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PATHOLOGICAL, IMMUNOHISTOCHEMICAL AND TOXICOLOGICAL STUDIES ON THALLIUM IN ALBINO RATS AND THE ROLE OF POTASSIUM IN PROTECTION.

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SUMMARY

The current study was carried out to evaluate the toxic effect of thallium on fertility of male albino rats as well as possible inhibition of adverse effects by using potassium as a prophylactic drug. The oral LD_{50} value of thallium was determined as 15.49 mg/kg B.wt.. In reproductive toxicity experiment, 90 male rats were divided into 6 groups the first group was a control group, the second group was given potassium chloride daily at a concentration level of 0.36 % in feed as a positive control group. The third and fourth groups (A, B) were administrated orally thallium at a concentration level 1/20 and 1/10 LD_{50} daily. The fifth and sixth groups (C, D) were intubated with 1/20 and 1/10 LD_{50} daily with the addition of potassium containing diet (0.36 %) for two months. The clinical symptoms observed were recorded. Five male rats were sacrificed at monthly intervals. The results revealed that thallium induced adverse effects in male reproduction in all thallium treated rats with or without potassium, whereas animals received thallium with potassium showed lesser effect. The effect was dose and time dependent and the most affection appeared in group (B) which was given 1/10 LD_{50}. These adverse effects were in the form of decrease in relative testes' weights; however, seminal vesicles and prostate glands weights were significantly increased in comparison to controls. Testicular sperm count, sperm motility, alive sperm percentages were significantly reduced, meanwhile, sperm cell abnormalities increased. Fertility was significantly reduced in groups' dosed thallium with or without potassium in that the number of females impregnated by them was significantly reduced to record 0% in group (B). Abortion was observed in group (A); resorption, significant drop in number of implants/litters and alive

percent were noticed. Fetal values indicated a significant decrease in their body weight and length However an increase in malformations percentage in group A&D was recorded; meanwhile, placental weights showed non significant variation. The positive control group showed non. significant changes in comparison to negative control group.

Pathological studies revealed dose and time dependent lesions in the male reproductive system. The male rats in group (A) at one month exhibited disorganization of the germ cells in the seminiferous tubules with release of immature germ cells into the tubular lumina, degeneration of the Sertoli cells and degenerative changes of the germ cells. Moreover, vacuolation in the epithelial linings of the epididymal ducts, prostatic acini and seminal vesicle alveoli were also detected. In group (B) at one month, severe degenerative changes were detected in both Sertoli and germ cells with the formation of multinucleated syncytial giant cells. Moreover, necrobiotic changes were observed in the epithelial linings of the epididymal ducts, prostatic acini and seminal vesicle alveoli. The group (A) at two months of intoxication showed maturation arrest of spermatogonial cells in most tubules with total exfoliation of germ cells into the tubular lumina and focal depletion of germ cells. In the group (B) at two months severe necrotic changes were seen in the germ cells and spermiostasis in the seminiferous tubules and rete testes with calcinosis in one case. Popillary hyperplasia of the prostatic acini and haemorrhage in the lumen of the seminal vesicle lobules and alveoli were also seen. In the group of male rats intoxicated with thallium plus potassium a relatively detectable reduction in tissue lesions were evident.

Immunohistochemical demonstration of P_{53} protein in normal and intoxicated rat testes revealed both cytoplasmic and nuclear detectable moderate immunoreactivity in degenerated germ cells and Sertoli cells with intense immunostaining reactions in necrotic and dead elongated spermatids meanwhile lack of immunoreactivity in both germ cells and Sertoli cells were observed in the testes of normal control rats.

In conclusion, these results strongly suggested that exposure to thallium showed overt disorders in male reproductive performance and induced testicular damage which corrupt spermatogenesis process. Potassium as a protective drug minimizes the deleterious effects of thallium but it couldn't give complete protection.

INTRODUCTION

Thallium and its salts have been incriminated as a highly cumulative poison that can produce multisystem toxicity in human and animals (Mulkey and Oehme, 1998 and Leung and Ooi, 2000).

Thallium is reported to be found as an environ-

mental pollutant in air, water and soil where it is released into the environment from various anthropogenic sources including mineral smelters, coal fire generating stations, brick works, cement factories or homicide attempts, as a rodenticide (Makridis and Amberger, 1989; Meggs et al., 1997; Mulkey and Oehme, 1998 and Arzate et al., 2000) Thallium is a heavy metal whose salts are used in the manufacture of optical lenses, semiconductors, scintillation counters, low temperature thermometers, and switching devices, green coloured fireworks, and imitation jewelry, and as chemical catalysts (Moore et al., 1993).

Both animal and human may be exposed to such substances through the fly ash emission into the atmosphere or waste water in industrial places (W.H.O. 1996) or through thallium load in different plant species (Schäfer et al., 1999). In farm animals, the intake of thallium occurs through contaminated fed (Hapke et al., 1980). Frerking et al. (1990) recorded thallium poisoning in cattle fed on silage from a contaminated areas and mentioned that thallium accumulated in kidneys, liver and testes.

The distribution of thallium in various organs is a time dependent process (Talas and Wellhner, 1983 and W.H.O. 1996) where it is completely absorbed through the respiratory system and gastrointestinal tracts (Schäfer et al., 1999).

Many studies on thallium toxicity have primarily focused on its effects on nervous system (Osorio-Rico et al., 1995 and Arzate et al., 2000). Meanwhile, other studies explored out that male reproductive system is the susceptible target site for toxic effects of thallium where it preferentially accumulates in animal testes and epididymis (Formigli et al., 1986 and Gregotti et al., 1992). It brings about impairment in reproductive performance which represents a significant economic problem in veterinary medicine (Osweiler, 1996). Moreover, higher concentrations of thallium have been reported to have mutagenic and antimitotic properties and also it affects on basic cellular activities such as disturbance in the function of mitochondria (WHO, 1996).

The mechanism by which thallium induced its toxicity is primarily in attribute to disturbance in the oxidant antioxidant systems in the cell where it inhibits glutathione- peroxidase activity with oxidative damage of the tissues (Arzate et al., 2000; Arzate et al., 2005 and Hanzel et al., 2005). Thallium enters cells by a unique process governed by its similarity in charge and ionic radius to potassium. Although the exact mechanism of toxicity has not been established, thallium interferes with energy production at essential steps in glycolysis, the Krebs cycle, and oxidative phosphorylation. Additional effects include inhibition of sodium-potassium-adenosine triphosphatase and binding to sulfhydryl groups (Hoffman 2003). Because thallium and potassium have the same charge and similar ionic radii, thallium

follows potassium distribution pathways and alters a number of potassium dependent processes (Mulkey and Oehme, 1998).

So, this study was designed as an effort to identify the gonadotoxic effect of thallium on male reproductive system of albino rats and its effects on male fertility as well as possible inhibition of toxicity by using potassium chloride as a prophylactic drug.

MATERIAL AND METHODS

Materials:

Thallium nitrate ($TINO_3$) Mol.W.266.40, produced by Merck, Germany.

Potassium chloride (kcl) produced by El- Nasr Pharmaceutical Chemicals Co. Egypt.

Experimental animals:

166 adult albino rats of Wister strain (106 males and 60 females), were used in this study.

Experimental design:

16 mature male albino rats were used for determination of the acute oral LD_{50}.

