ANTIFERTILITY EFFECT OF METHYLMETHANE SULPHONATE ON MALE REPRODUCTIVE ORGANS OF WILD INDIAN HOUSE RAT: HISTOLOGICAL AND HISTOCHEMICAL CHARACTERISTICS

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Objective. To study the effects of methylmethane sulphonate ($CH_3OSO_2CH_3$), on the testicular tissue of the adult wild Indian house rat (Rattus rattus).

Methods. A single intraperitoneal dose of methylmethane sulphonate (20 mg/kg) was administered and the effects were observed 2, 7, 15, 30 and 45 days later.

Results. Significant changes of the body, testes and accessory reproductive organs weight and a major depletion of the relative percentages of the spermatid and spermatozoa were noticed at 2, 7, 15 and 30 days after treatment. Gradual decrease in the seminiferous tubular area and Sertoli cell nuclear diameter was observed at 7, 15 and 30 days of treatment groups .The sperm population and sperm morphological abnormalities were also noticed in these three groups.

Histochemical studies clearly revealed that the intensity of staining of the acid and alkaline phosphatase within 7 and 15 days after treatment was decreased, while the quantity of lipid materials was increased especially on the 2nd and 7th day after treatment. However, no significant changes were noticed in the Δ^5-3β-HSDH and 17β-HSDH enzymatic activity in the treated animals.

Conclusion. These observations showed the antispermatogenic activity of methylmethane sulphonate on the testicular tissues and various accessory reproductive organs in the wild Indian house rat (*Rattus rattus*).

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Key words: Methylmethane sulphonate – Spermatogenesis - Wild Indian house rat - Dehydrogenase

Methylmethane sulphonate (one type of sulphonic esters), has been described as one of the antifertility compounds by its alkylation mechanism introduce the methyl group into biological material. A single intraperitoneal dose (50 mg/kg) produced complete sterility within 2 to 3 weeks after the single dose in mammals (Jackson et al. 1961). It is also reported that spermatozoa are affected via the entire epididymal pathway due to the administration of this sulphonic ester. Jackson et al. (1961) also reported that at low concentration (10 and 20 mg/kg) it caused a depletion of spermatozoa and spermatids for few days, but no detailed studies were recorded by this sulphonic ester on the wild rats.

While several aspects of action of other sulphonic esters on spermatogenesis in some mammalian species have been studied (Kar et al. 1968a; Aich and Manna 2001; Chakraborty and Manna 2001; Jackson 1959, 1966; Fox et al. 1963), and possible mode of action has been pointed out in the testes of mammals, but scanty reports are available concerning the action of methylmethane sulphonate on the wild mammalian as well as other vertebrate species.

Considering the lack of pertinent data, the present work was undertaken to investigate the time dependent effect following scheduled dose (20 mg/kg) of this sulphonic ester on the male wild Indian house rat (*Rattus rattus*).

Table 1

Changes in the body, testis, and major accessory glands weight after 2,7, 15, 30 and 45 days of Methyl methane sulphonate (20mg/kg. body wt.) tre adult male wild Indian house rat (*Rattus rattus*)

Groups	Body (gm)	Testis (mg)	Epididymis (mg)	Seminal vesicle (mg)	Coagulating gland (mg)	Vas deferens (mg)	Ve
Control (6)* A	100.00 ± 1.82**	1570.00 ± 48.57	474.52 ± 10.52	317.94 ± 22.22	63.98 ± 9.51	56.61 ± 6.70	9
2 Days(6) B	89.17 ± 6.76 N	1560.53 ± 72.62 N	317.68 ± 28.89 [a]	202.73 ± 33.48 [c]	34.60 ± 6.14 [d]	69.56 ± 8.53 N	6
7 Days(6) C	82.5 ± 8.83 N	1491.02 ± 72.72 N	354.91 ± 27.21 [b]	93.28 ± 11.28 [a]	30.11 ± 5.42 [c]	69.97 ± 7.38 N	6
15 Days(6) D	78.33 ± 4.07 [a]	1079.76 ± 141.56 [b]	243.50 ± 40.25 [a]	106.28 ± 20.95 [a]	15.8 ± 2.11 [a]	48.94 ± 5.40 N	2
30 Days(6) E	93.33 ± 3.33 N	1314.8 ± 35.53 [e]	292.83 ± 34.70 [a]	129.7 ± 30.60 [a]	34.06 ± 9.53 N	61.97 ± 2.98 N	4
45 Days(6) F	98.33 ± 5.11 N	1547.96 ± 76.42 N	301.34 ± 14.72 [a]	225.88 ± 10.86 [b]	42.33 ± 2.04 N	63.17 ± 5.53 N	9

