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Antifertility activity of *Millingtonia hortensis* in Male albino rats

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ABSTRACT

Though the present technology in the world can able to solve many problems that occur in different areas, still human life is unable to lead the good life. The population density in the India is increasing day to day because of that the basic needs are not meeting the poor. There are many approaches to cope up this problem. Medicinal plant extracts play an important role in coming over the problem as a contraceptive therapy in controlling the population. Since there are several medicinal plants proved for their pharmacology activities, the present study is also done to know the antifertility activity of the plant *Millingtonia hortensis* extracts in male albino rats. The methanol (MHMLE) and aqueous (MHALE) extracts of the plant were administered to male albino rats for 21 days by maintaining the control group. The decreased sperm count and motility were noticed in the extract administered rats. The serological results showed the increased serum levels of cholesterol, triglycerides, SGOT, SGPT, decreased protein, albumin, glucose levels. The Testis cross sections of extract administered rats also supported the results with degenerated interstitium. The testis and liver tissue glycogen and protein values also depleted in the extract administered rats.

Keywords: Antifertility, Spermcount, SGOT, SGPT, Fertility control.

INTRODUCTION

Civilization and technology are improving day to day. Though the technology is meeting the needs of the common man, it is only at one side of the coin. Because the other side the poverty and unemployment are questioning the existence of Human on the earth. Not only the government but some of the NGOS are also unable to cope up with this problem. Food, employment and health have become great problems for the country. One of the solutions to the above problems is controlling the population. Population control is not a simple task, actually to which every person should put forward his effort in succeeding that.

As in 2021 the population may mark near to the 140 crores in the India. The available reasons in country in all aspects may not reach the needs of poor and common middle-class people for that there is need to search in alternative option.

From ancient times onwards, our herbal practitioners have been depending on the plant extracts to treat for several ailments. Among them we can also search for the fertility controlling or birth controlling therapies. In that attempt, several research concepts were published in proving the antifertility activities of many plants [1].

The available synthetic chemical compounds may cause the side effects to the body. They may fetch the problems to the non-targeted parts of the body like. Liver, kidney, brain and heart can also affect the endocrine system even to reproductive organs [2].

Most of the works of the researchers have also proved that the herbal medicine usage in the form of anti-spermatogenic and spermicidal activities and some of them are proved for their safe and cheap method in controlling the population growth [3].

Plants like Neem,Amla, Ficus, Arjuna etc trees are already proved for their effective antifertility activity [4]. The present study on *Millingtonia hortensis* plant is done to unveil the antifertility activity.

MATERIAL AND METHODS

Collection of plant material

The medicinal plant *Millingtonia hortensis lin.* was collected from the village area of Gudur,

Mahabubabad District, Telangana State. It was observed and recognized by Prof. V.S. Raju (Retd.), Department of Botany, Kakatiya University, Warangal. The plant was stored in the herbarium of the lab by allocating voucher number (RPU/ZOO/MH/2015).

Preparation of Extract

The leaves of collected plant were dried in shade for about 15 days. The leaves were powdered with electrical grinder. The collected coarse powder then passed through No.20 plate mesh sieve and the fine powder was stored in airtight amber glass bottle and used for the preparation of extracts.

Maceration technique was employed to prepare the extracts from leaf powder of the plant. Solvents like methanol and aqueous were used to get the extract. 50g of powder was taken in Stoppard conical flasks; it was mixed with 250ml of solvent and allowed for 24hrs by shaking randomly at room temperature. This 1st filtrate was collected and the remaining marc dissolved in another 250ml of solvent with vigorous shaking and left for 24 hrs and collected the 2nd filtrate. Then the two filtrates were distilled for collecting extracts and were preserved in well closed amber glass containers at refrigerator temperature.

Solvents like Acetone, Diethylether, Ethanol, Methanol and water used to collect the extract with above maceration process, But among them Methanol and Aqueous leaf extracts were selected for the study of antifertility activity.