90 male rats were divided into 6 groups. The first group was the control group. The second group was given potassium chloride daily at a concentration level of o.36 % in feed as a positive control group (Henry et al., 1979). The third and fourth groups (A, B) were given orally thallium dissolved in water daily at a concentration level 0.78 and 1.55 mg/kg b.wt. equivalent to 1/20 and 1/10 LD_{50} for two months. The fifth and the sixth groups (C, D) were given orally 1/20 and 1/10 LD_{50} of thallium nitrate daily with the addition of K containing diet (0.36 %) for two months. All groups were kept under strict observation and 5 rats from each group were sacrificed after one and two months post administration for revealing the male reproductive toxicity studies as well as for histopathological evaluation. At the end of the experiment 5 rats from each group were paired with 60 healthy untreated female rats (1:2) for serial mating experiment.

Methods:

Determination of LD_{50}: was performed mathematically according to method described by Finney (1964).

Weight of sex organs: Testes, seminal vesicles and prostate glands were taken out and weighed; the relative weight was calculated according to the whole body weight.

Spermatozoal examination: Sperms were obtained according to (Blandau and Jordan, 1941) and examined for sperm cell concentration, motility percent, live and dead sperm percent and spermatozoal abnormalities (Bearden and Fluquary, 1980).

Serial mating technique: was performed according to Wand and Colin (1998).

Morphological examination of the obtained fertility: were carried out according to Cook and Fairweather (1968).

Histopathological studies:

Postmortem examination was performed and tissue specimens were collected from testes, epididymis, prostate glands and seminal vesicles for histopathological examination. These were immersed in 10% neutral buffered formalin solution for at least 24 hours, routinely processed by the standard paraffin embedding technique and stained with hematoxylin and eosin (Bancroft et al., 1994).

III- Immunohistochemical studies:

They were performed on normal rat testes and testes of intoxicated rats with 1/10 LD_{50} for two months for detection of P_{53} protein using avidin biotein immunoperoxidase complex method. The P53 protein accumulates in response to a number of cellular stresses such as DNA damage generating by many insults like chemical toxicants (Lakin and Jackson, 1999; Offer et al., 2002 and Abou Donia et al., 2003).

Biological reagents:

1- Mouse monocolonal antibody. Anti- P_{53} protein [D07] available by BioGenex Laboratories Inc. USA.

2- Antimouse biotinlated secondary antibody and streptavidin peroxidase were used as a conjugate.

3- Dab. Diaminobenzidine: a peroxidase precipitating substrate available by Lab. Vision. Co. USA.

Paraffin sections from normal control rat testes were used as positive controls.

Statistical analysis:

The obtained data were statistically analyzed using the Analysis of Variance (ANOVA). Two way classification according to SPSS11 (2002) computer program.

RESULTS

Determination of LD_{50}:

In the current work, the acute oral LD_{50} of thallium in thallium nitrate was calculated as 15.49 mg thallium /kg B.wt. in male albino rats (Table, 1).

Table (1): Determination of acute oral LD_{50} of thallium nitrate in adult male albino rats according to Finney (1964).

Groups	Dose (mg/kgB.wt.)	No. of rats in each group	No. of dead animals	Mortality%
1	5	4	0	0
2	10	4	2	50
3	20	4	3	75
4	40	4	4	100

Calculation of LD_{50}:

$M = x_1 + 1/2\ d - dr_1/N$

$\text{Log } LD_{50} = \text{Log } 40 + 1/2 \text{ Log} 2 - 9/16$

$= 1.60206 + 0.150515 - 0.5625$

$= 1.190075$

$LD_{50} = 15.49$ mg thallium /kg B.wt.

Clinical symptoms:

The observed clinical symptoms were alopecia, weakness and gastrointestinal disturbance. These symptoms were more obvious in groups that had received thallium only than those which had received thallium with potassium.

Male fertility and reproductive toxicity studies:

In the present work, male fertility was studied in albino rats administrated thallium (0.78 and 1.55 mg/kg b.wt.) alone or in combination with potassium (0.36 % in feed) for 2 months in comparison to -ve control as well as +ve control (potassium treated group). A positive control group showed non significant changes in comparison to -ve control group.

Table (2): showed a significant decrease ($P<0.05$) in testicular weights in group (B) at the first month. Also, at the second month decreased weights of the testes in group (A, B, D) were recorded, however, weights of prostate gland showed a significant increase in group (B) at the first and second months compared to control groups. The seminal vesicles showed a significant increase at $P<0.05$ using LSD in group (B) at the first month of the experiment, while, a significant increase in group (A, B, D) was recorded at the second month of the experiment compared to both controls.

Data displayed in table (3) showed a significant decrease at $P<0.05$ using LSD in sperm cell concentration in all treated groups. Also, it illustrated significantly Fischer exact probability test which proved that there was a significant difference at $P<0.05$ in control groups, group (C), group (A), group (B&D) at descending order in motility and live%. While the abnormality percentage showed a significant difference between control groups then group (C), then the other groups at the first month of the experiment. At the second month of the experiment, the motility percentage recorded significant difference at $P<0.05$ in control groups, group(C) then the other groups. Also, live percentage showed significance in control groups, group (A&C), then group (B&D). Meanwhile the abnormality percentage recorded a significant difference between control groups, group (C), group (A&D), group (B) in ascending order. The abnormalities were in the form of bent tail, fissured tail, bent abnormal mid piece, irregular mid-piece and detached head (Fig. 1).

Table (4), illustrated the effect of thallium alone or with potassium on fertility index of male albino rats. The pregnancy percent was recorded 0% in group (B), which received 1/10 LD_{50} thallium. While it was recorded 20% in group (D), 30% in group (A) and 50% in group (C). The abortion was observed in group (A) as 10%. The resorption was observed in group (A&D). Group (A&D) showed a significant decrease using LSD at ($P<0.05$) in number of implantation/litter, fetal body weight and length in comparison to control

groups. There was a decrease in alive fetii and an increase in dead fetii and malformed percentage in group (A), group (D), group (C) at the following manner. The malformed fetii were in the form of haematoma and stunt tail.

Table (2): Effect of thallium with or without potassium chloride on relative weight of sex organs compared to –ve & +ve controls. (Means±S.E)

		Testes	Seminal Vesicle	Prostate
1 month	-ve control	1.63 ± 0.112^{A}	0.49 ± 0.087^{A}	0.16 ± 0.016^{A}
	+ve control	1.60 ± 0.082^{B}	0.48 ± 0.031^{B}	0.156 ± 0.018^{B}
	Group(A) 1/20 LD_{50}	1.58 ± 0.096	0.465 ± 0.008	0.160 ± 0.015
	Group(B) 1/10 LD_{50}	1.36 ± 0.087^{ab}	0.45 ± 0.058^{ab}	0.185 ± 0.016^{ab}
	Group(C) 1/20 LD_{50}+ K	1.65 ± 0.083	0.46 ± 0.022	0.163 ± 0.017
	Group(D) 1/10 LD_{50}+K	1.62 ± 0.078	0.48 ± 0.019	0.165 ± 0.025
	F-calculated	11.321#	10.212#	5.541#
2 months	-ve control	1.64 ± 0.109^{A}	0.52 ± 0.092^{A}	0.162 ± 0.022^{A}
	+ve control	1.62 ± 0.091^{B}	0.51 ± 0.066^{aB}	0.161 ± 0.024^{B}
	Group(A) 1/20 LD_{50}	1.53 ± 0.073^{ab}	0.65 ± 0.039^{ab}	0.160 ± 0.021
	Group(B) 1/10 LD_{50}	1.30 ± 0.070^{ab}	0.68 ± 0.110^{ab}	0.173 ± 0.017^{ab}
	Group(C) 1/20 LD_{50}+ K	1.65 ± 0.110	0.60 ± 0.012^{ab}	0.158 ± 0.026
	Group(D) 1/10 LD_{50}+K	1.41 ± 0.056^{ab}	0.58 ± 0.013^{ab}	0.164 ± 0.024
	F-calculated	9.844#	11.714#	8.874#

Significant at $P < 0.05$

Aa, Bb Significantly different between two comparison groups against capital litter at $P < 0.05$ using LSD.