* : No. of animals in each group

** : Mean ± Standard error

[a]P<0.001; [b]P<0.01; [c]P<0.02; [d]P<0.05; [e]P<0.002; N-Not significant

Materials and Methods

Adult male wild Indian house rats (*Rattus rattus*) were trapped from various localities surrounding the Kalyani University campus, maintained in individual metallic cages and kept under 12.5:11.5 hr light:dark cycle. They were fed specially prepared rat food and water for 2 weeks prior to treatment. Thirty six rats of almost equal body weight were taken and randomly assigned to six groups (e.g. Groups A, B, C, D, E, and F). Each group contained equal number of individuals. Group A served as the control and the animals of groups B, C, D, E and F were treated with a single dose of methylmethane sulphonate (20 mg/kg) injected i.p. After 2, 7, 15, 30 and 45 days, the animals of respective groups B to F were weighed and sacrificed by decapiation under mild ether anesthesia. Immediately after autopsy, the right testis and other organs of interest were rapidly dissected, weighed on a torsion balance and fixed in freshly prepared Bouin's fluid. All the tissues were embedded in paraffin, 7 µm thick sections were prepared and stained by Cason's trichrome procedure. From the well stained sections observations were made and photomicrographs taken. Seminiferous tubular area, Sertoli cells nuclear diameter, different germ cell types were considered for quantitative histological study from the total count of about 500 tubular sections.

For the sperm parameter study, a small portions of cauda epididymis were minced in PBS solution (PBS 0.10 M, pH 7.0). Sperm number and sperm morphological abnormalities were studied according to Barratt et al.(1989); Didolkar et al. (1988); Nahas et al. (1989) and Aich (1995).

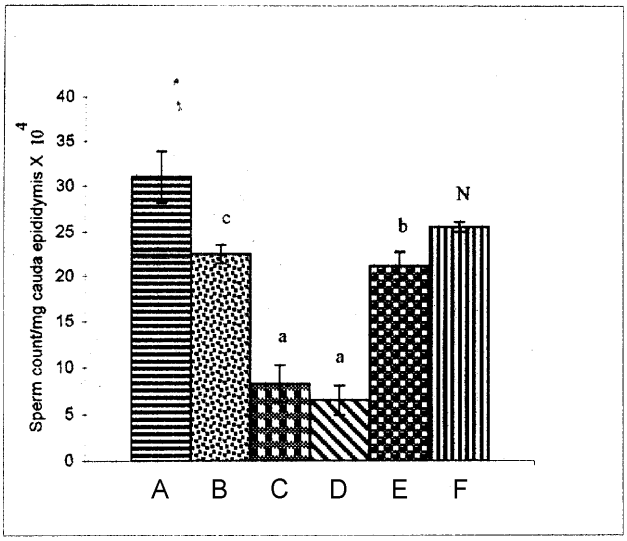

Fig.1 .Effect of methylmethane sulphonate on the sperm population of the wild Indian house rat (*Rattus rattus*)