Animal Models for the Study and Treatment

Male albino rats (Wistar strain) weighing about 200 to 240gr were brought from the Mahaveer enterprises, Hyderabad.

These rats were allowed to habituate to the required laboratory condition (25c, 60% humidity) as per the Institutional Animal Ethics committee protocol (IAEC/03/UPSC/KU/10). Rats fed with appropriate diet (Hypo feed for animals pure) and water ad libitum [5].

Studying the Toxicity of Extract

Different doses of extracts (150,200,250,300 mg/kg) were given to the rats (4 groups-6 animals in each group) and they were being observed for seven days. It was noticed that the toxicity of the extract had not seen in rats up to 300 mg/kg. So for the treatment of the extract the 150mg/kg dose was fixed.

Experimental Design

The rats were administered with MHALE (Group-II) and MHMLE (Group-III) with the dose 150 mg/kg through oral for 21 days with 8 rats in each group by maintaining a control (Group-I).

After 21 days the rats were sacrificed and blood sample were collected for serological tests. Important organs like Liver, Prostate, testis were separated for their weights and biochemical experiments. Testes were also processed for the histological studies.

Sperm Count and Motility

The rats were dissected and cauda epididymis were separated and teased finely in the 20ml of normal saline to know the sperm count and motility.

The Neubauer's haemocytometer was taken and cover slip was placed on it. With the help of a dropper 1 ml of saline was inserted into the Neubauer's haemocytometer then left a few minutes to settle the sample on the plate. The slide was placed under a microscope and an area of counting chambers was located with a low power objective. The sperm count was done by counting 5 squares (4 corners and 1 centre) of the plate.

Then the volume represents as

No. of Sperms /ml = No. of sperms x 0.1mm^3 x dilution rate x 1000.

% of Motility = Motile sperm/ Motile sperm+ Non motile sperm

The separated testis and liver were used for the tissue glycogen (modified Anthrone method) and protein content (Lowrey method). The testes were processed for the histological study.

Serological Tests

The blood samples which were collected from rats were centrifuged and processed for serological parameters like cholesterol, triglycerides total proteins, albumin, SGOT, SGPT. The tests were done with commercially available kits.

Biochemical Experiments

Liver and Testes tissue samples used to know the glycogen and protein content to know the adverse effect of the extracts. Histological sections of the testes were also taken to study the effect of the extracts of the plant.

Results and Statistical analysis

All values were expressed in mean ± SD (n=8), the values were analyzed with one way ANOVA followed by Dunnett Test. The results were assessed with significance of ** =p<0.01and *= p<0.05 and ns=p>0.05 compare to Group-I (Control).

RESULTS

The body weights of the treated rats were decreased in either extract treated rats, when compare to the control rats. The rats (treated with the extracts) vital reproductive organs weights and also other vital non reproductive organs weights were also decreased comparing to the control groups (Table-1).

Table 1: Body Weight

GROUP	BODY WEIGHT (g)	
	INITIAL	FINAL
Group-I (Control)	222.12 ± 3.68	230.37 ± 4.27
Group-II (MHALE 150 mg/kg)	217.25 ± 4.20*	224.25 ± 4.55*
Group-III (MHMLE 150 mg/kg)	218.75 ± 3.73^{ns}	227.25 ± 4.02^{ns}

The sperm count of the MHALE treated albino rats were significantly reduced (39.16 ± 2.04). The motility also greatly decreased in MHALE given rats (46.02 ±4.88), when compare with the control group rats (Table-2).

Table 2: Sperm count and Sperm motility

Group	Sperm Count (mil/ml)	Sperm Motility (%)
Group-I (Control)	54.93± 3.34	75.47 ± 4.81
Group-II (MHALE 150 mg/kg)	39.16 ± 2.04**	46.02 ± 4.88**
Group-III (MHMLE 150 mg/kg)	45.09± 3.56**	55.04 ± 4.32**

The serological analysis was also observed to notice the efficacy of the extracts, the serum SGOT, SGPT, values were observed to be increased in the MHALE given rats. This effect was less in the MHMLE given albino rats (Table-3).

