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## Evaluation of Anti-Fertility Activities of Leaves of *Artabotrys Hexapetalus* (Linn. F)

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### ABSTRACT

Population explosion is a major problem it is raising tremendously; this may affect the economic growth: so family planning has been promoted through several methods of contraception. a wide variety of synthetic contraceptive agents are available, but these cannot be used continuously due to their side effects. Thus, present study was to evaluate the effect of 70% hydroalcoholic extract (HAAH) of leaves of *Artabotrys hexapetalus* (Linn. f) on fertility of male wistar rats and female Sprague dawley rats and rabbits. Male wistar rats were orally administered with hydroalcoholic extract of the leaves of *Artabotrys hexapetalus* (Linn. f) (200, 400, 600mg/kg. p.o, 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. Progestogenic activity the number of viable fetus seen at the time of autopsy (on the 20th day) and the net success index was compared with control rats and HAAH treated rats. Clauberg Assay was performed to investigate the probable progestational or antiprogestational mechanism of antifertility in immature female rabbits. Progestational activity assessed by ability to produce endometrial proliferation by HAAH and anti- progestational activity is assessed ability to inhibit proliferation induced by the treatment with norethisterone and HAAH. The treatment caused decrease in weight of testis, seminal vesicles, epididymis and sperm count, serum testosterone testicular cholesterol and alkaline phosphates level and the histological examination of testis revealed that decrease in the diameter of seminiferous tubule sever hypercellularity of leydig cells. Pregnancy maintenance was assessed and it is non- progestogenic. Clauberg assay (endometrial proliferation) in immature it does not shown either progestational or anti-progestational activities.

**Keywords:** *Artabotrys hexapetalus*, antifertility, Antispermatic, Clauberg assay, Antiprogestational, Progestational

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## INTRODUCTION

The world's population has increased exponentially since the industrial revolution because of the modern technologies and medicines available. Thus, the population carrying capacity has increased. The planet is experiencing depletion of the world's net resources due to the requirement of food and other materials for sustenance. The increased use of fossil fuels presumably contribute to global warming resulting in the melting of glaciers, the rising ocean levels, and the more frequent hurricanes. Due to the ever-increasing demands for food and palatable water from the population increase, deaths due to hunger and thirst will potentially be at higher numbers if the demands are not met. However, the lands are becoming infertile because of over cropping and urbanization, considering that the constructions as well as the inflow of household wastes and industrial effluents increase the acidity of the soil [1].

Population explosion is alarming; it results in the exploitation of natural resources and affects the economic growth of a country. Population control is the practice of curtailing the population increase, usually by reducing birth rate [2-4]. Many plants/ plant extract have been used as antifertility agents in folklore and traditional medicines without producing apparent toxic effects. As part of this research programme, we present in this paper antifertility efficacy of leaves of *Artabotrys hexapetalus* (Linn. f)

## MATERIAL AND METHODS

### Plant Material

The leaves of *Artabotrys hexapetalus* (Linn. F) were collected from Gandhi Krishi Vignana Kendra, Bangalore, Karnataka, India. The plant was identified, and authenticated by Dr. M. Vasundara, Professor, Division of Horticulture, University of Agricultural Sciences, GKVK, Bengaluru, Karnataka (No. 13/Hort/MADP/2011-12 (Authentication No 1).

### Preparation of Extract

The leaves were shade dried and coarse powder by using a mechanical grinder. The powdered material was packed in Soxhlet apparatus and extracted with 70% (v/v) ethanol. The extract was concentrated and dried. The dried extract (HAAH) stored in air tight container in refrigerator <math>10^{\circ}</math> C and percentage of yield calculated.

### Preliminary Qualitative Phytochemical Analysis [5]

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 weighing between 150-200g were procured from the Biogene, Bangalore, Karnataka. Sprague-Dawley rats of female and male sex weighing between 200-250 g were procured from IN VIVO Biosciences, Bangalore and Rabbits are procured from the Rabbit Farm, Shivakote, Bangalore. All animals were acclimatized for seven days under laboratory conditions. The animals were fed with commercially available pelleted diet (Amruth) feeds & foods, Bangalore). Water was allowed *ad libitum* under strict hygienic conditions. The study protocols were duly approved by the Institutional Animal Ethics Committee (IAEC/018/12/2010) (Approval no: 491/01/c/CPCSEA) of Gautham College of Pharmacy, Bangalore. Studies were performed in accordance with the CPCSEA guidelines.