$^{a}P<0.001$; $^{b}P<0.01$; $^{c}P<0.02$; N-Non significant

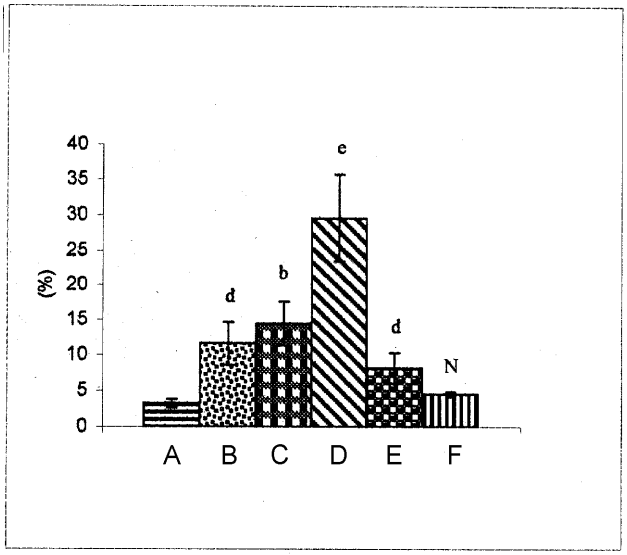

Fig.2. Effect of methylmethane sulphonate on the sperm abnormality of the wild Indian house rat (*Rattus rattus*)

$^{b}P<0.01$; $^{d}P<0.05$; $^{e}P<0.002$;N-Non significant

Immediately after autopsy, left testis was quickly taken out, made free of extraneous tissues, rinsed in liquid nitrogen for a few minutes and kept in the Cryocut Chamber at –18 °C. After proper thawing in the cryocut chamber, 15 µm thick serial sections were made, taken on the grease free slides, kept in room temperature for half an hour and processed for the cytochemical localization of the neutral lipids (KAY and WHITEHEAD1941), D^{5}3b-hydroxysteroid dehydrogenase (WATTENBERG 1958), 17b-hydroxysteroid dehydrogenase (PEARSON and GROSE 1959 as modified by BILASPURI and

GURAYA 1984), acid phosphatase (BITENSKY 1963) and alkaline phosphatase (BUTCHER and CHAYAN 1966).After processing, the intensities of reactions were recorded with the aid of light microscope.

All the quantitative data were analysed statistically using Students t-test procedure..

Results

Gravimetry. Gravimetric changes in the body and various organs weight of control and all groups of experimental rats are shown in Table 1. The most remarkable changes in the weight of the testis and other reproductive organs were noticed 7, 15 and 30 days after treatment. However, the weight of organs had returned to the control level at 45 days of post treatment.

Histology. The control testis showed a large number of seminiferous tubules with wide lumen containing abundant spermatids and spermatozoa. One or two layers of spermatogonial cells, two to four layers of primary and secondary spermatocytes are present. Bundles of spermatozoa are found within each seminiferous tubules (Fig. 3). Spermatozoa are attached mainly at the neck and apex of the Sertoli cells.

Methylmethane sulphonate treatment. After 2 days (Group B) the testis showed mild reduction in Sertoli cell nuclear diameter (Table 2; $P<0.01$). Few germ cells have lost their percentages (Table 3) and the number of spermatozoa decreased ($P< 0.001$). Spermatogonial and Sertoli cell population have increased ($P<0.001$) (Table 3).

After 7 days(Group C) the seminiferous tubular area and Sertoli cells nuclear diameter were decreased (Table 2). The number of spermatogonial cell increased ($P<0.001$) and only few spermatozoa were found ($P<0.001$; Fig. 4). Sertoli cells population increased significantly ($P<0.001$).

After 15 days (Group D) spermatogonial cell populations increased. However, the number of spermatozoa and spermatids decreased ($P<0.001$; Fig. 5).

