus, but not proliferation of endometrium and endometrial glands(uterus mucosa) .

Group- II

Section of immature rabbits uterus treated with HAMH 200mg/kg p.o showing grade 1 type of proliferative changes in the endometrium. i.e. Slight proliferation of endometrium and endometrial glands (uterus mucosa).

Group- III

Section of immature rabbits uterus treated with HAMH 400mg/kg p.o showing grade 2 type of proliferative changes in the endometrium. i.e. slight proliferative changes in the endometrium and endometrial glands (uterus mucosa).

Group- IV

Section of immature rabbit uterus treated with HAMH 600mg/kg p.o showing grade 3 types of proliferative changes in the endometrium i.e. pronounced proliferation changes in the endometrium and endometrial glands (uterus mucosa).

Group- V

Section of immature rabbit uterus treating with norethisterone at the dose of 0.75 mg /kg showing grade 3 type of proliferation changes in the endometrium i.e. pronounced proliferation of the endometrium and endometrial glands (uterus mucosa).

Group- VI

Section of immature rabbits uterus treated with norethisterone at the dose of 0.75 mg/kg and HAMH 200mg/kg p.o showing grade 3 type of proliferation changes in the endometrium i.e.does not inhibit proliferation changes in the endometrium (uterus mucosa).

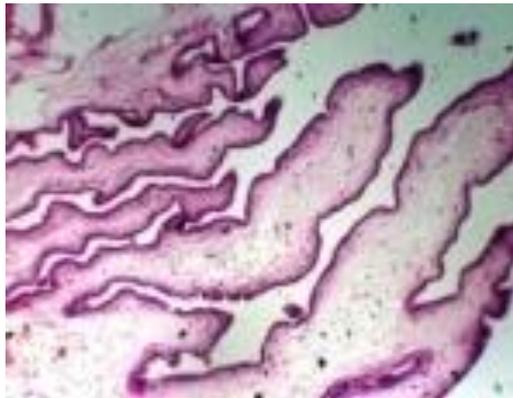
Group- VII

Section of immature rabbit uterus treated with norethisterone at the dose of 0.75

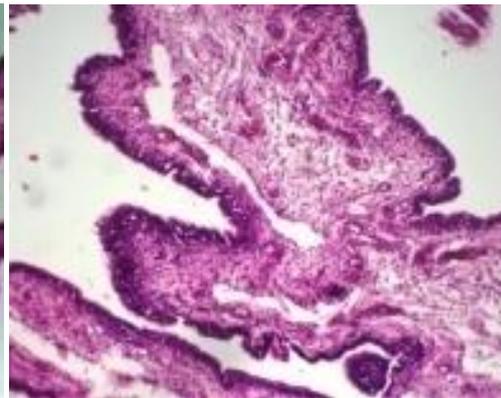
mg /kg and HAMH 400mg/kg p.o showing grade 3 type of proliferation changes in the endometrium i.e. does not inhibit proliferation changes in the endometrium. (uterus mucosa).

Group- VIII

Section of immature rabbit uterus treated with norethisterone at the dose of 0.75 mg/kg and HAMH 600mg/kg p.o showing grade 3 type of proliferation changes in the endometrium i.e.does not inhibit of proliferation changes in the endometrium (uterus mucosa)



