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Effects of Heat and Lead on the Reproductive System of Male Wister Rat

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Abstract: ***Wistar rats*** **are an outbred strain of albino rats belonging to the species** ***Rattus norvegicus*****. Histopathological study of the testis was carried out to determine the effects of heat and lead on male fertility by evaluating some andrological parameters of the wistar rat such as mophology of spermatozoa, sperm count and motility. The main objective of this research is to compare the deleterious effect of lead and heat on the male reproductive system of a wistar rat.**

Fifteen adult male rats were randomly divided into 3 groups; group A were the control group; group B were kept in hot environment above 42 degrees Celsius and group C are administered 0.18mls of lead acetate solution per grams body weight once daily for 14 days.

The gonadotoxins caused morphological alterations of sperm cells in this study; including head, tail and middle defects which are indications of interference with maturation stage of spermatogenesis in the somniferous tubules. The deleterious effect of the gonadotoxins on the somniferous tubules is corroborated by histopathology which revealed degeneration of epithelium of the somniferous tubules. The degree of sperm cell motility was significantly lowered in animals exposed to gonadotoxins compared to animals in the control group and also the sperm cell count was lowered ($P<0.05$) in the animals exposed to gonadotoxins in comparison with animals in the control group, as a result of decline in the production of normal, viable sperm cells in the test animals.

It was concluded that the gonadotoxins exert toxic effect on the somniferous tubular epithelium with concomitant reduction in the reproductive abilities of the male rats and as such exposure to heat and high level of lead should therefore be avoided both in animal and man.

Keywords: **Gonadotoxins, Heat, Lead Acetate, Spermatogenesis, Somniferous Tubules.**

1. INTRODUCTION

The human cells are exposed to various types of environmental contaminant at different stages of his life span, majority of them are harmful. One of the oldest and harmful agents to mankind is lead. It came into use very early in the history of civilization and its poisonous effects were soon discovered (Imran, Muhammad & khalid, 2003).

Lead is a soft heavy metal with a specific gravity of 11.34 and atomic weight of 207.21. It's bluish in color that tarnishes to dull gray. Although lead might be harmful in so many ways, but still used by human being for several purposes including: making bullets, iron construction, acid battery, plant refinery and fuel combustion (Cleveland et al, 2008).

Normal daily intake of 3.5mg taken for a few months results in toxicity (Neathery & Miller, 2007); the blood lead value of 80mg or above is considered as an unequivocal instance of lead poisoning. It was also suggested that prolonged lead exposure initially produces a direct testicular toxicity followed by hypothalamic or pituitary disturbances on long exposure. Therefore, it is concluded that lead exposure during growing period when spermatogenesis is proceeding might result in reproductive impairment during adulthood (Pant et al, 2003; Rodamilans et al, 1998). In the literature, it has been reported that lead intoxication may cause oxidative stress (Schafer et al, 2005; Tchernitchin, 2002)

The testes are cooled by countercurrent exchange of heat between the venous and arterial blood in the spermatic cord (Setchell, 1998). Heat is lost from the testis and scrotum to the environment through the scrotal skin, which is well endowed with sweat glands (Banks et al, 2005). The temperature on the surface of the scrotum is lower at its base than near the neck, but the temperature inside the testis is almost uniform, even slightly warmer at the base (Kastelic et al, 1996; Sailer et al, 1997).

Regarding histological studies, it has been concluded that pachytene spermatocytes and early spermatids are the cells in the testis which are more sensitive to heat. These findings have been confirmed using flow cytometry and confocal microscopy of isolated seminiferous tubules after making the testis cryptorchid in hamsters (Kandeel et al, 1998; Karabinus et al, 1997 Kastelic et al, 1996).

However, even when these cells are not killed, there is no evidence that some of them may complete their development, but appeared as spermatozoa with damaged DNA, as revealed by COMET and sperm chromatin structure analyses (Brito et al, 2004; Arman et al, 2006).

Objectives: The main objective of this research was to compare the deleterious effect of lead and heat on the male reproductive system of a wistar rat.