Table 2

Effect of the Methylmethane sulphonate on the area of the Seminiferous tubules and the nuclear diameter of the Sertoli cells of the adult male Indian house rat (*Rattus rattus*)

Groups	Seminiferous tubular area (Cm^2)	Sertoli cells Nuclear diameter (υm)
Control (6)* A	21.32 ± 0.42**	2.87 ± 0.10
2days (6) B	20.02 ± 2.40 N	2.55 ± 0.03 [b]
7days (6) C	14.34 ± 0.81 [a]	2.36 ± 0.09 [b]
15days (6) D	11.88 ± 0.67 [a]	2.13 ±0.04 [a]
30days (6) E	14.52 ± 0.66 [a]	2.43 ± 0.05 [b]
45days (6) F	18.17 ± 0.40 [a]	2.89 ± 0.08 N

* :No. of animals in each group

** :Mean ± Standard error

[a]P<0.001; [b]P<0.01; N-Not significant

Seminiferous tubular area and Sertoli cells nuclear diameter were significantly reduced (P<0.001). There was also a significant increase in Sertoli cell population (P<0.001).

After 30 days (Group E) the seminiferous tubular area (P<0.001) and Sertoli cells nuclear diameter (P<0.01) have been different from the control group. Although there was some decrease in spermatogonial cell population, the number of spermatids and spermatozoa have increased as compared to groups B, C, and D (Fig. 6).

After 45 days (Group F) the seminiferous tubular areas have increased. Spermatid and spermatocyte population show the normal percentage.

Epididymal sperm count and sperm abnormality. The sperm population strikingly decreased after 2 to 45 days of post treatment, but maximum reduction occurred in the Group C and D (P<0.001) (Fig.1).Sperm abnormality was also noticed almost in all the groups i.e., B, C, D and E (Fig.2).

Histochemical observations. In controls, the intensity of the sudanophilic lipid materials within the interstitial cells and basement membrane was quite lower (Fig. 11). In the basement membrane of the seminiferous tubules the intensity of acid (Fig. 7) and alkaline (Fig. 9) phosphatase was very high. Moderate Δ^5-3β-HSDH and 17β-HSDH enzymatic activity within the interstitial cells was also observed.

Methylmethane sulphonate treatment. A significant depletion of acid (Fig. 8) and alkaline phosphatase (Fig. 10) has been noticed within the testis of 7 and 15 days post treated animals. Lipid granules have been significantly increased especially in the groups B and C (Fig.12). There was no significant change of Δ^5-3β-HSDH and 17β-HSDH enzymatic activity within the interstitial cells of the treated animals.

Discussion

The results show that in the wild Indian house rats (*Rattus rattus*) administered with methylmethane sulphonate, the weight of the body, testis and important accessory sex reproductive organs has been decreased. The spermatogenesis was greatly suppressed. This sulphonic ester affected only on the mature spermatid and spermatozoa, while the Leydig cells were not affected. However, the nuclear diameter of the Sertoli cells was reduced. On the 7th and 15th day after treatment, abnormal spermatozoa

