Research article Available online www.ijrpsonline.com ISSN: 2249–3522

International Journal of Research in Pharmacy and Science

Effect of *Ceric Sulphate* on Gonads of Male Albino Rats

Babu Senthil Kumar*, Gopalsamy Panneer Selvi

Vinayaka Mission's Kirubananda Variyars Medical College and Hospital,
Salem - 636308, India

ABSTRACT

Chemicals have become important needs of our day to day life. Many industries that are concerned with manufacturing various products use variety of chemicals. The deleterious effect of these chemicals causes certain occupational diseases. One of such chemicals (*cerric sulphate)* which are widely used in industries such as ceramic, glass and pharmaceuticals was taken in this study and its effects on gonads of male albino rats were analyzed. Twenty healthy male albino rats were used in the study and the *cerric sulphate* solution was administered subcutaneously to 10 experimental rats and its effect on gonads were analyzed using various parameters such as sexual behavior (Mounting index and total sexual behavior), weight of animals, dimension of testes, hormonal analysis, semen analysis, histological analysis of testes and diameter of seminiferous tubules with that of 10 control rats. The experimental rats had deleterious effect on gonads except the testosterone hormone level and mounting index which only showed a slight variation when compared to that of control rats and all the other parameters showed much difference and was also statistically significant. *Ceric sulphate* was found to be inducing sterility in male rats which has been confirmed after analyzing all the parameters of the study**.**

KEYWORDS - Albino rats; Ceric suphate; Seminiferous tubule; Sterility; Testes.

*** Corresponding Author**

Dr. B. Senthil Kumar
Tutor, Department of Anatomy,
Vinayaka Mission's Kirubananda Variyars Medical College and Hospital,
Salem - 636308, India
Email – skdrchinu88@gmail.com
Contact – 09894398677 & 08489793038

INTRODUCTION

In our day-to-day life, change in culture and food habits, adulteration, exposure to various radiations, chemical hazards, pollution, smoking, and alcohol, various diseases like hypertension, diabetes mellitus, obesity, and resultant grave side effects of medication for these conditions, impacts the human species with dangerous and unexpected complications in life. With the growth of the chemical industry a number of new compounds are attaining wide use. Some of them have been found to have deleterious effects on the reproductive organs. Cerium is attaining wide application in ceramic and glass industries, medicine, as catalytic agent and in nuclear technology.[1] The presentation of our reports shows some interesting results on the effect of *Ceric sulplate* on the fertility of male albino rats.

MATERIALS AND METHODS

Animal preparation

20 healthy male Wister albino rats with an average weight of 130 – 140 gm were utilized in this study. The rats are randomly allocated 10 for control and 10 for experimental and housed separately 2 rats per cage with the maintenance of adequate ventilation and 12:12 hr light/dark cycle at room temperature. The animals were fed with standard pellet diet and provided water ad libitum.[2] The study involved only healthy animals without any fur loss or wound in skin and normally active. *Ceric sulphate* was purchased from CHENCHEMS Chennai which was used in the study. The experimental protocol was approved by Institutional Animal Ethical Committee of Saveetha Medical College, Saveetha University, Chennai (Letter no - ANAT MSc 003–2010) with CPCSEA Registration No - 865/ac/04/CPCSEA.

Preparation of millimolar solution of *Ceric sulphate* [1]

Millimolar solution was prepared by mixing *Ceric sulphate* and distilled water (Table 1). Molar solution in which the number of gram of dissolved substance per liter equals to its molecular weight i.e., a solution of molarity1M.

Administration of *Ceric sulphate* solution:

Dose -1 ml of 1m.mole/ 100 gm body weight [1]

Dosage calculation:

Weight of young rats of both groups was 130-140gm; its average was 135gm

100 gm body weight = 1ml of 1m.mole of *Ceric sulphate*

Therefore 135 gm (average) body weight of rats =1/100×135gm

= 1.35ml of 1m.mole of *Ceric sulphate*

Table No. 1: "Preparation of millimolar solution of *Ceric sulphate*"

Molecular weight of *Ceric sulphate*	332.24
1Molar solution	332.24g/1000 in 1000ml
1Molar solution	0.332g/ in 1000ml
1 millimolar	0.332g/100 in 100 ml
1millimolar solution	0.03g in 100ml

The above dosage was calculated according to the weight of the animal and administered subcutaneously for 7 days (1week) once a day in the morning at a fixed time. The site of injection had to be changed frequently to avoid local swelling and indurations. The pH of the solution was also assessed, using a pH meter and it was found to be slightly acidic (6.4). The control rats were also administered 1.35 ml of sterile water for placebo. The animal was fed with the standard diet and water as usual. The weight of the animal was also monitored on the first and last day of drug administration and a rest period of 5 days was given for all rats.