2. MATERIALS AND METHODS

Procedures for Animal Euthanasia and Management of Experimental Animals:

Fifteen adult Wistar rats were used in this research. The animals were housed in hutches and fed with rabbit pellets. They had access to feed and water ad libitum. They were acclimatized for three weeks under standard conditions of temperature and illumination (12 h light and 12 h dark). The rats were randomly divided into three groups of 5 animals each (A, B, C). Group A was the control and received 0 mg/kg body weight of lead acetate, but were given normal saline for the period of the experiment. Group B were exposed to a hot environment of above 35 degree Celsius twice daily in a specially designed cage (Kastelic et al, 1997). Group C received 6 mg/kg body weight of lead acetate, with normal daily food ration and clean water (Imran, Muhammad & khalid, 2003).

The animals were sacrificed using the cervical dislocation method, after which a Y-skin incision was made over their perineum and the epididymis and testes were exposed and harvested. The epididymis was crushed and mixed with some drops of normal saline and immediately analyzed for sperm count, sperm motility and morphology while the testes was immediately fixed in bouin's fluid

Method of Administration:

Lead acetate was procured from British Drug (BDH, UK). 5 g of lead acetate was dissolved in 1 L of water and 6 mg/kg body weight of lead acetate (Imran et al., 2003) was administered to the three groups of animals using an insulin hypodermic injection (40 units to 10 mls).

The techniques for studying the effects of heat on scrotal testes were to expose the whole animal to a hot environment intermittently (Sailer et al, 1997) or continuously twice daily and the temperatures also were recorded (see table 2).

Sperm Analysis:

Sperm analysis was done using new improved Neubauer's haemocytometer (Deep 1/10 mm, LABART, Germany) and the results were recorded.

Histological studying:

The testes were fixed in Bouin's fluid until processed. Thereafter, they were stained using the haematoxylin and eosin method for routine histological studies. Then, the slides were viewed on a light microscope and the photo-micrographs were taken.

Statistical analysis:

The results were statistically analyzed using the Prism 5 for Windows (version 5.02, © GraphPad Software, Inc). The mean, standard error of mean and standard deviation of the data were calculated. In addition, the student t-test was used to check the significance of the results. The differences of means were considered significant at $P < 0.05$.

3. RESULTS

Results reviewed include the weight changes during the duration of study, sperm viability assessments and histological photomicrograph.

The lead treated animals (Group C) for four weeks lost 12.4% of body weight. This significant drop in weight was due to the loss of appetite and gastrointestinal disturbances (Neathery & Miller, 2007). Majority of animals also developed ulcerative lesions at the sites of injections due to necrotic changes induced in skin by this metal, which failed to respond to any conservative treatment.

Lead acetate greatly affected the microscopic count of Group C because there was an observed significant ($P < 0.05$) reduction in the microscopic count values as presented in Table 2 and Figure 2.

It was observed that prolonged administration of lead for two weeks significantly ($P < 0.05$) reduced the microscopic count as observed in Group C (Imran, Muhammad & khalid, 2003; Kandeel & Swerdloff, 1998).

The sperm count of the control group shows significant difference that is the highest in the group lead-treated group. As documented by Rodamilans et al (1998), lead acts as a spermicidal agent in case of high exposure and has an adverse effect on sperm count and retards the activity of live sperm (Kandeel & Swerdloff, 1998). (See table 4).

In heat-exposed group, there was also a significant decrease in sperm count. This finding is confirmed by several studies (Brito et al, 2004; Karabinus et al, 1997; Ross, 2008) that the spermatozoa appeared scanty with damaged DNA as revealed by sperm chromatin structure analyses after heat exposure. Comparing the different groups, group C had the least sperm count showing that lead has more destructive effect on the testes than heat.

Normal and head defect sperm cells of the control group show significant change to all other treated groups. All other treated groups show significant decrease in normal spermatozoa, when compared to the lead-treated group control group (see table 3).

In the lead and heat group, the tail and the middle piece shows no significant damage when compared to the control group and this is consistent with sperm analysis that revealed the morphological abnormalities of sperms mainly, the tail abnormality (Kandeel & Swerdloff, 1998)

Rapid and slow progressive sperm cells of control group show a significant change to all other treated groups. Comparing group 1 and group 2, it shows that lead has more destructive effect than heat. The non-progressive and the dead sperm cells of the control shows no significant difference to all other treated groups showing that heat and lead do not totally kill sperm cell after the study (see table 4).