Table (3): Effect of thallium and thallium with potassium chloride on semen analysis.

		Count x106/ epididymis	Motility %	Live %	Abnormality%
1 month	-ve control	58.2 ± 2.18^{A}	92 a	95 a	8 c
	+ve control	56.0 ± 2.34^{B}	90 a	92 a	11 c
	Group(A) 1/20 LD_{50}	48.5 ± 3.52^{ab}	62c	75c	28a
	Group(B) 1/10 LD_{50}	41.6 ± 3.67^{ab}	50d	68d	35a
	Group(C) 1/20 LD_{50}+ K	50.2 ± 3.67^{ab}	72b	80 b	20b
	Group(D) 1/10 LD_{50}+K	45.2 ± 2.96^{ab}	55d	70d	32a
	F-calculated	10.261#			
2 months	-ve control	57.6 ± 2.29^{A}	88 a	93 a	10 d
	+ve control	58.6 ± 2.71^{B}	90 a	92 a	9 d
	Group(A) 1/20 LD_{50}	43.7 ± 3.14^{ab}	40c	60b	34b
	Group(B) 1/10 LD_{50}	38.2 ± 2.14^{ab}	35c	50c	50a
	Group(C) 1/20 LD_{50}+ K	46.4 ± 3.68^{ab}	60b	70b	27c
	Group(D) 1/10 LD_{50}+K	40.6 ± 3.16^{ab}	40c	55c	37b
	F-calculated	10.112#	8.974#	11.654#	12.354#

Significant at $P < 0.05$

Aa, Bb Significantly different between two comparison groups against capital litter at $P < 0.05$ using LSD.

a, b, c, d Significantly different against different letter using Fischer Exact Probability Test at $P < 0.05$

Table (4): Effect of thallium and thallium with potassium on fertility index of adult male albino rats and in maternal fetal manifestations.

	-ve Control	+ve Control	Group(A)	Group(B)	Group(C)	Group(D)	F-calculated
Pregnancy %	100	100	30	0	50	20	
Abortion %	0	0	10	0	0	0	
No. of implantation/litter	9.0 ± 0.65^{A}	8.9 ± 0.71^{B}	5.6 ± 0.84^{ab}	-	8.0 ± 0.71	6.3 ± 0.96^{ab}	9.214#
No. of resorption/ litter	0	0	1.8 ± 0.23	-	-	0.7 ± 0.18	-
Body weight(g)	3.94 ± 0.43^{A}	4.12 ± 0.37^{B}	2.85 ± 0.15^{ab}	-	3.36 ± 0.086	3.17 ± 0.13^{ab}	8.154#
Length(cm)	3.70 ± 0.105^{A}	3.76 ± 0.112^{B}	2.78 ± 0.128^{ab}	-	3.18 ± 0.118	3.06 ± 0.103^{ab}	11.324#
Alive feti%	98	98.6	80	-	90	85	
Dead feti%	2	1.4	20	-	10	15	
Malformed%	0	0	4	-	3	3.6	
Placental wt.(g)	0.825±0.026	0.836±0.028	0.827±0.019	-	0.816 ± 0.030	0.830 ± 0.026	

Significant at $P < 0.05$

Aa, Bb Significantly different between two comparison groups against capital litter at $P < 0.05$ using LSD.

Pathological studies:

Thallium treated animals:

I- Gross pathology:

At one month of intoxication:

The testes were oedematous, swollen and soft in consistency with prominent congestion of the blood vessels. The epididymis was slightly enlarged and the lobes of both the prostate glands and seminal vesicles exhibited oesdematous swellings.

At two months of intoxication:

The testes were reduced in size, relatively hard in consistency and on cut section, it had coarse granular appearance.

In one male rat, the two lobes of the seminal vesicles were reddish in colour with minute petechial haemorrhages on its surface. On cut section, free blood oozed out.

II- Microscopic pathology

In group (A) at 1 month:

I- Testes:

The testicular blood vessels were widely dilated and engorged with blood and the presence of focal small areas of haemorrhage in the interstitial tissue.There was a marked oedema of the interstitial tissue with intratubular faintly eosinophilic fluid in the lumina of some seminiferous tubules.

The most prominent changes in the testes were disorganization of the seminiferous epithelium associated with a release of immature dislodged germ cells into the tubular lumina (Fig. 2A).

Some seminiferous tubules revealed degenerative changes of the sertoli cells emphasized by the appearance of large pleomorphic cytoplasmic vacuoles of definite borders (Fig, 2B). Meanwhile, other tubules exhibited reduced numbers of the spermatocytes and round spermatids with inhibited release of elongated spermatids. Irregular contorus of the basal lamina were evident in most tubules. In few seminiferous tubules, there were marked a degenerative changes of the spermatocytes emphasized by deeply eosinophilic cytoplasm with pyknosis, of the nuclei. Meanwhile degeneration of round spermatids was manifested by peripheral condensation of the nuclear chromatin (round ring nuclei) (Fig, 3A).

Furthermore, other seminiferous tubules showed necrosis of the spermatocytes and round spermatids represented by strongly eosinophilic cytoplasm, karyolysis of the nuclei and exfoliation into the tubular lumina (Fig. 3B). The endothelial cells of some testicular blood vessels appeared swollen with intracytoplasmic vacuoles of variable sizes and pyknotic nuclei. In few instances, there were wide dilatations of the intra testicular rete testes beneath the tunica albuginea.

II- Epididymis:

There was a wide dilatation of the epididymal blood vessels with perivascular mononuclear cell

ggregations (Fig, 4A). Immature spermatogenic pithelial cells were detected in the lumen of some epididymal ducts (Fig, 4B). Most of the epididymal ducts exhibited irregular contours. The epithelial linings of some epididymal ducts showed vacuolations of its cytoplasm. In some instances, cystic dilatations of some epididymal ducts were evident.

III- Prostate glands:

There were mild degenerative changes of the epithelial linings of the prostatic acini.

IV. Seminal vesicles:

There were small areas of haemorrhage in the lumen of the seminal vesicle lobules admixed with few lymphocytes.

Degenerative changes of the epithelial linings of some seminal vesicle alveoli were noticed in many instances emphasized by the appearance of intracytoplasmic vacuoles of variable sizes.

In group (B) at 1 month:

I- Testes:

There were marked degenerative changes of the Sertoli cells manifested either by severe vacuolation of its cytoplasm (Fig, 5A) or by the appearance of faintly eosinophilic cytoplasm with pyknosis or karyolysis of its nuclei (Fig, 5B).