## **Acute Toxicity Studies [6]**

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 HAAH (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 8<sup>th</sup> and 14<sup>th</sup> day. The same procedure was repeated with another set of animals to nullify the errors.

## **Antifertility activity in Male Rats [7-9].**

The animals were divided in to four groups of six animals each Group I Received only tween 80 of 0.5 ml (p.o daily) for 45 days and served as control. Group II,III,IV Received HAAH at 200, 400, 600 mg mg/kg body weight (p.o daily) for 45 days. On the 45<sup>th</sup> 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 was preserved in neutral formalin buffer 10% for 24h.

### **Pregnancy Maintenance Test [10-12]:**

Progestational activity was assessed by pregnancy maintenance test. Mature Spraque-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 8<sup>th</sup> 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 0.1µg of Estradiol valerate (s.c) from 8<sup>th</sup> to 19<sup>th</sup> day, serves as the control; Group II Received Progesterone 3mg/rat/day, s.c, and 0.1µg of Estradiol valerate (s.c) from 8<sup>th</sup> to 19<sup>th</sup> day served as reference standard; Group III, IV, V Received HAAH at the 200, 400, 600 mg/kg body weight (p.o daily) and 0.1µg of Estradiol valerate (s.c) from 8<sup>th</sup> to 19<sup>th</sup> day.

On the 20<sup>th</sup> day, the animals were autopsied, presence or absence of the implantation sites and the number of live embryos were recorded.

### Clauberg Assay [10-12]:

Progestational and anti-progestational activity was assessed in New Zealand rabbits using Clauberg assay. Immature rabbits weighing 550-650g were used for experiment. The animals were maintained under standard experimental conditions. The animals were grouped into eight groups of three animals each. All animals were injected, s.c, with estradiol valerate at the dose of 8.3 µg/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 5days. Group II Received norethisterone 0.75 mg/kg (p.o daily) for 5 days; Group III, IV,V, Received norethisterone 0.75 mg/kg and HAAH at the 200, 400, 600mg/kg (p.o. daily) for 5 days; Group VI, VII,VIII Received HAAH at the 200, 400, 600mg/kg (p.o daily) for 5 days.

The animals were sacrificed on the 15<sup>th</sup> 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 24hr. Uterus is processed for histopathology.

## RESULTS

### Extraction

Extraction of *Artabotrys hexapetalus* (Linn. f) was carried out by using the Soxhlet apparatus with 70% (v/v) ethanol solvent the percentage yield of extract is given below in Table No 1.

Table No 1: Extractive Yield and Percentage Yield of *Artabotrys hexapetalus* (Linn. F)

Extract	Yield in gms	Percentage yield
Hydroalcoholic	34.51	9.08%

### Preliminary Qualitative Phytochemical Analysis

Table No 2: Preliminary Phytochemical Screening of Extracts

Sl no	Test	HAAH
1	Alkaloids	+
2	Carbohydrates	+
3	Flavonoids	+
4	Glycosides	+
5	Phytosterols/Terpens	+
6	Proteins	+
7	Tannins	+
8	Saponins	+
9	Lipids	-

+ Indicates presence

- Absent

## Acute Toxicity Studies

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

## Antifertility Activity in Male Rats

### Effect of Extract on Total Sperm Count

Reduction in Total Sperm Count was observed in all treatment groups when compared with Group- I are shown in Table No- 3. The respective levels in Groups- II, III and IV were  $29.33 \pm 1.05$ ,  $24.67 \pm 0.66$  and  $22.67 \pm 0.88$  versus  $44.33 \pm 1.28$  in Group- I. Oral administration of HAAH at the dose of 200, 400 and 600mg/kg showed very significance ( $P < 0.001$ ) reduction in total sperm count. Most significance reduction of total sperm count  $22.67 \pm 0.88$  was observed at the dose of 600mg/kg.