The administration of lead acetate significantly reduced the number of rapid progressive sperm cells, ($P < 0.05$) as seen in Group C. Also the number of non-progressive sperm cells was increased in the Groups B and C with the highest number in Group C. It can be observed that lead administration significantly reduced ($P < 0.05$) the number of normal sperm cells and increased the number of head defects as seen in Groups B and C when compared to the control in Group A as documented by Imran, Muhammad & Khalid (2003).

From the photomicrograph, comparing the 3 groups of tissue plate, fig 5B-C and fig6B-C are presented with a generalized degeneration of the interstitial spaces. The treated groups also show some form of mild-moderate testicular atrophy when compared with the control group (Roy, 2004). Roy, 2004, reported the Leydig cells which are the principal cells of the interstitial supporting tissue that syntheses and secretes testosterone in the intact interstitial space that suffer from this atrophy. The sperm morphology were affected in fig 5B-C and fig 6B-C with defects in the tail most obvious has documented in Priya & Reddy (2012). The major function of testosterone in its target tissues including initiation and maintenance of spermatogenesis and the promotion of sexual maturation at puberty, in group B and C, these functions were absent or reduced as corroborated by Imran, Muhammad & khalid (2003).The symptoms in this research are still mild and will be worse on prolonged administration.

4. DISCUSSION

Plucking and stringing phenomenon were absent, suggesting the degeneration of somniferous tubules, thickening of basement membrane and condensation of the stroma (Imran, Muhammad & Khalid, 2003). The pale color of the testicular tissues was suggested to be to reduced vascularity. It is indicated that reduced demand is due to arrest of spermatogenesis

that was later proved on microscopy (Imran, Muhammad & Khalid, 2003). These findings are corresponded with the observations made by (Grant, 2009; Ross, 2008) that lead acts as a spermicidal agent in case of high exposure.

The number of blood vessels in the interstitial tissue was reduced and most of them were collapsed as was evident by their reduced diameter. The population of Leydig cells had dispersed and they were rarely seen in groups or clumps (see plate 3A and 3B). The nucleoli in majority of Leydig cells, rich in rRNA had disappeared suggesting an atrophy of these cells. These findings are agreed with findings of previous study (Grant, 2009; Saxena et al, 2001). All these scientists described variety of toxic changes induced by lead in the testes depending upon dose and duration of the treatment. At the end, it is concluded that lead has more deleterious effect than heat on spermatogenic and Leydig cells.

5. CONCLUSION

The result of this study established the gonadotoxic effect of lead on male rats as well as extended and supported previous findings implicating lead-related male infertility and the effects of heat may not be confined to cell death in the testis and the consequent fall in sperm numbers in semen, but the produced sperm may be less capable of fertilization and subsequently cannot produce normal embryos. In conclusion, this result shows that lead has more destructive effect than heat as a gonadotoxin. In these times of global warming, these effects may have more serious consequences for human population as well as for domestic and wild animals.

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Table 1: Weight of animals in groups

	Control	Heat	Lead
Initial Weight	204.38 ±5.74	142.94 ± 8.51	151.68 ± 3.80
Final Weight	210.5 ± 5.46	136.84 ± 8.2	134.94 ± 4.08
Weight Differences	6.12	-6.1	-16.74
% Differences	2.9%	-4.46%	-12.4%

B: Means with the same superscript on the same row are significant at the level of P<0.05

The mean ± standard error of mean values for initial and final weight of animals was calculated as shown above.

Table 2 . Sperm count analysis result

	Control(group a)	Heat(group b)	Lead(group c)
SPERM COUNT MEAN ±SEM	78.14 ± 7.844^{ab}	38.34 ± 2.349^{a}	32.28 ± 3.270^{b}

NB: Means with the same superscript on the row are significant at the level of p<0.05

The mean ±standard error of mean values of sperm count analysis is given above.

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Table 3: Sperm morphology grading analysis:

	Control	Heat(group A)	Lead(group b)
Normal Spermatozoa	79.00± 2.915[ab]	50.00 ± 3.162[a]	52.00 ±3.742[b]
Head Defect	10.00 ± 2.236[ab]	40.00 ± 3.162[a]	38.00 ± 3.742[b]
Tail Defect	6.000 ± 2.236	5.00 ± 0.00	5.00 ± 0.000
Middle Defect	5.000 ± 0.000	5.00 ± 0.00	5.00 ± 0.000

NB: Means with the same superscript on the same row are significant at the level $p<0.05$

The row mean ±standard error of mean values of sperm morphology grading is given above.