In most seminiferous tubules, the spermatogenic epithelium revealed degenerative and/ or necrotic changes with absence of normal architecture.

The necrotic germ cells were exfoliated into the tubular lumina and exhibited strongly eosinophilic cytoplasm with pyknosis or karyolysis of its nuclei (Fig, 6A). Meanwhile, degenerated round spermatids revealed peripheral condensation of the nuclear chromatin (round ring nuclei).

Sometimes, the exfoliated germ cell obliterated the tubular lumen and admixed with cellular debris or organized in the form clumped necrotic material with numerous bucklings of the basal lamina (Fig, 6B).

Sometimes, the degenerated round spermatids coalsce with each other to form multinucleated syncytial giant cells with few numbers of the germinal epithelium (Fig, 7A).

In few instances, there were abnormal configurations of elongated spermatids which were displaced and scattered in all layers of the spermatogenic epithelium (Fig, 7B).

One of the most important findings in this group was the detection of misshaped elongated spermertids retained deep in the seminiferous epithelium at the periphery of the tubules (Fig, 8A).

In one male rat, there was a moderate accumulation of sperms in the dilated intra testicular rete testes beneath the tunica albuginea admixed with exfoliated immature germ cells within its lumina (Fig, 8B).

II- Epididymis:

The epithelial linings of the epididymal ducts appeared vacuolated with oedema and mononuclear cellular infiltrate in the interstitial tissue (Fig, 9A).

In few instances, necrobiotic changes were detected in the epithelial linings of some epididymal ducts and desquamated into the ductal lumina (Fig, 9B). Immature germ cells were seen in the lumen of some epididymal ducts.

III- Prostate glands:

The interstitial blood vessels were widely dilated and engorged with blood, Focal necrobiotic changes of the epithelial linings of some secretory acini that were desquamated into the acinar lumina. (Fig, 10A). Moreover, mononuclear cell infiltrate were detected in the interstitial tissue.

IV- Seminal vesicles:

The interstitial blood vessels were widely dilated and engorged with blood. The epithelial linings of some alveoli were vacuolated meanwhile necrosis and exfoliations of the lining epithelium of other alveoli were evident (Fig, 10B).

In group (A) at two months:

The same changes encountered in the group of rats intoxicated for 1 month in addition to:

Hyaline thickening of the epithelial basement membrane of most of the seminiferous tubules with absence of radial orientation of germ cells.

There were degenerative and necrotic changes of the spermatogenic epithelial cells emphasized by dislodged cells, irregular cell borders, strongly eosinophilic cytoplasm and karyolysis of its nuclei.

In some instances the lumina of some seminiferous tubules were obliterated with exfoliated necrotic germ cells admixed with cellular debris (Fig, 11).

Cytoplasmic vacuolation of some germ cells were detected in some tubules meanwhile an arrest of maturation of spermatogonial cells was evident in other tubules particularlly at the spermatocyte phase (Fig, 12A).

Marked undulation of the basal lamina with the appearance of immature germ cells within its lumina particularly leptolene and pachytene spermatocytes was also seenin some tubules.

One of the most important pathologic entities was the focal germ cell depletion represented by the appearance of large vacuoles and/ or spaces retained deep in the spermatogenic epithelium with sharp irregular outlines in some seminiferous tubules (Fig 12 B and 13A).

In few tubules, a marked decreased cellularity of the germ cells which were totally exfoliated into the tubular lumina (Fig, 13B) was recorded.

II. Epididymis:

The changes were nearly similar to group (A) at one month. Moreover, necrosis of the epithelial linings of some epididymal ducts was more evident.

III. Prostate gland:

The changes were nearly similar to group (A) at one month. Moreover, focal hyperplasia of the epithelial linings of some secretory acini was seen.

IV. Seminal vesicle:

The changes were nearly similar to group (A) at one month.

In group (B) at two months:

I- Testes:

There were severe degenerative and necrotic changes of the spermatogenic epithelial cells with reduced numbers of the germ cells.

The necrotic spermatogenic epithelial cells were exfoliated into the tubular lumina.

Moreover, the lumina of some seminiferous tubules showed accumulation of sperms within its lumina (Fig., 14A) as well as in the lumina of the intratesticular rete testes (spermiostasis).

In one male rat, focal deposition of calcium salts were detected in the rete testes with spermiostasis (Calcinosis) (Fig., 14B). Marked atrophy of some seminiferous tubules was evident and they were lined by sertoli cells only and few spermatogenic epithelial cells (Fig., 15A). Meanwhile, in few tubules, most of the seminiferous epithelium disappeared and they were lined by remnants of germ cells (Fig 15B).

II. Epididymis

The same changes detected in the group (A) at two months, moreover, homogenous eosinophilic oedema fluid was observed in the lumina of some epididymal ducts.

III. Prostate glands:

Papillary hyperplasia of the epithelial linings of the prostatic acini (Fig., 16 A) was detected in some secretory acini.

IV- Seminal vesicles:

The changes were nearly similar to group (B) at one month. Moreover, in one male rat, extensive areas of haemorrhage were detected in the lumen of some lobules and alveoli as well as in the interstitial tissue (Fig, 16B).

The epithelial linings of some alveoli were necrotic and exfoliated into the tubular lumina.

Thallium and potassium treated animals:

I- Gross pathology:

At one month of intoxication with thallium plus potassium:

The testes, epididymis, prostate and seminal vesi-

cles were slightly odematous and enlarged.

At two months of intoxication with thallium plus potassium:

The testes were relatively reduced in size.

II- Microscopic pathology

The group (C) at 1 month:

I- Testes:

The testes revealed few degenerative changes in round spermatids and spermatocytes.

There was distortion of the normal sequence of the spermatogenic epithelial cells in some tubules, moreover, vacuolation of the Sertoli cells were also detected in other tubules. There was redundant basal lamina with mild thickening.

II. Epididymis:

Cystic dilatations of the epididymal ducts were evident.

III- Prostate glands:

Mild degenerative lesions of the prostatic acini associated with oedema and mononuclear cell infiltration in the interstitial tissue.

IV- Seminal vesicles:

Mild degenerative changes were observed in the epithelial linings of seminal vesicle alveoli.

In group (D) at 1 month:

I- Testes:

The changes were nearly similar to those recorded in group (C) at one month.

Some seminiferous tubules exhibited disarrangement of the germ cells with appearance of vacuoles of variable sizes in the spermatogenic epithelium and Sertoli cells. Meanwhile, spermatogenesis was proceeding in other tubules.

II. Epididymis:

There were focal mononuclear cell infiltrations in the interstitial tissue and few spermatogenic cells were detected in the lumen of some epididymal ducts. Proliferation of the epithelial linings of some epididymal ducts was observed.

III- Prostate glands:

Small focal degenerative lesions of the epithelial linings were evident in some prostatic acini. Perivascular mononuclear cell infiltration was detected.

IV- Seminal vesicles:

There were vacuolation of the epithelial linings of some alveoli.

In group (C) at two months:

Testes:

Mild degenerative changes were detected in the spermatogenic epithelium of some tubules with desquamation of immature germ cells into the tubular lumina.

Epididymis:

Showed the same changes like that in group (D) at 1 month.

prostate glands:

showed focal hyperplastic lesions of the epithelial linings of the prostatic acini.