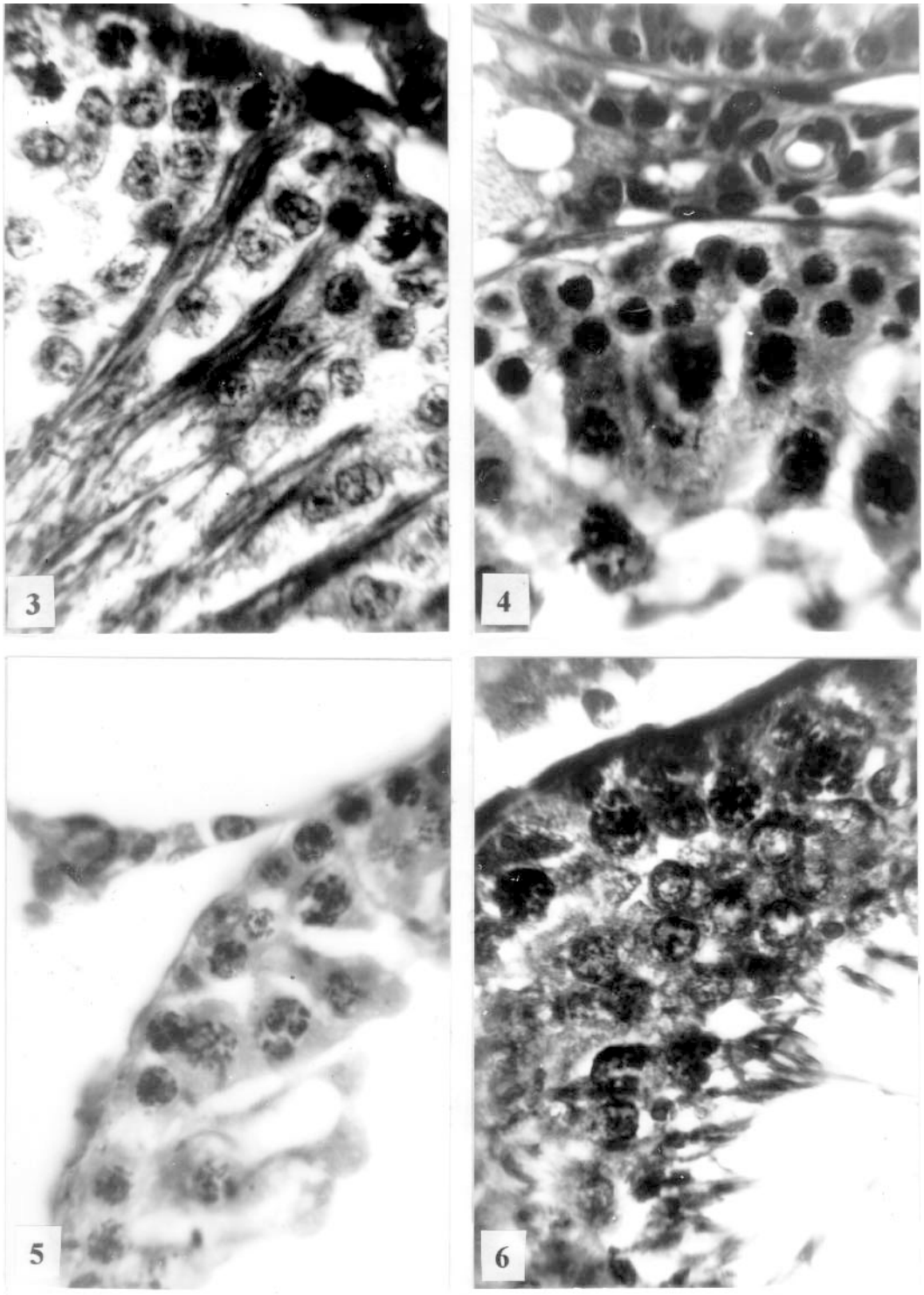

Fig 3. Control testicular section of adult rat showing various germ cell types and abundant spermatozoa. (x1000)
Fig 4. Methylmethane sulphonate treated testicular section showing only spermatogonia, primary and secondary spermatocytes and few spermatid within the seminiferous tubules after 7 days of post treatment. (x1000)
Fig 5. The testicular section of 15 days Methylmethane sulphonate treated rat showing complete absence of mature spermatid and spermatozoa. (x1000)
Fig 6. Recovery of spermatogenesis is noticed within the testicular section after 30 days of post treated rats. (x1000).