Table 4: Sperm motility grading

	Control(group a)	Heat(group b)	Lead(group c)
Rapid Progressive Motility (%)	82.00 ± 2.00[a]	64.00 ± 6.00[a]	66.000 ±6.78
Slow Progressive Motility (%)	8.00 ± 2.00	14.00 ±2.449	12.00 ± 2.000
Non-Progressive Motility (%)	5.00 ± 0.00	10.00 ± 2.739	11.00 ± 3.674
Dead Sperm Cells (%)	5.00 ± 0.00	10.00 ± 2.739	11.00 ±3.674

NB: Means with the same superscript on the row are significant at the level of $p<0.05$

The row mean ±standard error of mean values of sperm motility is given above.

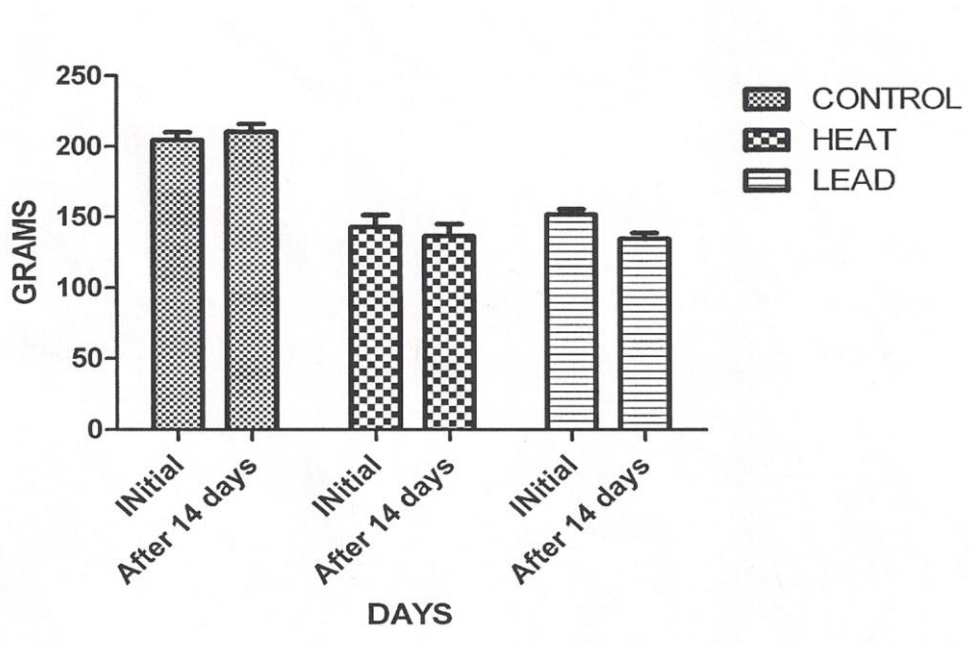

Fig 1: The relationship between initial and final weights of all groups

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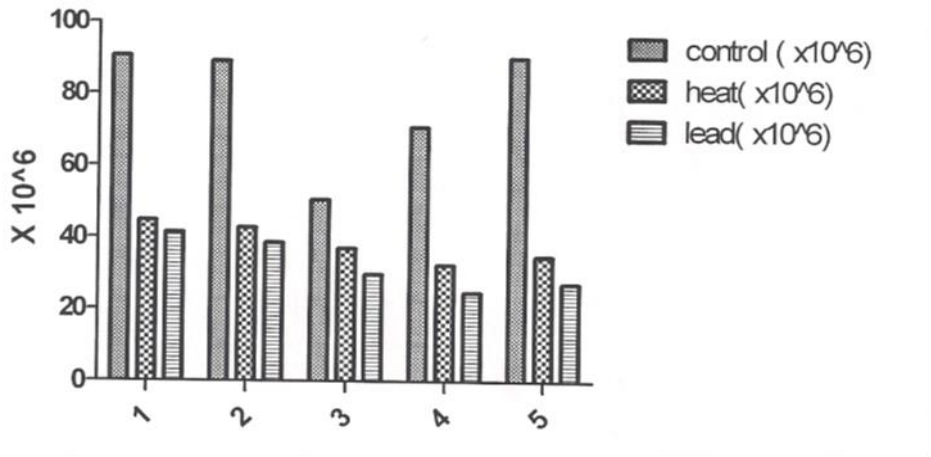

Fig 2: shows the graphical representation of the sperm count analysis.