Table 3: SGOT and SGPT

GROUP	SGOT (U/L)	SGPT(U/L)
Group-I (Control)	30.37 ± 1.40	31.18 ± 0.94
Group-II (MHALE 150 mg/kg)	32.63 ± 1.44**	32.06 ± 0.73*
Group-III (MHMLE 150 mg/kg)	28.76 ± 0.46*	29.97 ± 0.32**

Serum cholesterol and triglycerides concentrations were significantly increased in the MHALE and MHMLE given albino rats (Table-4). The effect was observed highly in the MHALE administered rats. The depleted values of serum protein and albumin, glucose were noticed in the extracts given rats (Table-5).

Table 4: Cholesterol and Triglycerides

Group	Cholesterol (mg/dl)	Triglycerides (mg/dl)
Group-I (Control)	144.25 ± 3.27	87.17 ± 4.20
Group-II (MHALE 150 mg/kg)	164.65 ± 2.31**	91.15 ± 2.66*
Group-III (MHMLE 150 mg/kg)	150.79 ± 3.63*	94.83 ± 2.42**

Table: 5 Serological tests Total Protein & Albumin

Group	Protein (gr/dl)	Albumin (gr/dl)
Group-I (Control)	7.30 ± 0.28	3.33 ± 0.26
Group-II (MHALE 150 mg/kg)	7.01 ± 0.19*	2.95 ± 0.12**
Group-III (MHMLE 150 mg/kg)	7.77 ± 0.20**	2.98 ± 0.25*

The Biochemical tests like tissue proteins, glycogen estimation were also performed to know the effect of extracts in testes and liver. These results were also proved the tissue degenerative effect of the MHALE and MHMLE. The glycogen and protein content of testes and liver tissues were drastically decreased in the MHALE and MHMLE given rats (Table-6 & 7). The degenerated intersitium of the testis and germinal epithelium also observed in the extracts administered rats (Fig.01-03).

Table 6: Liver Glycogen and Testis Glycogen

Group	Liver Glycogen (µg/100mg)	Testis Glycogen (µG/100MG)
Group-I (Control)	700.09 ± 13.47	385.84 ± 10.82
Group-II (MHALE 150 mg/kg)	602.08 ± 16.11**	260.22 ± 17.27**
Group-III (MHMLE 150 mg/kg)	618.51 ± 9.17**	344.03 ± 15.14**

Table 7: Liver Protein and Testis Protein

Groups	Liver Protein (mg/gr)	Testis Protein (mg/gr)
Group-I (Control)	184.21 ± 3.50	186.14 ± 4.60
Group-II (MHALE 150 mg/kg)	165.94 ± 5.00**	187.85 ± 3.25ns
Group-III (MHMLE 150 mg/kg)	173.25 ± 5.74**	181.02 ± 3.28*

All values were expressed in mean ± SD (n=8), the values were analyzed with one way ANOVA followed by Dunnett Test. ** =p<0.01and *= p<0.05 and ns=p>0.05 compare to Group-I (Control).

Cross Section of Testis

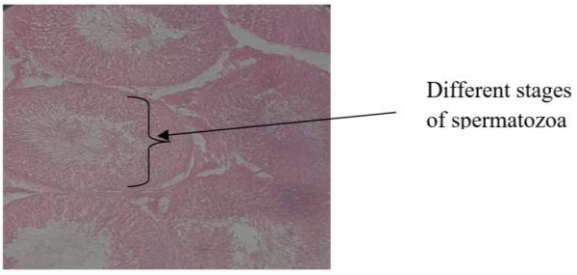

Figure 1: Testis cross section of control rat Group-I – (Control)