Table 3
Percentage of different germ cell types due to Methyl methane sulphonate treatment in the adult male house rat (*Rattus rattus*)

Groups	Spermatogonia (%)	Primary spermatocytes (%)	Secondary spermatocytes (%)	Spermatid (%)	Spermatozoa (%)	S
Control (6)* A	7.39±0.40**	12.37±1.25	22.11±0.86	20.27±0.42	34.70±0.99	[illegible]
2 Days (6) B	15.94±1.1 [a]	19.84±2.37 [d]	27.23±2.74 N	14.17±3.22 N	16.7±2.13 [a]	6
7 Days (6) C	18.65±1.49 [a]	31.90±2.90 [a]	19.39±2.72 N	18.43±3.71 N	3.44±1.05 [a]	8.
15 Days (6) D	23.97±1.75 [a]	35.85±3.46 [a]	25.13±2.68 N	2.13±0.99 [a]	4.74±1.56 [a]	8.
30 Days (6) E	10.19±1.32 N	12.18±0.78 N	14.62±0.77 [a]	34.75±2.62 [a]	24.12±2.13 [e]	4
45 Days (6) F	6 .91±0.67 N	9.00±0.37 [d]	24.88±0.81 [d]	20.63±0.68 N	35.42±0.42 N	[illegible]

* : No of animals in each group

** : Mean±Standard error

[a]P<0.001; [d]P<0.05; [e]P<0.002;N-Not significant

with broken head and tail were also noticed. Partington et al.(1964) reported a selective degeneration of spermatids during the Golgi-cap phase of development due to the administration of methylmethane sulphonate. Their studies also indicate that methylmethane sulphonate does not affect the early spermatogoneal stages at the lower dose level (50 mg/kg). Although a reduction in type A cells to approximately 50 percent of normal number was recorded, no corresponding fall in type B spermatogonia, resting spermatocytes was noticed.

Histochemical reaction revealed the increased level of lipid granules within the interstital cells, spermatogonial cells and decrease in acid and alkaline phospatase activity on the 2, 7 and 15 days after treatment. Increased lipid material within the testis was also reported in the Busulphan treated gerbil testis (Singh et al. 1968). The decreased level of testicular phosphatase due to the suppression of functional changes in the testicular cell types was also described earlier (Guha et al.1980; Males et al.1971; Tice et al.1963). However, there was no significant alteration in the Δ^5-3β-HSDH and 17β-HSDH activity. It is also reported that the changes in lipid materials within the testis are an indication of a derangement of steroid biosynthesis at a time of low gonadotrophin output from the adenohypophysis (Lofts 1962 a,b; Lacy and Lofts 1965)

Due to the administration of methylmethane sulphonate there is some structural change within the Sertoli cells. It is known that the alteration of Sertoli cells affects the production of ABP which in turn lead to the arrest of spermatogenesis .There is also evidence that the disturbance of Sertoli function results in the damage of spermatogenesis (Born et al.1988).