IV- Seminal vesicles:

Mild to moderate necrobiotic changes were observed in the epithelial linings of some alveoli.

In group (D) at two months:

The same observations were seen as in group (C) at two months.

Immunohistochemical studies:

They were carried out on both normal and intoxicated rat testes (with 1/10 LD_{50} for two months) to determine the prevalence of P_{53} protein expressions.

In normal rat testes, there were lack of P_{53} protein expressions in the spermatogenic epithelial cells and Sertoli cells (Fig, 17A).

In the testes of intoxicated rat with 1/10 LD_{50} for 2 months there were mild to moderate immunoreactivity for P_{53} protein in both cytoplasmic and nuclear compartments of the degenerated germ cells particularly spermatocytes and round spermatids (Fig., 17 B).

Furthermore, cytoplasmic immunostaining reactions were evident in the degenerated sertoli cells (Fig., 18A). Meanwhile, immunoreactivity for P53 protein was also demonstrable in the necrotic exfoliated germ cells (Fig., 18 B). In some instances, the degenerated round spermatids exhibited nuclear membranous immunostaining reactions paralleled to the distribution of chromatin within its nuclei in H and E sections.

In some tubules detectable immunoreactivity was clearly evident in the nuclei of denuded necrotic rounded or elongated spermatids (Fig., 19 A & B).

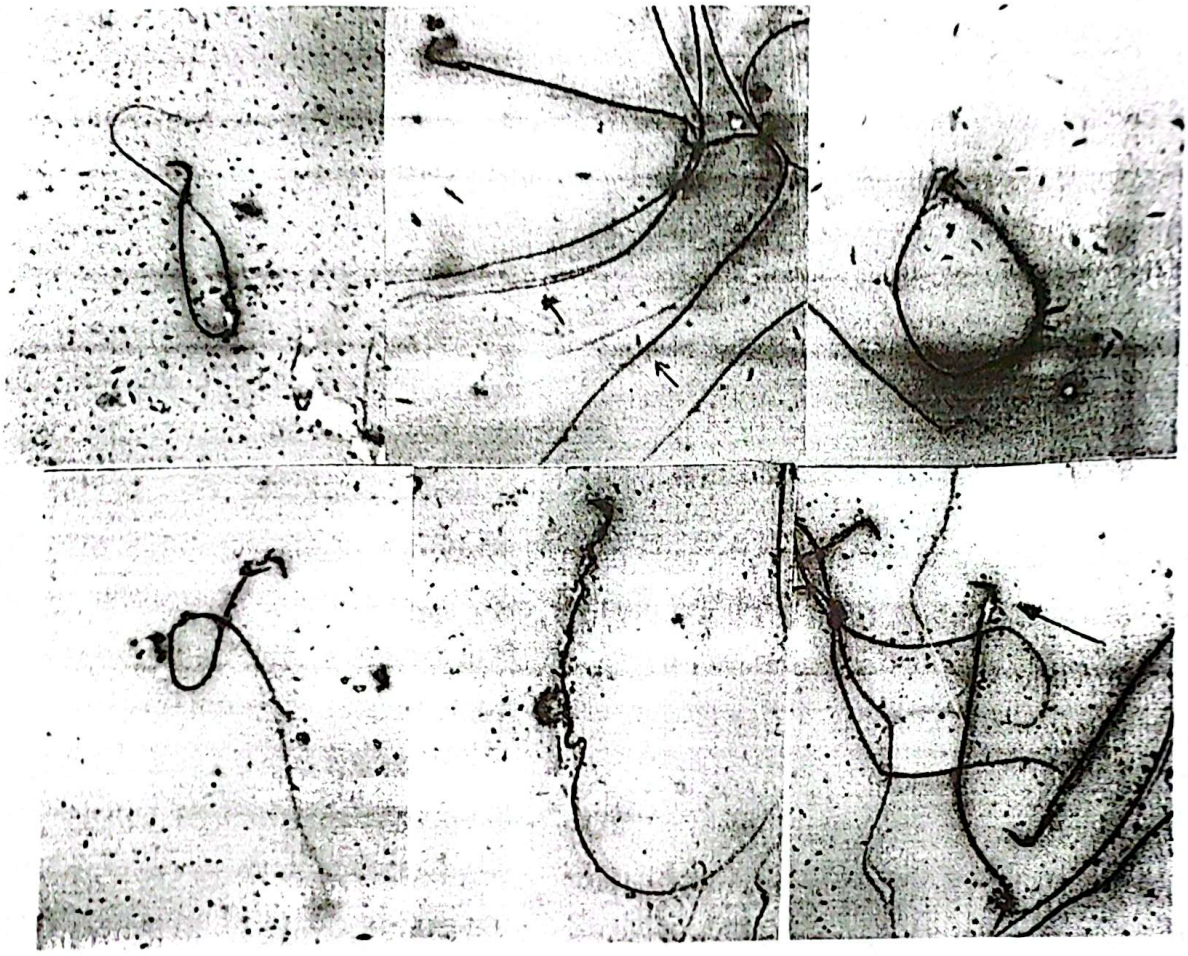

Fig. (1): Illustrating morphological deformities in the sperms shape represented by:

A- Bent tail.

B- Fissured tail. (Arrows)

C&D-Bent abnormal mid piece.

E- Irregular mid-piece.

F-Detached head. (Arrow)

Eosin nigrosin stain (X400)

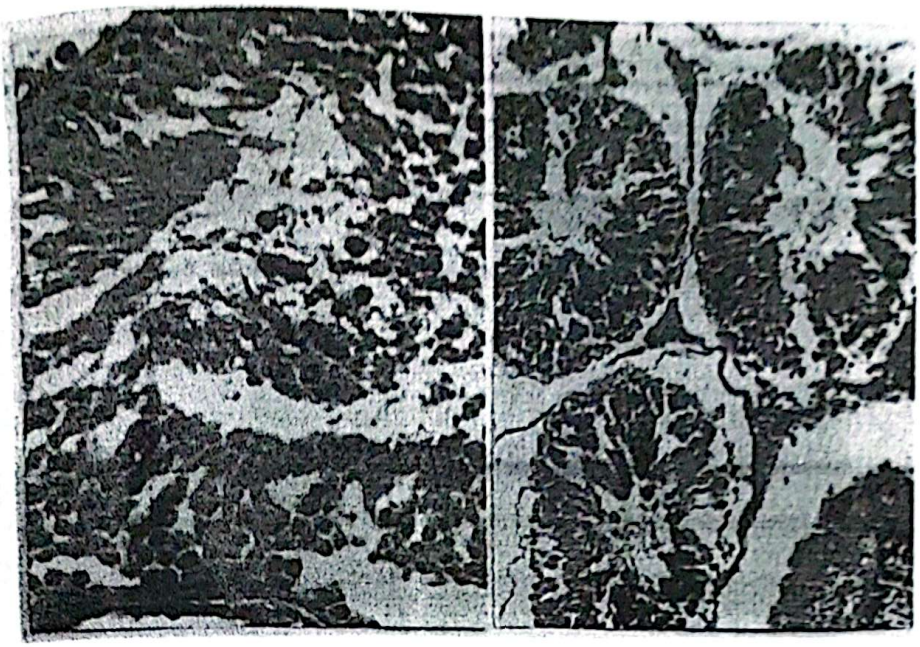

Fig (2): Testis of male rat intoxicated with 1/20 LD_{50} of thallium nitrate for one month illustrating:

A- Immature dislodged germ cells in the tubular lumen.