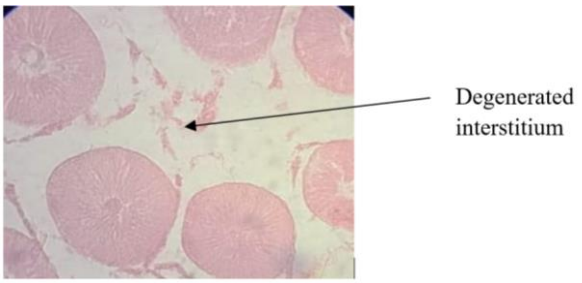

Figure 2: Testis cross section of aqueous extract (Group-II- MHALE 150mg/kg)

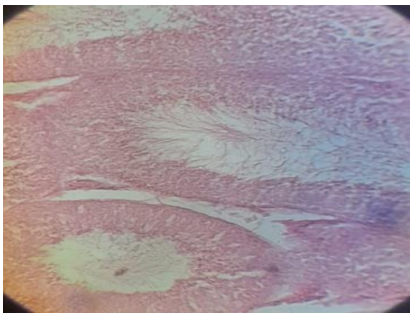

Figure 3: Testis cross section of methanol extract. (Group-III- MHMLE 150mg/kg)

DISCUSSION

MHALE extracted rats were shown reduced body weights might be the effect of the extracts caused the reduction in the body weights of rats when compare to the control (Table-1). The vital organ weights were also decreased in the both methanol and aqueous extracts given rats. But the effect of aqueous extract was observed to be more than to the methanol extract. Similar results were observed in the experiments performed in the rats treated with ethanolic extracts of *Leonotis repetifolia* [6].

The sperm count and motility values were also decreased significantly in the MHALE administered rats. The testosterone hormone may not be released from the leydig cells (of the testicular interstium) in the extracts given rats, which caused the low sperm count and motility. The reduced vital reproductive organs weights may also the possible reason in observing the lower number of sperms and also motility. The results were comparable with the observation in male rats, which were treated with *Martynia annua* root extract on male rats [7].

It was noticed that the serum parameters like SGOT, SGPT were increased in the MHALE given albino rats. These are generally considered as the markers for the liver functioning. The increased values may be because of the marginal toxicity of the extracts. But this effect is not observed in the MHAME given albino rats because of the presence of potential phytochemicals like flavanoids, phenols. The effect of MHMLE is not much when compare to the MHALE.

The SGOT, SGPT increased values may be because of the slight liver damage caused by the extracts. The serum cholesterol and triglycerides were increased in the extract administered rats. Similar reports were noticed in the rats treated with *Viscum album* with its crude extracts [8].

Low proteins and albumin levels were observed in the extract treated rats this may be because of the liver dysfunction, malnutrition, nephrosis, malabsorption, diarrhea etc [9].

The decreased blood glucose levels may be the stress effect of the rats towards the drug (extract) MHALE.

Liver proteins, glycogen including the testis protein, glycogen content were also depleted in the MHMLE administered rats than to the MHMLE and control rats. This might be because of the stress and adverse effect of extract on the testis and liver, which also supported with the serum results.

The seminiferous tubules of the testis in the MHALE administered rats were noticed to loss of germinal epithelium, the degeneration of the seminiferous tubules may be the reason of reducing the spermcount in the MHALE given rats than to the MHMLE and control rats. These results correlate with the findings of *Caryca papaya* aqueous extracts treated rats [10].

CONCLUSION

The MHMLE and MHALE extracts administered rats were observed with reduced spermcount, motility and increased serum cholesterol, triglycerides, SGOT, SGPT, decreased protein, albumin, glucose levels. Tissue glycogen and protein contents also reduced in the extracts given rats when compare to the control rats. These results were noticed with antifertility activity in MHALE than to the MHMLE. So, with these results it is to conclude that MHALE has antifertility activity.

Conflict of Interest

None declared.

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None declared.

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