

**EFFECT OF H-1 ANTIHISTAMINE PROMETHAZINE ON
FERTILITY IN MALE ALBINO RATS.****DR. GAJANAN PURUSHOTTAM KULKARNI*, DR. YOGITA LAXMIKANT KULKARNI,
DR. PADMANABHA T. S. AND DR. MOHAMMED MATEENUDDIN***Department Of Pharmacology Bidar Institute Of Medical Sciences Bidar, Karnataka, India***ABSTRACT**

Present study was undertaken to evaluate effects of Promethazine, a commonly used H₁ Histamine antagonist on fertility in adult Male Albino rats. Although there are evidences of histaminergic system in the testis and spermicidal action of H₁ receptor antagonists in vitro, there is complete lack of information about its effects on sperm count, sperm motility and histopathology of testes in vivo⁽¹⁾. Male albino rats were segregated in 4 groups of 24 animals each. Group 1 received Promethazine 6 mg / Kg / day orally daily for two weeks period, group II received Promethazine 6 mg / Kg orally for six weeks, group III and IV which received 2ml normal saline daily orally for two and six weeks respectively served as control. Twenty four hours after the last treatment animals were anesthetized and laparotomy was conducted. Epididymis removed & minced in 1ml phosphate buffer solution (PBS PH 7.2) and epididymal sperms were studied for sperm count and sperm motility. Sperm count was done using neubars chamber and sperm motility was studied after staining with 1% aqueous eosin^{(2),(9)}. Histopathology of testes was done after scarification of rats. Data were analysed using Mann whitney 'U' Test. Sperm count and sperm motility in Promethazine treated groups was significantly reduced, as compared to control groups. Promethazine reduced sperm count by interfering spermatogenesis and decreased motility, probably affecting intracellular Ca⁺ concentration. Hemorrhage and necrosis of seminiferous tubules was seen in histopathology of promethazine treated rats. But more studies are necessary to reveal exact mechanism of action.

KEY WORDS: Promethazine, Sperm Count, Sperm Motility, Histopathology of Testes, Rats.**DR. GAJANAN PURUSHOTTAM KULKARNI**
Department Of Pharmacology Bidar Institute Of Medical Sciences Bidar,
Karnataka. India**Corresponding author*

INTRODUCTION

Modern medicines always work as a double edged sword. They are life saving on one side, while on the other side have serious adverse effects. Adverse effects of drugs on male fertility occur more often than recognized. Many drugs and chemicals have been found to interfere with the process of spermatogenesis. Among these substances are sex hormones like androgens, antiandrogens, estrogens, progestogens, antifungal drugs, anticancer drugs, NSAIDS antihypertensives, barbiturates, immunosuppressants, anticonvulsants, alcohol, aldosterone⁽¹⁾⁽¹¹⁾. H1 antagonists like Promethazine which have established and valued place in symptomatic treatment of various immediate hypersensitivity reactions shown spermicidal action in vitro. It produces irreversible loss of sperm viability in the concentrations much lower than which is achieved on oral administration⁽³⁾. Despite of these studies there is a scarcity of evidence of the effect of Promethazine on sperm count and motility in Vivo. Hence the present study was undertaken to explore the effect of Promethazine on sperm count, and sperm motility in male albino rats.

MATERIALS AND METHODS

Healthy adult Wistar male albino rats with body weight between 175 to 250 gm were used. Animals were acclimatized in the laboratory conditions for a period of one month, before starting the experiment and were fed pellet chow and water ad libitum. The acclimatized animals were randomly divided into following four groups consisting 12 rats in each group.

Group I Control (Normal Saline 2ml / day for 2 weeks).

Group II Promethazine (6 mg / Kg / day for two weeks).

Group III Control Normal Saline 2ml / day for 6 weeks.

Group: IV Promethazine, 6 mg / Kg / day for 6 weeks.

Dose of Promethazine was obtained by using surface area conversion table and given in 2ml volume of orally. 24 hours after cessation of treatment, the rats were sacrificed, testis and epididymis were removed and each epididymis was minced in the small Petri dish containing 1ml phosphate buffer solution maintained at 37°C⁽³⁾.

Evaluation of Sperm Motility

To evaluate sperm motility a small drop of spermatozal suspension was placed on microscope slide, pre warmed approximately to 37°C & then covered with a coverslip. Motility was checked for progressive forward movements according to the laboratory manual procedure (4). In order to calculate percent motile sperm at least 100 sperms were checked in 10 different fields^{(4),(9)(11)}.