B- Cytoplasmic vacuolation of the Sertoli cells with irregular contorus of the basale lamina of the seminiferous tubules. (A and B H&E. AX 400; BX 160).

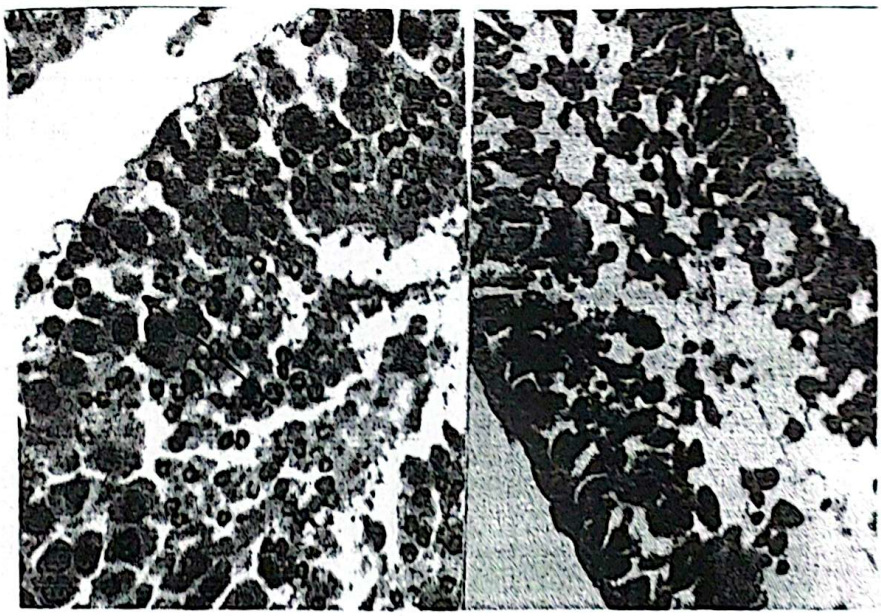

Fig (3): Testis of male rat intoxicated with 1/20 LD_{50} for 1 month revealing:

A- Degenerative changes of the spermatocytes with karyorhexsis and karyolysis of the nuclei (arrow) and degeneration of round spermatids with peripheral condensation of the nuclear chromatin (round ring nuclei).

B- Necrosis and exfoliation of germ cells into the tubular lumen.

(A and B H & E. X 400)

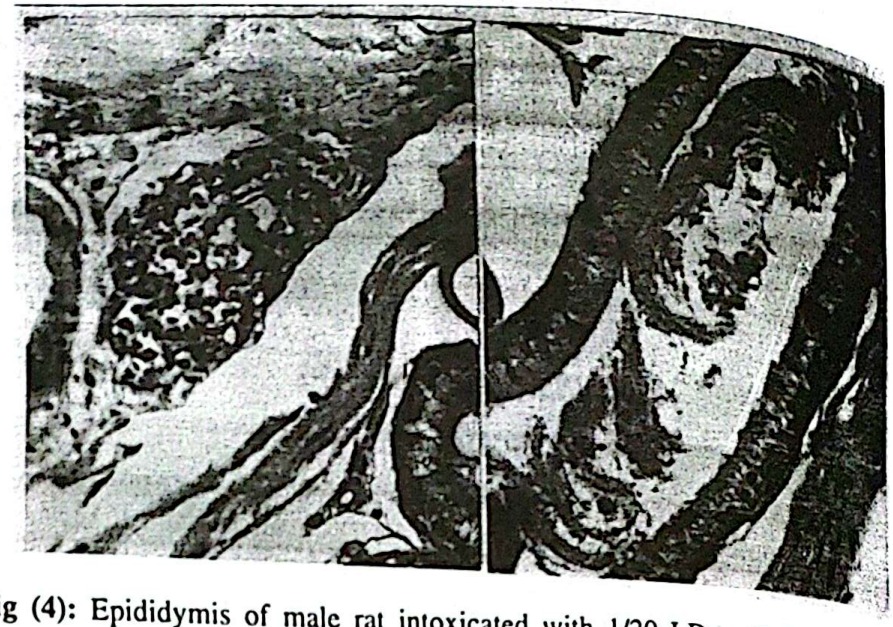

Fig (4): Epididymis of male rat intoxicated with 1/20 LD_{50} of thallium nitrate showing:

A- Preivascular monouclear cell xaggregations.

B- Immature germ cells in the lumen of the epididymal duct.

(A and B H & E. X 400)

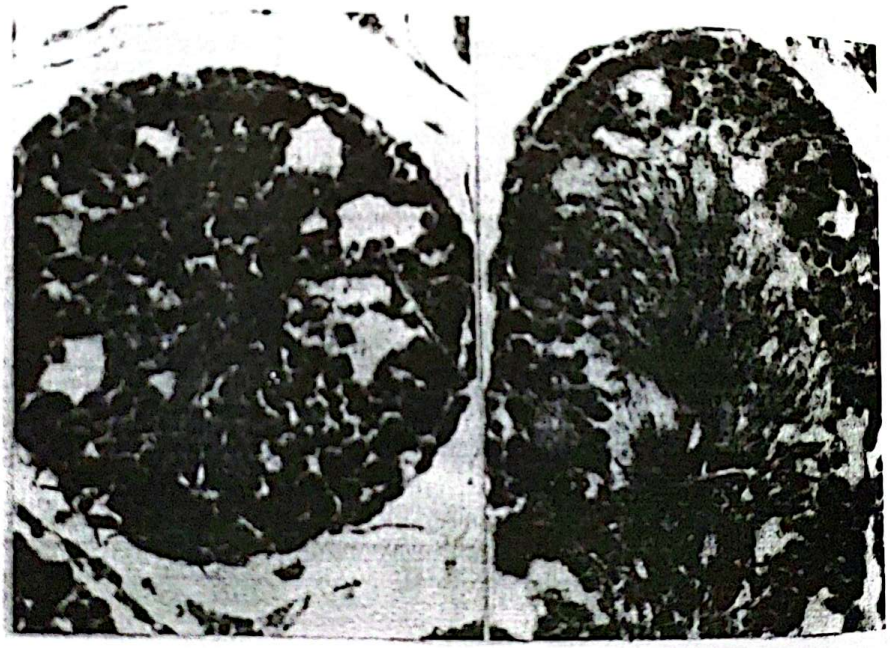

Fig (5): Testis of male rat intoxicated with 1/10 LD_{50} of thallium for 1 month revealing:

A- Severe vacuolation of the Sertoli cell cytoplasm.

B- Degeneration of the Sertoli cells emphasized by faintly eosinophilic cytoplasm with pyknosis or karyolysis of the nuclei.