Procedure to check sexual activity of rats:

Mounting Index (MI):

The experiment was carried out in a specially designed box with a dim light measuring 50×30×30 cm. Two female rats at proestrus period were kept in the box and to the same one male rat were

introduced. [2] The rats were identified by picric acid marking. After a 15 minutes acclimatization period, frequency of mounting was observed for 60 minutes and the number of mounts counted.[3-5]

Total sexual behavior (TSB):

Male sexual behavior such as genital grooming and anogenital sniffing at females was visually monitored for 60 minutes and recorded. Both mount test and TSB were done and data were calculated. [3-5]

Collection of samples:

After observing the sexual activity animals were anaesthetized using Xylazine & ketamine.[6] Skin over the ventral surface of the neck was shaved off and incised. The Jugular vein was traced and using a 22 gauge needle and disposable 3 ml syringe, the vein was punctured and the blood was withdrawn. The serum was collected and the hormone analysis was carried out using ELISA analyzer. [1] The rat was cut open by midline thoraco-abdominal incision and transcardial perfusion was done using 4% Para formaldehyde in 0.1 M phosphate buffered solution. Pre-scrotal incision was made and the testicles were removed from scrotum. One side testes with epididymis were fixed in Bouin's fluid for 48 hrs, dehydrated in ascending grades of alcohol, cleared in xylene and embedded in paraffin wax. Sections were taken of about 5µm thick, which were stained with haematoxylin and eosin.[7, 8] the other side testis were then separated from the epididymis with the scalped blade.[9, 10] The lengths, breadth, height of the testes were measured using vernier caliper and the testes were weighed. The volume of the testis were calculated using the Lambert's formula.[11] The gonado-somatic index (GSI) were calculated.[12,13] The semen samples were there after collected from the epididymis and semen analysis were carried which includes sperm count and sperm morphology. The morphological characteristic of the sperm cells in all the smears were observed under oil immersion (100 X). The following abnormalities were noticed both in the control and experimental groups as described by Oyeyemi et al.[9] The sperm morphology data's were analyzed by Chi-Square test.

RESULTS

The data's were collected for each parameter which was tabulated and expressed as Mean ± SEM of 10 animals in each group. Comparison was done between the control group and the experimental group using student's t test and found to be statistically significant for all parameters (Table 2).

Table No. 2: "Various Parameters of the Study"

S.no	Parameters	Control rats (n = 10) Mean±SEM	Experimental rats (n = 10) Mean±SEM
1	Mounting Index(MI)	9.95 ± 1.68	9.84 ±1.59#
2	Total sexual behavior (TSB)	264.00 ± 0.967	260 ± 0.984**
3	Weight of rats (gm)	136.50 ± 1.18	132.50 ± 0.96**
4	Volume of testes (cu.cm)	0.89 ± 0.03	0.75 ± 0.06*
5	Weight of Testes (gm)	1.05 ± 0.02	0.62 ± 0.04***
6	Gonado somatic index (GSI)	0.769 ± 0.02	0.467 ± 0.01***
7	Sperm count (millions/ ml)	19.34 ± 0.75	16.31 ± 0.56**
8	Testosterone hormone level (ng/ml)	2.1 ± 0.21	1.94 ± 0.14#
9	Diameter of seminiferous tubule (µm)	251.4 ± 0.93	208.2 ± 0.72***

*P<0.05, **P< 0.01, ***P<0.001, # - Statistically not significant, n – number of animals, SEM – Standard Error Mean

DISCUSSION

Mounting Index (MI):

The mounting index has shown only a slight variation among the control and experimental rats and it proves that there is mild effect of *Ceric sulphate* on the male sexual activity also. The data's proved to be statistically not significant (Table 2).

Total sexual behavior (TSB):

The total sexual behavior of the rats were observed and the sexual activity was less in experimental when compared to the control rats resulting in moderately significance (Table 2).

Weight of rats

The weight of control rats were more when compared to the experimental rats. The reason for reduction in weight for experimental rats is due to the effect of *Ceric sulphate* on the rats which is moderately significant (Fig 2).

Volume and Weight of testes:

The results pertaining to the weight and volume of the testes and GSI were analyzed which showed a marked difference between the Control's and experimental rats respectively. The testes of experimental rats showed seminiferous tubules with much shrinkage and loss of their tubular appearance and the

lumens appear empty (Fig 4). Whereas the control rats have normal seminiferous tubules (Fig 3) and these changes caused the reduction in weight, volume of testes and GSI in experimental rats (Fig 1) and the data's were statistically significant (Table 2).