Determination of Sperm Count

The suspension of sperm was thoroughly shaken to evenly disperse the sperms. 0.05 ml of the suspension was diluted to 1ml with formalin bicarbonate solution using W.B.C. pipette. The formalin bicarbonate solution effectively immobilize the sperm cells to facilitate the counting of sperms with accuracy. Sperm count was done on Neubauer's hemocytometer using the W.B.C. counting chambers⁽²⁾. Statistical analysis of all values were expressed as mean \pm SD. Drug treated groups were compared with control groups using Mannwhitney 'U' test.

Histopathological Examination of Testes

After removal testes were sent for histopathological examination. The microtomic sections of the organs were studied under the microscope.

RESULTS

Sperm Motility

Motility of spermatozoa was observed for one hour duration for all animals. The groups treated

with Promethazine (6ml / Kg / day) for 6 weeks showed highly significant decrease in sperm motility ($P < 0.01$). The inhibition of sperm motility by Promethazine for two weeks was less significant than for 6 weeks ($P < 0.05$).

Sperm Count

There was significant difference between the number of sperms of treatment groups as compared to the control group. Epididymal sperm count was significantly reduced in male rats treated with Promethazine for 6 weeks & 2 weeks duration ($P < 0.001$).

Histopathology of Testes

Animals treated with promethazine for 2 weeks showed normal histopathology of testes.

However with 6 weeks treatment promethazine produced following changes:

- i) Necrotic changes in seminiferous tubules
- ii) Areas of hemorrhage in the interstitium
- iii) Atrophied tubules.

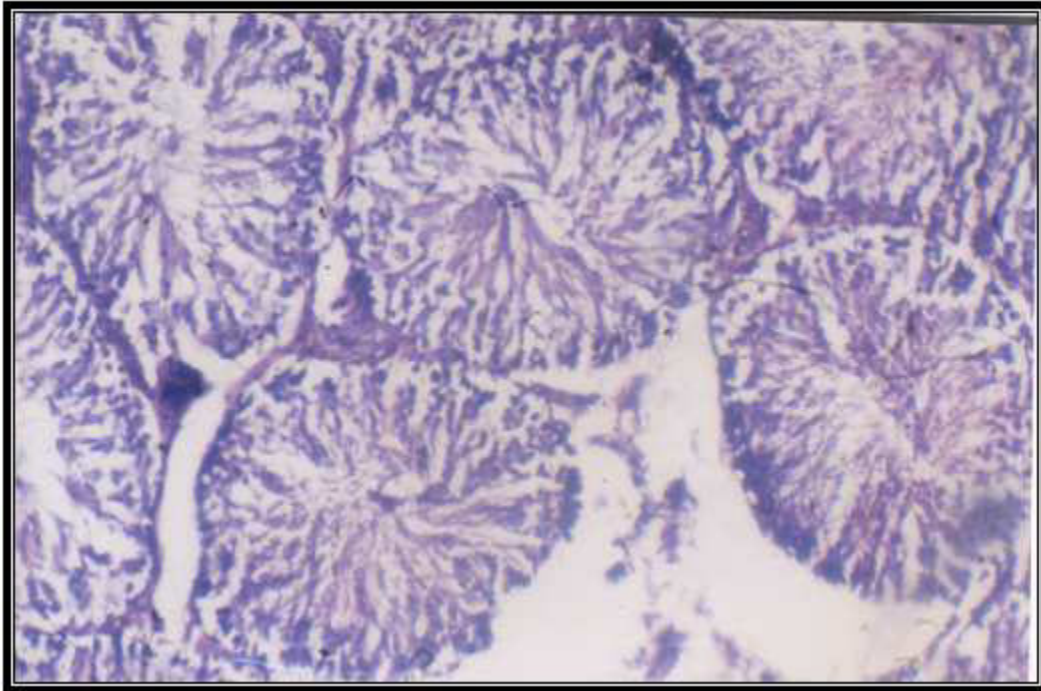
DISCUSSION

In the present study H_1 antihistamines namely Promethazine significantly affected the spermatogenesis in the form of decreased sperm count. There are several evidences to show the involvement of histamine in spermatogenesis. NorisG et al shown that histamine directly stimulates GnRH secretion & this effect was blocked by H_1 receptor blocker like Promethazine in a dose dependent manner. In another study kantzher J. found that H_1 antagonists affected the histopathology of rats testicles. This may possibly play a role in reduction of sperm count by Promethazine⁽⁵⁾. Promethazine found to reduced motility of rat