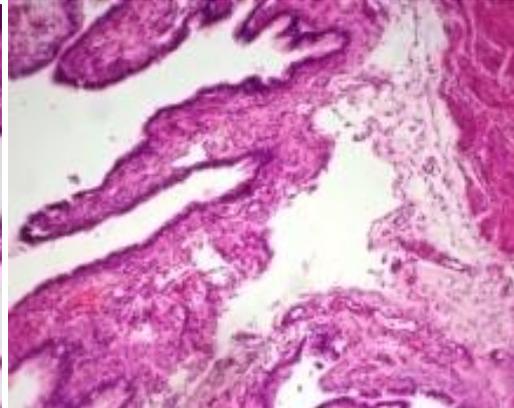
Group- I



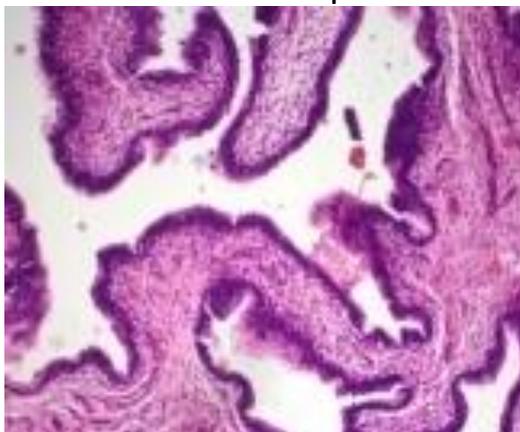
Group- II



Group- III



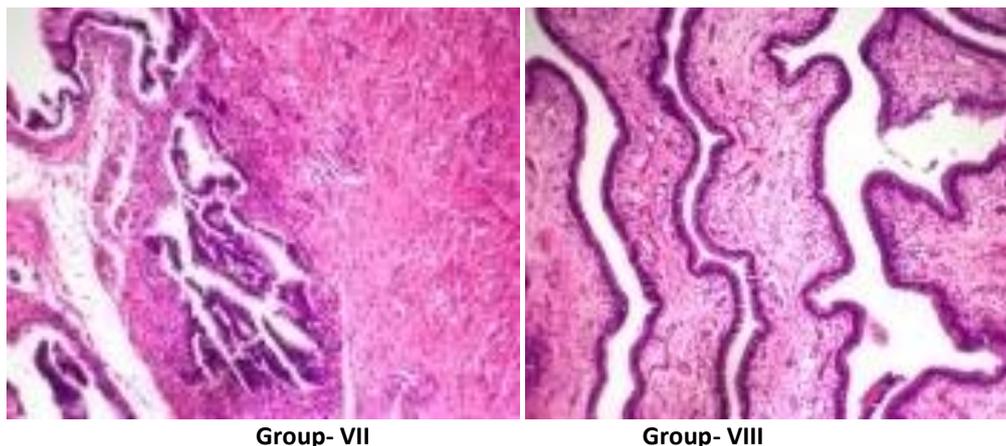
Group- IV



Group- V



Group- VI



Figuer No 6: Effect of Extract on Histopathological Changes in the Uterus of Immature Rabbits after the Treatment.

DISCUSSION

Antispermatic Effect

The antispermatic affects results in the cessation of spermatogenesis. It is indicated by the decrease in sperm count, histopathological observations like cytolytic lesions in the germinal layer, invasion of gonial elements in to the lumen of seminiferous tubules, disintegration of luminal gonial elements and sperm resulting in the accumulation of an edematous fluid, the absence of intact sperm in seminiferous tubules and epididymis and increase in the activity of ALP. ALP is widely distributed in the testes and is important in the sperm physiology. Increase in the activity of ALP is indicative of suppression of spermatogenesis, suppression of exchange of materials between germinal and sertoli cells and extensive lytic activity [16].

The results of the present study showed that administration of HAMH at the dose of 200, 400 and 600mg/kg p.o effect the sperm count and the ALP activity. And histopathology of testes showed distorted seminiferous tubules with disorganised population of spermatogenic and supporting cells. The spermatocytes within the lumen are very few with evidence of reduction spermatogenesis hence the extract have antispermatic activity.

Antiandrogenic Activity

The antiandrogenic activity is reflected by the regression and disintegration of leydig cells, regressive and degenerative changes in the cauda epididymis, seminal vesicles and prostate gland. And hence reduction in the weight of testes, cauda epididymis, seminal vesicle and prostate gland [17,18]. Cholesterol is the precursor for testosterone biosynthesis. Accumulation of cholesterol in the testes is a direct evidence for antiandrogenic action. Testosterone are required for the maintenance of accessory sex organs functions, hence reduction in the serum level of testosterone will lead to the atrophy of sex organs [11]. Leydig cells secrete testosterone. As the population of the leydig cells increases, the level of

testosterone also increases.

Administration of HAMH at the dose of 200, 400 and 600 mg/kg p.o decrease the weights of the accessory sex organs and decrease in cholesterol level in the testes. The serum testosterone level was significant decrease at a dose of 200, 400 and 600 mg/kg and the histopathology of testes showed mild and severe hypercellularity of Leydig cell. Hence, HAMH shows antiandrogenic activity.

Progestational and Antiprogestational Activity

Pregnancy Maintenance Test and Clauberg Assay was performed to investigate the probable progestational or antiprogestational mechanism of antifertility of *Melothria heterophylla* (Lour.) Cogn. in females.

Pregnancy Maintenance Test

The pregnancy maintenance test is a historical bioassay for progestational activity. In the rat, bilateral ovariectomy performed during the first half of pregnancy results in termination of gestation [14]. When ovariectomy is performed during the second half of pregnancy, abortion may not necessarily occur, particularly when a high placenta:fetus ratio exists. This is due to the capacity of placenta to produce progesterone and estrogen [19]. Although abortion or resorption which generally follows ovariectomy after mid-term, occasionally appears to be part of a surgery, it must be concluded that the principle cause for termination of pregnancy is inadequate placental production of progesterone and estrogen. And the ovary should be functional and is necessary throughout normal pregnancy in rat. It has been shown that pregnancy can be successfully maintained in rats ovariectomized during the first half of pregnancy by administration of sufficient quantities of exogenous progesterone alone or progesterone and estrogen [14].

In the present study administration of estrogen and standard progesterone maintained pregnancy, whereas administration of estrogen and Hydro alcoholic extract of *Melothria heterophylla* (Lour.) Cogn. at the dose of 200, 400 and 600 mg/kg p.o maintain the pregnancy. Hence, HAMH at the dose 200, 400 and 600 mg/kg p.o has progestational activity.

Clauberg Assay

Clauberg assay is another historical bioassay of progestational and antiprogestational activity. The histological changes in the uterus, i.e., the endometrial proliferation in estrogen pretreated immature rabbits after the administration of progestational or antiprogestational compounds are assessed here. Progestational activity of the compound was assessed by its ability to produce endometrial proliferation, whereas the antiprogestational activity was assessed by its ability to inhibit the endometrial proliferation produced by norethisterone, a progesterone analog [20]. Administration of estrogen alone to the rabbits caused ramification of the uterus, but not proliferation [14].