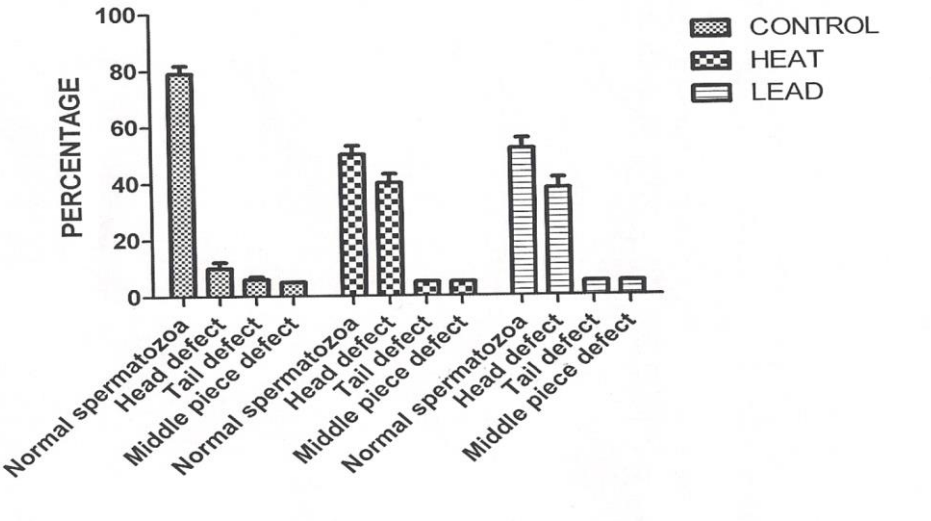

Fig 3: Graphical representation of the morphology grading of sperm for the groups