(A and B H & E. X 400)

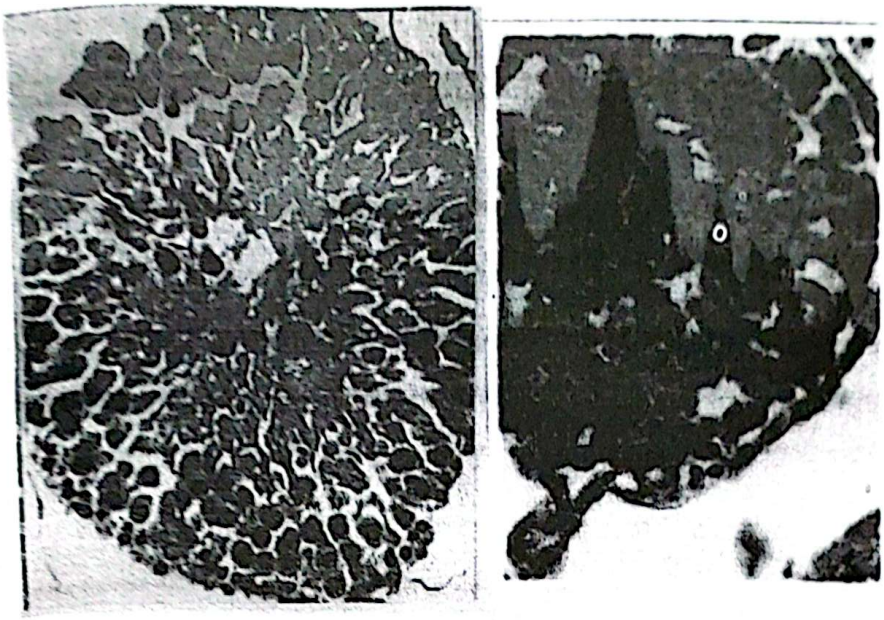

Fig (6): Testis of male rat intoxicated with 1/10 LD_{50} for 1 month illustrating:
A- Desquamation of necrotic germ cells into the tubular lumen.
B- The necrotic germ cells were organized into clumped necrotic material with numerous bucklings of the basale lamina (arrows).
(A and B H & E. X 400).

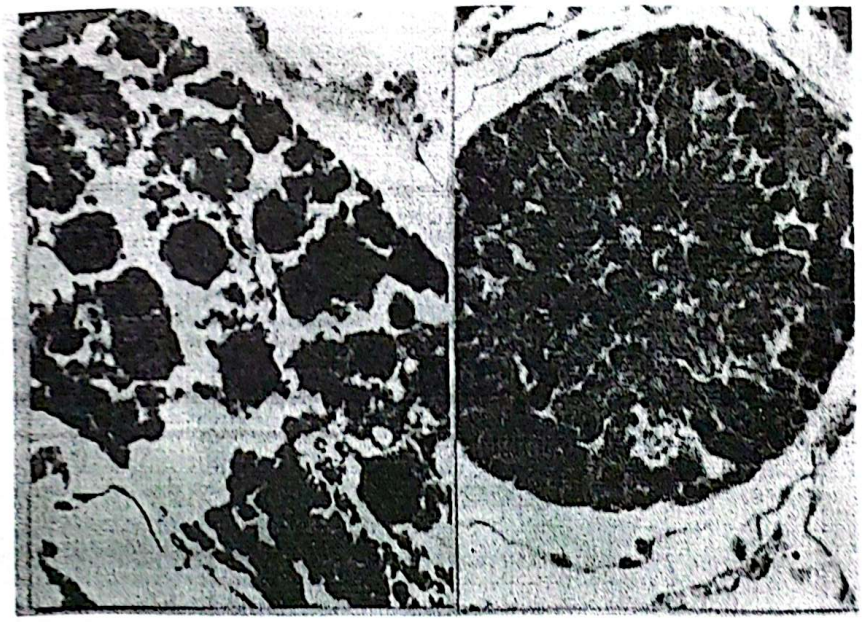

Fig (7): Testis of male rat intoxicated with 1/10 LD_{50} for 1 month Showing:
A- Multinucleated syncytial giant cells in the lumen of the seminiferous tubules.
B- Abnormal configurations of elongated spermatids which appeared scattered in all layers of the seminiferous epithelium.
(A and B H & E. X 400)

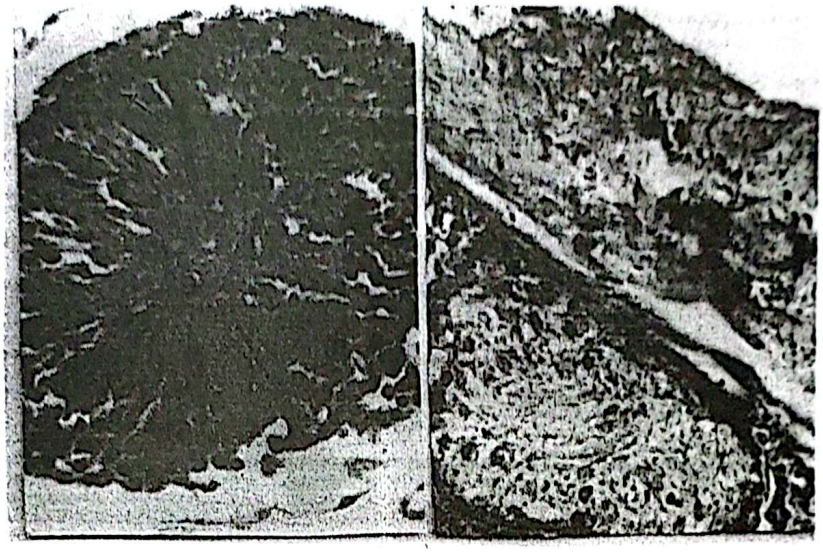

Fig (8): Testis of male rat intoxicated with 1/10 LD_{50} for 1 month demonstrating:

A- Misshaped elongated spermatids retained deep in the germinal epithelium (arrow).

B- Moderate accumulation of sperms in the dilated intra testicular retetestes and mixed with immature germ cells.

(A and B H & E. X 400).

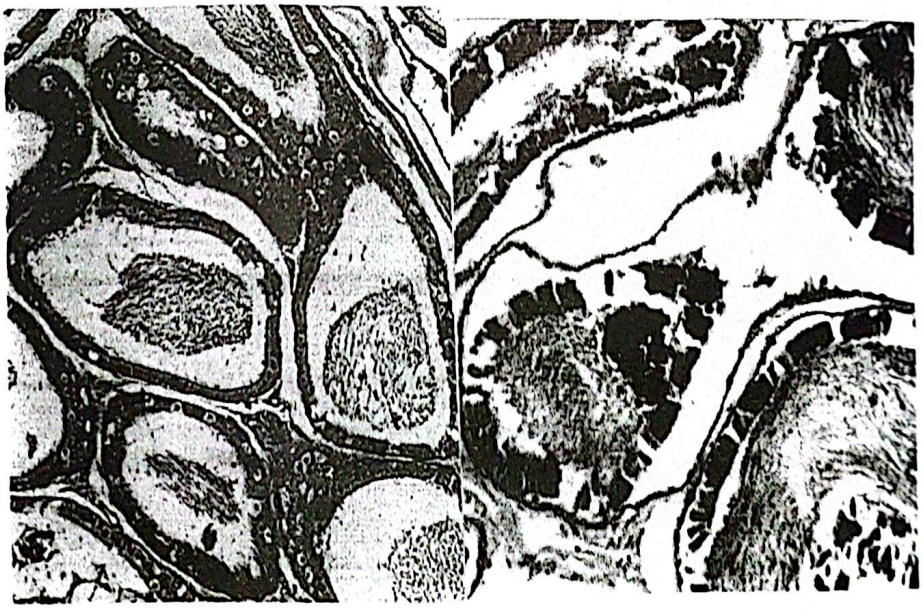

Fig (9): Epididymis of male rat intoxicated with 1/10 LD_{50} for 1 month showing:

A- vacuolation of the epithelial linings of the epididymal ducts with few mononuclear cell infiltration in the interstitial tissue.