From the present results it is very difficult to conclude that the methyl-

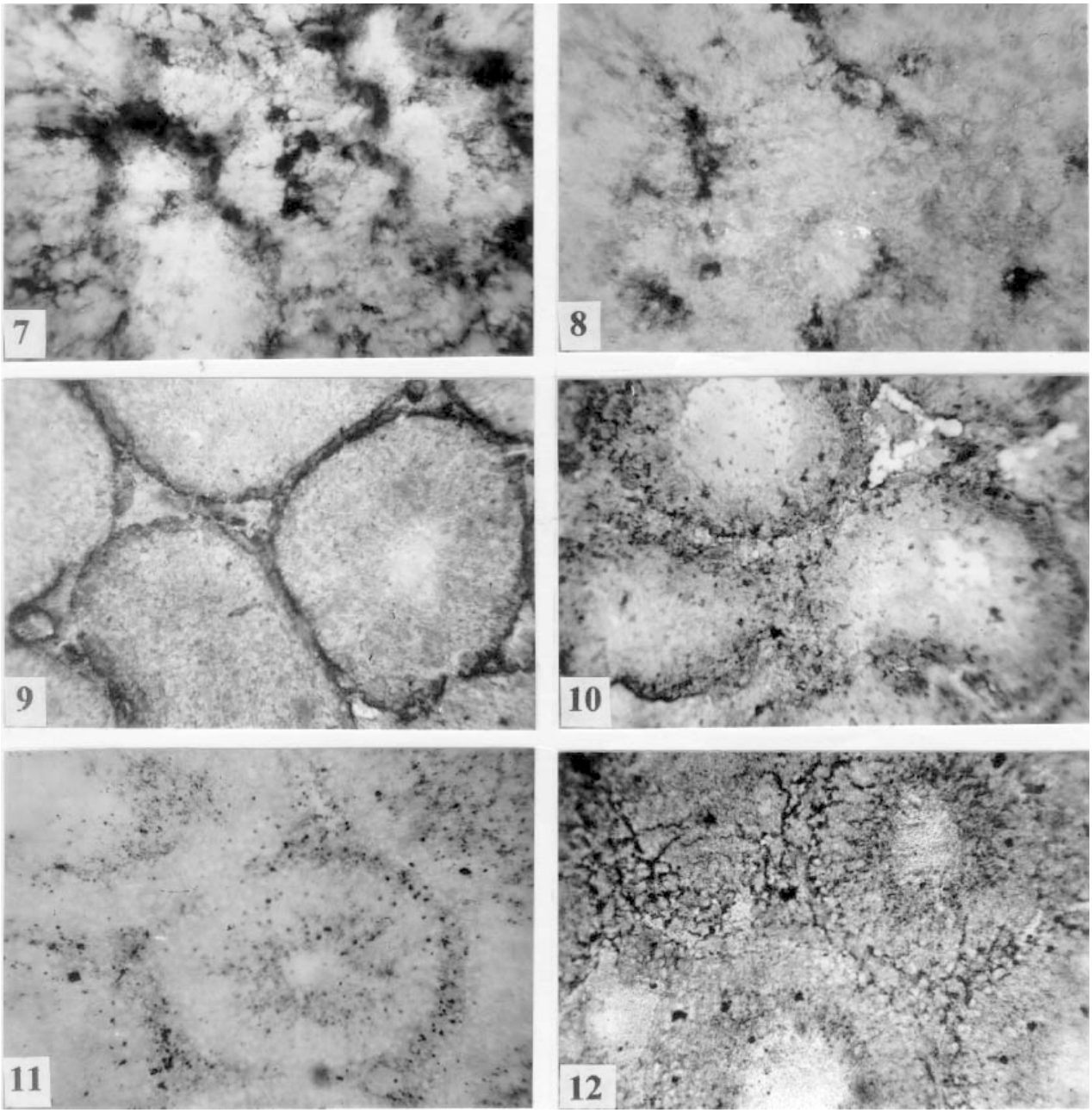

Fig. 7 Section of the control testis showing strong acid phosphatase reaction. (x200)
Fig. 8 Low acid phosphatase activity within the testicular section after 15 days of Methylmethane sulphonate administration. (x200)
Fig. 9 Intense alkaline phosphatase reaction in the testes of the control rat. (x200)
Fig. 10 Reduced alkaline phosphatase reaction within the seminiferous tubules after 15 days of Methylmethane sulphonate administration. (x200)
Fig. 11 Control testicular section of rat showing weak deposition of Sudanophilic lipid granules within the seminiferous tubules. (x200)
Fig.12 Methylmethane sulphonate treated testis of 2 days rats, showing an increase accumulation of lipid granules within the seminiferous tubular cells. (x200)

methane sulphonate has any remarkable role in the alteration of the androgenic hormones. It has also been observed that this specific ester interferes with the spermatogenesis within the seminiferous tubules of the testis of the male wild Indian house rat. Whether the action of this particular ester acts directly on the testicular tissue or via the pituitary gland is not yet clear, although SAHA and GHOSH (1982) and AICH and MANNA (2001) mentioned that the action of busulphan (a potent group of sulphonic ester) on the testicular tissue may be via the pituitary–gonadal axis. From these results, however, the question of possible pituitary involvement in the action of methylmethane sulphonate cannot be clarified and thus further rigorous work is required.

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