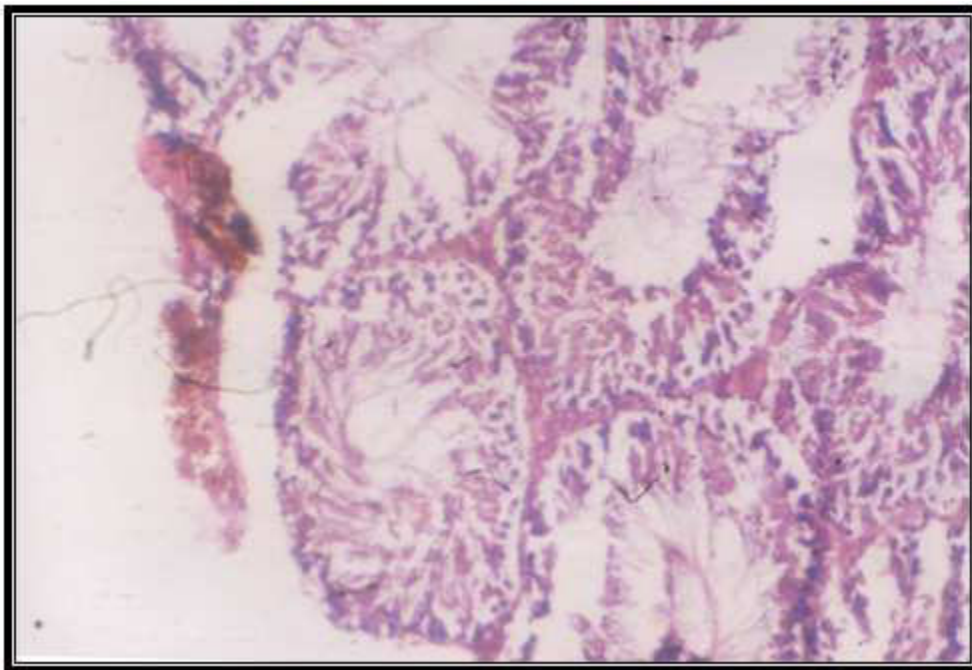
sperms on short term as well as long term administration. According to Gupta et al histamine causes elevation of intrasperm Ca^{+2} . This action was antagonized by low concentration of H_1 antagonists., This strongly suggested presence of H_1 receptors on sperm cells⁽⁶⁾. They also showed that the concentration required for spermicidal actions were lower than those administered orally. In a similar study Molly Thomas also found that H_1 antihistamines, chlorpheniramine reduced human sperm motility in vitro, but not cimetidine suggesting the presence of selective H_1 receptor on sperms⁽⁷⁾.

These observations that classical antihistamines decreased sperm motility & presence of histamine, N methyl transferase in the sperm as reported by Janson & Sastry et al point to the possibility that in the initiation of sperm motility, histamine plays important role⁽⁸⁾.

In addition to the membrane stabilizing effect of classical H_1 antihistamines, their cholinergic action may also contribute. To know the exact cause and changes in the histology of testes, histopathology of testes of rats was done, it revealed almost normal picture with short term administration of promethazine. But its long term administration (6 weeks) caused necrotic changes in seminiferous tubules. Few areas of hemorrhage and atrophic changes in the interstitium were also seen. These findings match with previous findings of Kautzner J. who found that classical H_1 antihistamines produce interstitial hemorrhagic changes in the rat testes⁽¹⁰⁾. Thus it was found that H_1 antihistamines promethazine significantly reduced the sperm count, sperm motility and affected histology of testes possibly resulting in decreased fertility.



Photograph showing atrophied seminiferous tubules in rats treated with promethazine for 6 weeks.



Photograph showing the areas of hemorrhage in Histopathology of testes in rats treated with promethazine for 6 weeks

TABLE-1
Effect of Promethazine on sperm motility of male Albino Rats.