**Table No 3: Effect of HAAH on Total Sperm Count of Male Rats.**

Groups	Treatment	Epididymal Sperm count ( $\times 10^5$ )
Group I	Saline	$44.33 \pm 1.28$
Group II	HAAH 200mg/kg	$29.33 \pm 1.05^{***}$
Group III	HAAH 400mg/kg	$24.67 \pm 0.66^{***}$
Group IV	HAAH 600mg/kg	$22.67 \pm 0.88^{***}$

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. HAAH- Hydro alcoholic extract of *Artabotrys hexapetalus*.

### 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-2. Oral administration of HAAH at the dose of 200mg/kg showed significance at  $P < 0.01$  and 400, 600mg/kg showed very significance ( $P < 0.001$ ) decrease in testis and epididymis.

Administration HAAH at the dose of 200, 400 and 600mg/kg, p.o showed very significance ( $P < 0.001$ ) decrease in seminal vesicle weights. Most significance reduction of testis, seminal vesicle and epididymis weights  $1.165 \pm 0.02$ ,  $0.443 \pm 0.08$  and  $0.246 \pm 0.004$  respectively was observed at the dose of 600mg/kg.

**Table No 4: Effect of HAAH 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	170.3±2.71	211.5± 3.81	1.63±0.02	0.387±0.015	0.672±0.01
Group II	HAAH 200mg/kg	176.8±5.68	219.7 ±3.29	1.50±0.01**	0.331±0.015**	0.570±0.005***
Group III	HAAH 400mg/kg	173.3±4.41	222.2± 5.88	1.36±0.01***	0.274±0.004***	0.486±0.01***
Group IV	HAAH 600mg/kg	166.5±3.54	224.8±5.73	1.165±0.02***	0.246±0.004***	0.443±0.08***

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 compare with Group1. HAAH- Hydro alcoholic extract of *Artabotrys hexapetalus*.

**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 are shown in Table No-5. The respective levels in Groups- II, III and IV were 5.815 ± 0.07, 5.115 ± 0.02 and 5.097 ± 0.05 versus 11.10 ± 0.015 in Group- I. Oral administration of extract at the dose of 200, 400 and 600mg/kg showed very significance (P<0.001) reduction in serum testosterone levels. Most significance reduction of serum testosterone level 5.097 ± 0.05 was observed at the dose of 600mg/kg.

**Table No 5: Effect of HAAH on Serum Testosterone Level of Male Rats.**

Groups	Treatment	Testosterone (ng /mL)
Group I	Saline	11.10 ± 0.015
Group II	HAAH 200mg/kg	5.815 ± 0.07***
Group III	HAAH 400mg/kg	5.115 ± 0.02***
Group IV	HAAH 600mg/kg	5.097± 0.05***

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. HAAH- Hydro alcoholic extract of *Artabotrys hexapetalus*.

**Tissue Biochemistry**

**Effect of Extract on Testicular Cholesterol and ALP Levels in Male Rats.**

Increasing in testicular Alkaline phosphates levels and decreasing in the testicular cholesterol levels was observed in all treatment groups when compared with Group- I are shown in Table No-6. The respective levels in Groups- II, III and IV were 682.1± 3.48, 760.9± 4.00 and 844.1 ± 6.34 versus 522.8± 8.24 in Group- I. Administration of extract at the dose of 200, 400 and 600mg/kg, p.o showed very significance (P<0.001) increase in testicular ALP levels. Most significance increase of testicular ALP level 844.1 ± 6.34 was observed at the dose of 600mg/kg.

The respective cholesterol levels in Groups- II, III and IV were  $9.668 \pm 0.62$ ,  $6.307 \pm 0.93$  and  $4.752 \pm 0.701$  versus  $16.75 \pm 0.87$  in Group- I. Administration of HAAH at the dose of 200, 400 and 600mg/kg showed very significance ( $P < 0.001$ ) decrease in testicular Cholesterol levels. Most significance decrease of testicular Cholesterol level  $4.752 \pm 0.701$  was observed at the dose of 600mg/kg.