In the present study administration estrogen and Hydro alcoholic extract of *Melothria heterophylla* (Lour.) Cogn. at the dose of 200 , 400 and 600 mg/kg p.o show slight and pronounced proliferation changes in the endometrium and endometrial glands. Hence, HAMH at 200, 400 and 600 mg/kg, p.o doses have progestational activity.

Administration of estrogen and norethisterone showed pronounced proliferation of endometrium and endometrial glands. Whereas, administration of esrtogen, norethisterone and Hydro alcoholic extract of *Melothria heterophylla* (Lour.) Cogn. did not inhibit the proliferative changes caused by the norethisterone. Hence, HAMH at 200, 400 and 600 mg/kg, p.o doses may not have antiprogestarional activity.

The results of the Pregnancy maintenance test and Clauberg assay showed that the mechanism of antifertility activity of the Hydro alcoholic extrat of *Melothria heterophylla* (Lour.) Cogn. may be due to progestational activity.

CONCLUSION

The antifertility effect of ethanolic extract of *Melothria heterophylla* (Lour.) Cogn was confirmed by following measures .A great decline in the weights of testis, epididymis and seminal vesicle were observed in all treatment groups when compared with control. Reduction in serum testosterone levels was observed in all treatment groups when compared with control. Decrease in testicular cholesterol levels was observed in all treatment groups and increasing in testicular alkaline phosphates levels was observed in all treatment groups when compared with control. Distorted of seminiferous tubules with disorganised population of spermatogenic and supporting cells. The spermatocytes within the lumen are very few with evidence of reduction spermatogenesis in the histopathological observation. All above parameter indicate that HAMH at 200, 400 and 600 mg/kg, p.o doses have male antifertility activity. The pregnancy maintenance test and Clauberg assay showed that the mechanism of antifertility activity of HAMH may be due to progestational and may not be antiprogestational.

REFERENCES

- [1] Current World Population [Internet]. Cited 2012 January 26. Available from: <http://geography.about.com/od/obtainpopulationdata/a/worldpopulation.html>.
- [2] Khuranan S, Suresh P, Kashi R. Health Education and Community Pharmacy. India: S Vikas and Co; 1996. p. 45-65.
- [3] Herfinadale ET, Gourelly DR. Text book of Therapeutics drugs and disease management. 7th ed. USA: Lippincotts Willams and Wilkins; 2000.p. 2019-30.
- [4] Herb, fertility and contraception [Internet]. Cited 2012 January 7. Available from: <http://www.Healthy.net/scr/Article.asp?id=1207>.
- [5] Neem – A herbal contraceptive for men [Internet]. Cited 2011 July 14. Available from: <http://www.Sisterzeus.com/herbalcontraception.Html>.
- [6] Gu ZP, Mao BY, Wang YX et al. Asian J Androl 2000; 2(4):283-287.
- [7] Cameron SM, Waller DP, Zaneveld LJ. Fertil Steril 1982; 37(2):273-274.
- [8] Kokate CK, Khandelwal KR, Pawar AP, Gohale SB. Practical Pharmacognosy. 3rd ed. Pune: Nirali Prakashan; 1995.p.137-139.

- [9] OECD 2001-guideline on acute oral toxicity (AOT), Environmental health and safety monograph series on testing and adjustment, No. 425.
- [10] Sathiyaraj K et al. Int J Pharma Biol Archives 2011; 2(4):1179-1182.
- [11] Mali PC, Ansari AS, Chaturvedi M. J Ethnopharmacol 2002; 82:61-7.
- [12] Ajeesh Elizabeth A. Evaluation of antifertility activity of roots of *Momordica cymbalaria* (dissertation) Visveswarapura Institute of Pharmaceutical Sciences, Bangalore. Submitted to RGHUS, Karnataka, India, 2005.
- [13] Raju Koneri CD, Saraswati R, Balaraman, Ajeesha EA. Indian J Pharmacol 2007;39: 90-96
- [14] Vogel HG, Wolfgang Vogel H, editors. Drug discovery and evolution pharmacological assays. Springer Verlag Berlin Heidelberg Publications, 2^{ed}, 1997. p.1167-70.
- [15] Gupta SK editors. Drug screening methods. Jaypee Brothers Medical Publishers, ed 2.p.135-137.
- [16] Anjali R, Joshi R, Nazeer Ahamed R. Indian J Exp Biol 1996 Nov; 34:1091-4
- [17] Hiremath SP, Badami S, Swamy HK, Patil SB, Londonkar RL. J Ethnopharmacol 1997 Mar;56(1):55-60
- [18] Vijaykumar B, Sangamma I, Sharanabasappa A, Patil SB. Asian J Androl 2004 Mar;6(1):67-70
- [19] Haterius HO. Am J Physiol 1935; 6(4):399.
- [20] Suganthan D, Santhakumari G. Indian J Med Res 1979 Sep; 70:504-16.