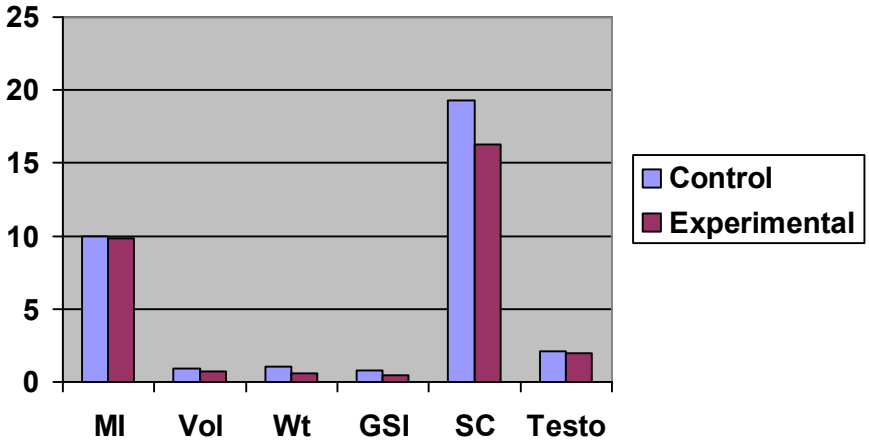

Fig 1 Graph showing various parameters

MI- Mounting index, Vol– Volume of testes, Wt– Weight of testes, GSI– Gonado somatic index, SC– Sperm count, Testo– Testosterone hormone level.

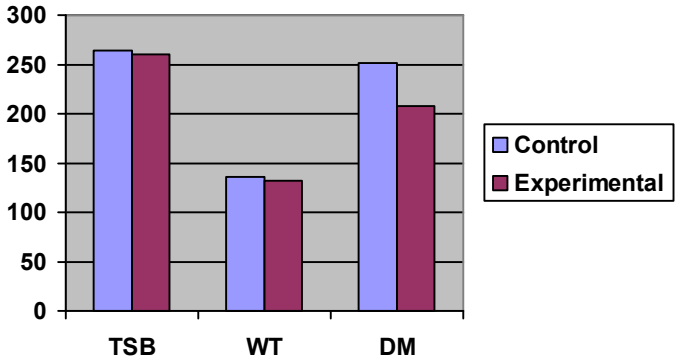

Fig . 2 Graph showing various parameters of the study

TSB– Total sexual behavior, Wt– Weight of rats, DM– Diameter of seminiferous tubule

Sperm count

Reduction in number of spermatozoa in experimental rats than that of control was observed.[1] spermatocytes showed severe reduction in their number and are significant. The morphology of the spermatozoa of experimental rats (31.72%) had more number of abnormal spermatozoa than the

control rats which showed a marked decrease (8.69%). Further analysis of the spermatozoal count, using Chi-square test was found to be highly significant (0.001).

Histological analysis:

The experimental rat testes were compared to that of the control group of young rats. The control rat testes showed normal in appearance Fig 3. Histological damage was observed in 45 % tubules and some tubules seemed to be completely empty without the spermatozoa in experimental (Fig 4). The basement membrane was very thin on which a few unaffected germ cells were attached. The seminiferous tubules showed much shrinkage, some tubules completely lost their tubular appearance and looked under liquidation, loaded with many nuclei while in others the lumen were empty or filled with fluid. The diameter of seminiferous tubule showed marked variation in experimental than control group (Fig 2). The Leydig cells were normal but the stroma was considerably reduced.[1]

Serum Testosterone Hormone Level

The testosterone hormone level of control rats and experimental rats showed not so marked variation (Fig 1). The Leydig cell which found to be normal in experimental rat seminiferous tubule and that was responsible for secretion of testosterone in experimental rats as well as control rats with only a slight variation. The hormone levels of both groups were statistically significant. *Ceric sulphate* induces sterility only by acting on the seminiferous tubules but not on the interstitial cells of Leydig.[1] Testosterone level was only slightly altered between the groups when compared to other parameters (Table 2).

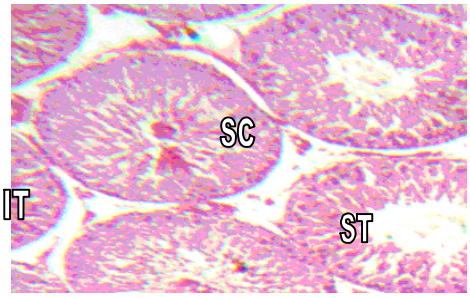

Fig. 3 Testis of Control Rats

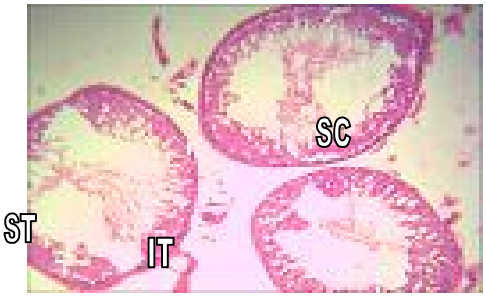

Fig. 4 Testis of experimental Rats

IT-Interstitial tissues, ST-Seminiferous tubule, SC-Sertoli cells.

CONCLUSION

Ceric sulphate was proved to have some deleterious effect on the gonads of male albino rats which ended in sterility of rats. The various parameters involved in our study showed significant variations between 2 groups of rats. The study has to be further extended using various modern aids and also the effect of the chemical on vital organs. If proved to be harmful to mankind, various precautions and safety measures can be implemented to the workers handling this chemical in various industries.

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