**Table No 6: Effect of HAAH on Testicular Cholesterol and ALP Levels in Male Rats.**

Groups	Treatment	Alkaline Phosphates	Cholesterol
Group I	Saline	$522.8 \pm 8.24$	$16.75 \pm 0.87$
Group II	HAAH 200mg/kg	$682.1 \pm 3.48^{***}$	$9.668 \pm 0.62^{***}$
Group III	HAAH 400mg/kg	$760.9 \pm 4.00^{***}$	$6.307 \pm 0.93^{***}$
Group IV	HAAH 600mg/kg	$844.1 \pm 6.34^{***}$	$4.752 \pm 0.701^{***}$

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

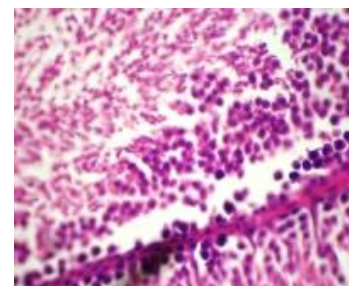
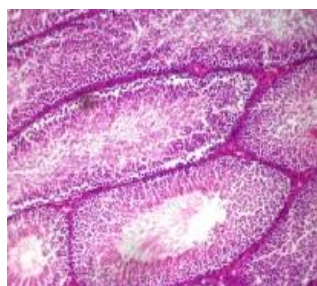
**Histopathology Study of Testis of Rats (Figure 1)**

Group –I (Normal Control + Saline) : Section of testis of rat treated with saline (control) showing normal spermatogenic activity in the seminiferous tubules, normal population of the leydig cell.

Group II- HAAH 200mg/kg: Section studied shows that slightly reduction in the diameter of seminiferous tubules, with reduced layering, with less spermatozoa, showing mild hypercellularity of leydig cells.

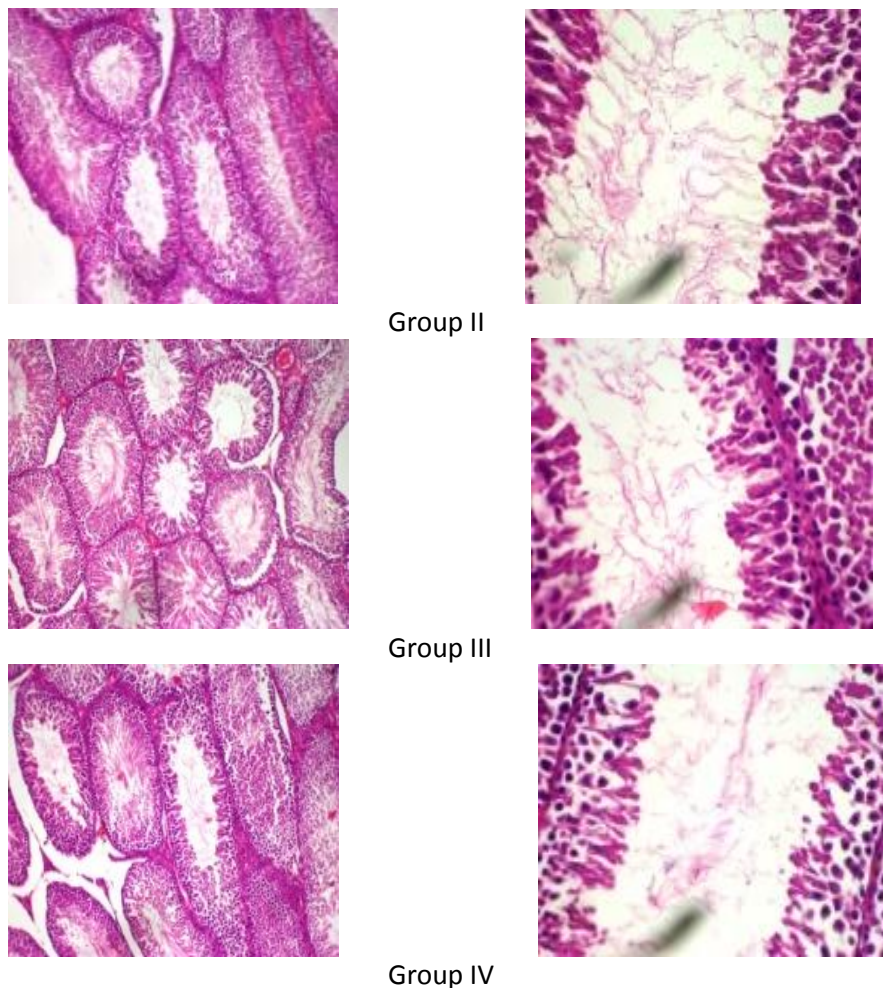
Group III- HAAH 400mg/kg: Section studied shows that significantly reduction in diameter of seminiferous tubules, immature spermatozoa, less number of spermatozoa, showing sever hypercellularity of leydig cells and presences of large multinucleated cells.