Groups	% Sperm Motility	
	2 Weeks	6 Weeks
Control		
N.S. 2 ml / day	70.45 ± 2.12	65.27 ± 1.15
Promethazine 6mg / kg / day.	# 60.45 ± 4.65	## 43.13 ± 1.38

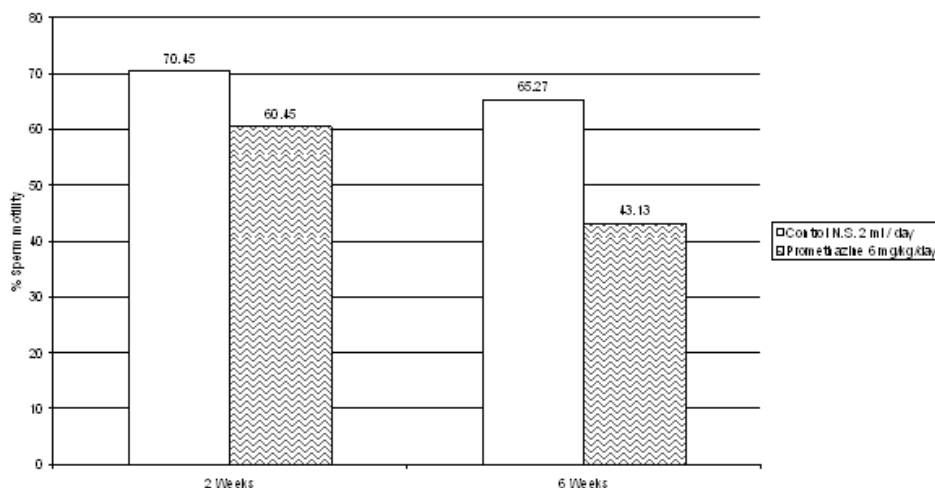
P < 0.01 as compared to control.
P < 0.05 as compared to control.

Table-2
Effect of Promethazine on sperm count of male Albino Rats.

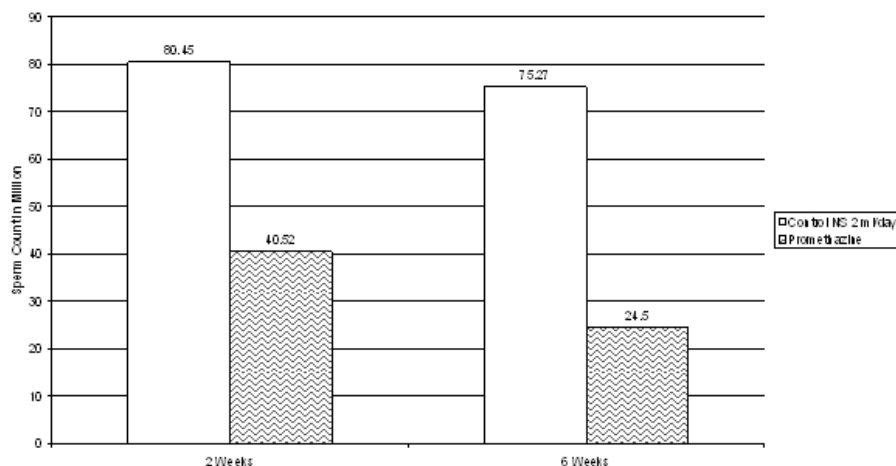
Groups	Sperm Count in Millions (Mean ± SEM)	
	2 Weeks	6 Weeks
Control		
N.S. 2 ml / day	80.45 ± 3.36	75.27 ± 1.15
Promethazine 6mg / kg / day.	### 40.52 ± 2.88	### 24.50 ± 2.42

P < 0.001 as compared to control.

Graph-1: Effect of Promethazine on sperm motility of male Albino rats



Graph-2: Effect of Promethazine on sperm count in epididymal sperm suspension



CONCLUSION

In the present study the effect of H₁ antihistamine Promethazine on fertility in male Albino rats was done using sperm count, sperm motility and histopathology of testes as parameters. On short term administration (2 weeks) Promethazine doesn't have a significant effect on all the parameters tested. But on long term administration (6 weeks) it significantly reduced sperm count, sperm motility and

resulted in significant changes in histopathology of rat testes. Possible mechanism of action for these effects is due to the effect on spermatogenesis and factors governing sperm motility. However in order to find exact mechanism of action further studies such as ion transport in sperm, acrosomal reaction, zona pellucida penetration, gonadal hormonal levels will be required.

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