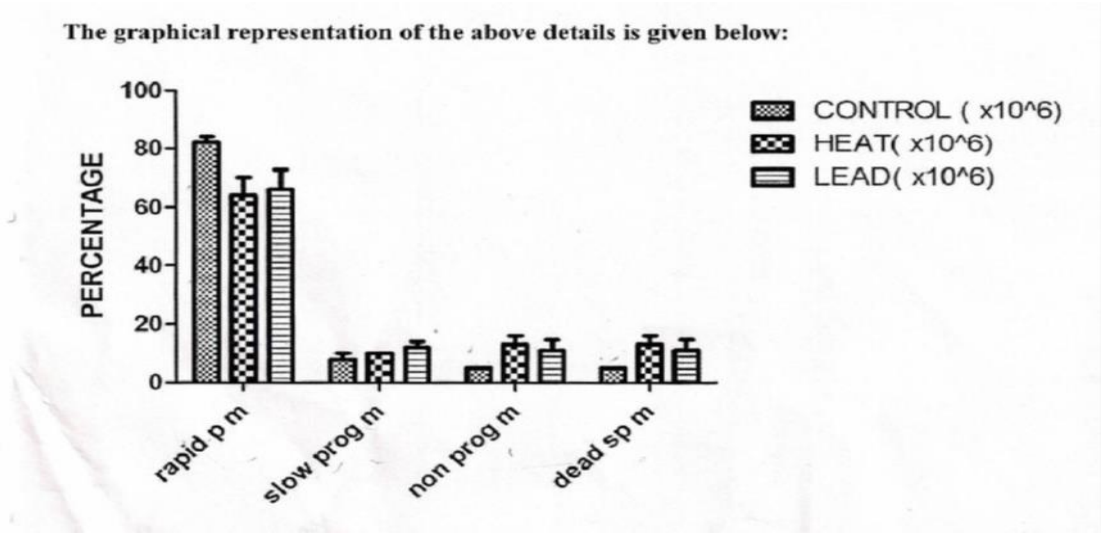

Fig 4: Graphical representation of the motility grading of the groups

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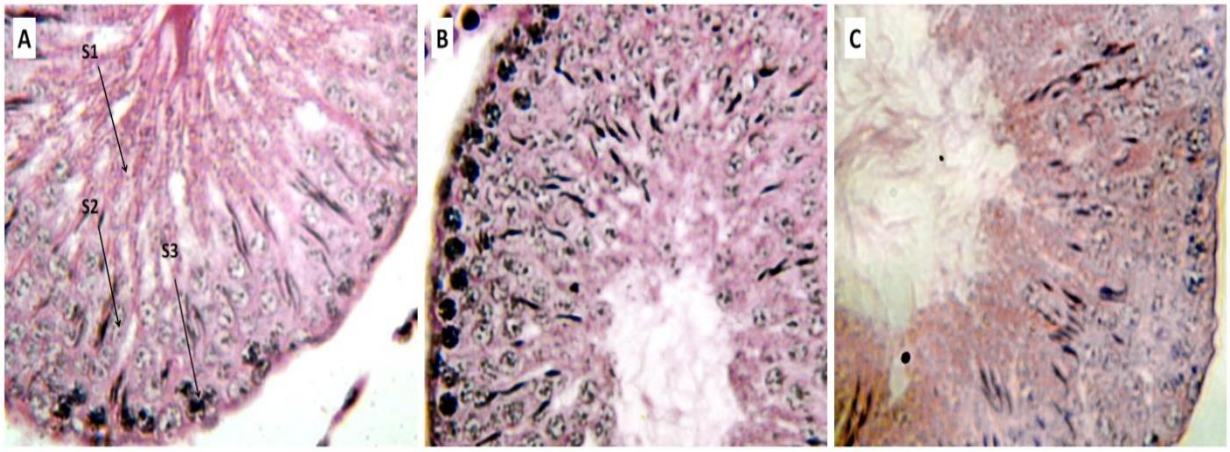

Fig 5: A) Photomicrograph of a transverse section of testis of control group-A rats.

(A) Transverse section of the control group. B) Photomicrograph of a transverse section of testis from group B (heat group) showing few number of germ cells, sertoli cells and spermatozoa. C) Photomicrograph of transverse section of testis of lead treated group-C rats. 3B shows a transverse section of the lead. H&E stain (×400). S1= spermatozoa, S2= spermatids and S3=spermatogonia.

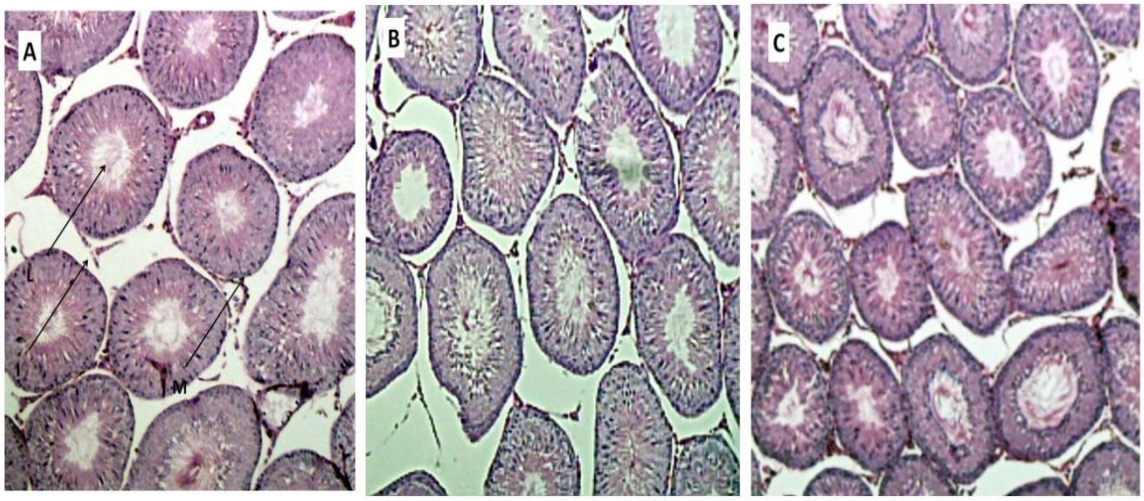

Fig 6: A) Photomicrograph of a transverse section of testis of control group-

A wistar rats, showing normal seminiferous tubules. The transverse section of the control group using. B) Photomicrograph of transverse section of testis of group B (heat group) showing decreased diameter of seminiferous tubules and increased in interstitial space. C) Photomicrograph of the transverse section of testis of lead treated group-C rats. 3A H&E stain (×40). I = interstitial space, L = lumen and M = basement membrane