Group IV - HAAH 600mg/kg: Section studied shows that significantly lesions of seminiferous tubules, significantly reduction in diameter of seminiferous tubules, immature and less number of spermatozoa, showing sever hypercellularity of leydig cells, less spermatozoa and empty interstitial spaces and presences of large multinucleated cells.



Group I





**Fig No 1: Histopathology of Testes**

**Progestational and Antiprogestational Activity**

**Table No 7: Effect of Extract on Maintenance of Pregnancy in the Rats Ovarietomized on the 8th day of Pregnancy.**

Groups	Treatment	Mean Viable Feotus	Net Success Index (%)
Group I	Saline + Estradiol 0.1µ g/rat /day s.c. (control)	0	0
Group II	Estradiol 0.1µ g/rat /day s.c.+ Progesterone 3mg/rat/day s.c. (reference standard)	7±1.46	63.56±13.27
Group III	Estradiol 0.1µ g/rat /day s.c. + HAAH 200mg/kg	0	0
Group IV	Estradiol 0.1µ g/rat /day s.c. + HAAH 400mg/kg	0	0
Group V	Estradiol 0.1µ g/rat /day s.c. + HAAH 600mg/kg	0	0

Administration of estradiol 0.1µg/rat/day s.c. and progesterone 3mg/rat/day s.c for 13 days to the rats after ovarietomizing the rats (on the 8th day of pregnancy) maintained

pregnancy. The number of viable fetus seen at the time of autopsy (on the 20th day) and the net success index of pregnancy maintenance was statistically very significant (\* $P < 0.001$ ), when compared to the animals in the control group (Table No 7). In the control group the animals had complete abortion and the pregnancy was not maintained. Administration of estradiol 0.1 $\mu\text{g}/\text{rat}/\text{day}$  and hydro-alcoholic extract of *Artabotrys hexapetalus* (Linn. F) at the dose of 200, 400 and 600 mg/kg/ day, p.o to the rats did not maintain pregnancy. The effect of the drug extract was similar to that of the control group.

### **Clauberg Assay**

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

Group I: (Estradiol valerate [8.3  $\mu\text{g}/\text{kg}$ ]+ Saline)

Section of immature rabbits uterus treated with 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.

Group II : (Estradiol valerate [8.3  $\mu\text{g}/\text{kg}$ ] + Norethisterone[0.75 mg/kg])

Section of immature rabbit uterus treating showing grade 3 i.e., medium pronounced proliferation of the uterus.

Group III: (Estradiol valerate [8.3  $\mu\text{g}/\text{kg}$ ] + Norethisterone [0.75 mg/kg] + HAAH [200mg/kg])

Section of immature rabbit uterus treating showing grade 3 i.e., medium pronounced proliferation with convolution of glands.

Group IV: (Estradiol valerate [8.3  $\mu\text{g}/\text{kg}$ ] + Norethisterone [0.75 mg/kg] + HAAH [400mg/kg])

Section of immature rabbit uterus treating showing grade 3 i.e., medium pronounced proliferation of the uterus mucosa, slight additional ramification.

Group V: (Estradiol valerate [8.3  $\mu\text{g}/\text{kg}$ ] + Norethisterone [0.75 mg/kg] + HAAH [600mg/kg])

Section of immature rabbit uterus treating showing grade 3 pronounced proliferation of the uterus mucosa.

Group VI: (Estradiol valerate [8.3  $\mu\text{g}/\text{kg}$ ] + HAAH [200mg/kg])

Section of immature rabbits uterus treated showing grade 0 type of proliferative changes in the endometrium. i.e. ramification of the uterus, but not proliferation of endometrium and endometrial glands.

Group VII: (Estradiol valerate [8.3 µg/kg] + HAAH [400mg/kg])

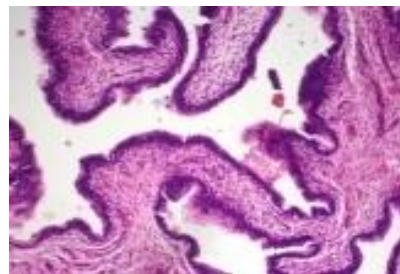
Section of immature rabbits uterus treated showing grade 0 type of proliferative changes in the endometrium. i.e. ramification of the uterus, but not proliferation of endometrium and endometrial glands.

Group VIII: (Estradiol valerate [8.3 µg/kg] + HAAH [600mg/kg])

Section of immature rabbits uterus treated showing grade 0 type of proliferative changes in the endometrium. i.e. ramification of the uterus, but not proliferation of endometrium and endometrial glands.



Group I



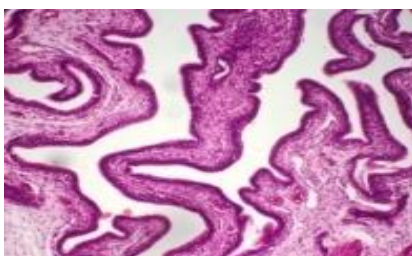
Group II



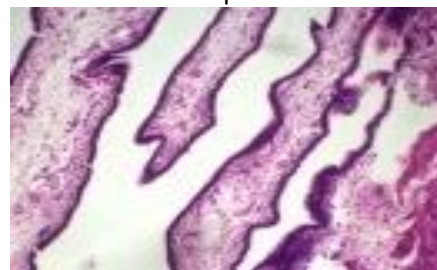
Group III



Group IV



Group V



Group VI



Group VII



Group VIII

**Fig No 2: Histopathology of Rabbit Uterus**

## DISCUSSION

### Antispermatic Effect

The antispermatic effects results in the cessation of spermatogenesis. It is indicated by the decrease in sperm count, histological observation 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, decrease in diameter in seminiferous tubules, the absence of intact sperm in seminiferous tubules, presence of multi nucleated cells, hypercellularity of leydig cells, 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 [13,14]. The results of the present study showed that oral administration of HAAH at the dose of 200, 400 and 600mg/kg effect the sperm count and the ALP activity. Histopathology of testes showed abnormal, immature spermatogenic activity in the seminiferous tubules, decrease in diameter in seminiferous tubules, presence of nucleated cells etc. 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. Cholesterol is the precursor for testosterone biosynthesis. Accumulation of cholesterol in the testes is a direct evidence for antiandrogenic action[13]. 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[7]. Leydig cells secrete testosterone. As the population of the leydig cells increases, the level of testosterone also increases. Administration of HAAH at the dose of 200, 400 and 600mg/kg decreases 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

600mg/kg and the histopathology of testes showed mild and severe hypercellularity of Leydig cells (which may be dose dependent). Hence HAAH shows antiandrogenic activity.

### **Progestational and Antiprogestational Activity**

Pregnancy Maintenance Test and Clauberg Assay were performed to investigate the probable progestational or antiprogestational mechanism of antifertility of HAAH in female Sprague-Dawley rats and rabbits.

#### **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 [11]. 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 [15]. Although abortion or resorption which generally follows ovariectomy after mid-term, occasionally appears to be part of 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 [16]. 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 [15]. In the present study administration of estradiol valerate and standard progesterone maintained pregnancy, whereas administration of estradiol valerate and HAAH at the dose of 200, 400 and 600mg/kg did not maintain the pregnancy. Hence, HAAH does not show 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 [17]. Administration of estrogen alone to the rabbits caused ramification of the uterus, but not proliferation [15].

In the present study administration of estrogen and HAAH at the dose of 200, 400, and 600mg/kg showed ramification of the uterus but not proliferation, which was similar to that of estrogen and vehicle alone. Hence the HAAH at the dose of 200, 400 and 600 mg/kg may not have progestational activity. Administration of estrogen and norethisterone

showed pronounced proliferation of endometrium and endometrial glands. Whereas administration of estrogen, norethisterone and HAAH did not inhibit the proliferative changes caused by the norethisterone. Hence, HAAH at the dose of 200, 400 and 600mg/kg do not show antiprogestational activity.

In conclusion, the present study indicate that hydro-alcoholic extract of *Artabotrys hexapetalus* (Linn. f) possess male antifertility activity may be due active principles present in the extract and Pregnancy maintenance test and Clauberg assay showed that the mechanism of antifertility activity in female rats and rabbits of the HAAH was not progestational or antiprogestational.

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