B- Necrosis and exfoliation of the epithelial linings of the epididymal ducts into the ductal lumina.

(A and B H & E AX 160. BX 400)

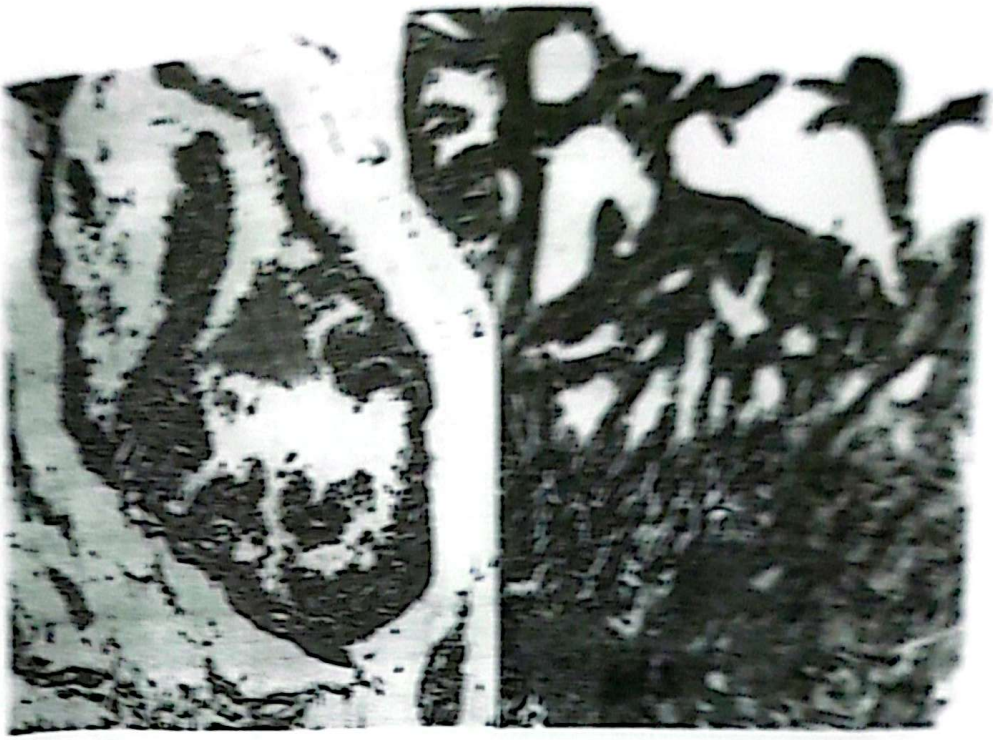

Fig (10): Prostate gland and seminal vesicle of male rat intoxicated with 1/10 LD_{50} for 1 month revealing:

A- Focal degeneration of the epithelial linings of the prostatic acinus.

B- Necrobiotic changes of the epithelial linings of some alveoli and mononuclear cell infiltration in the interstitial tissue.

(A and B H & E. X 160)

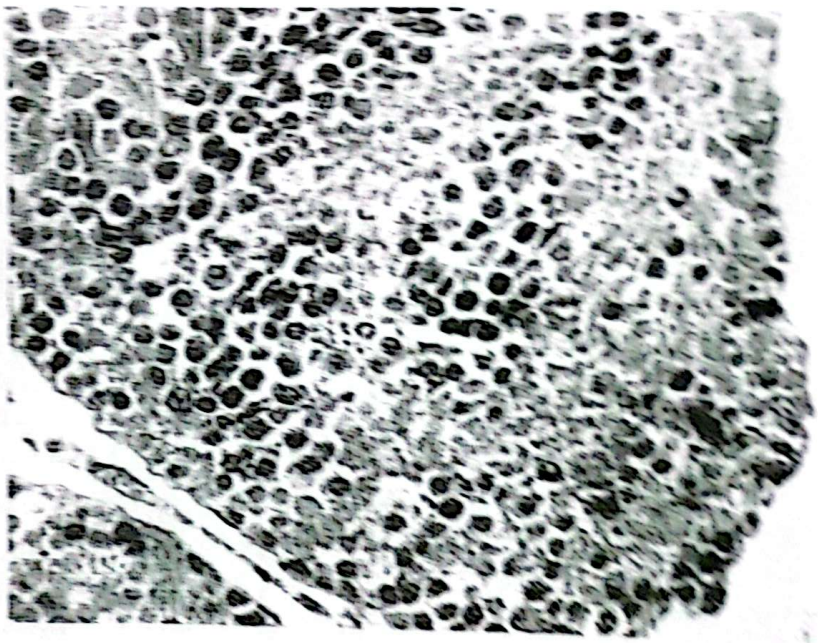

Fig (11): Testis of male rat intoxicated with 1/20 LD_{50} of thallium nitrate for two months revealing absence of radial orientation and severe necrotic changes of the spermatogenic epithelium which desquamated and obliterated the tubular lumen: (H & E. X 400)

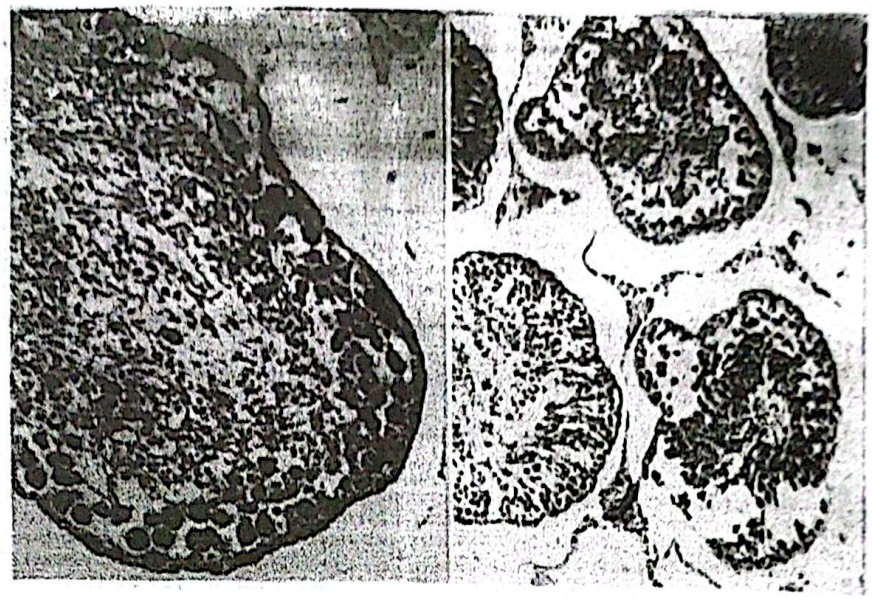

Fig (12): Testis of male rat intoxicated with 1/20 LD_{50} for two months illustrating:

A- Reduced numbers of the spermatogenic epithelium and maturation arrest of spermatogonial cells.

B- Appearance of large spaces or vacuoles in the spermatogenic epithelium with irregular contorus of the basal lamina.

(A and B H & E. AX 400, BX 160)

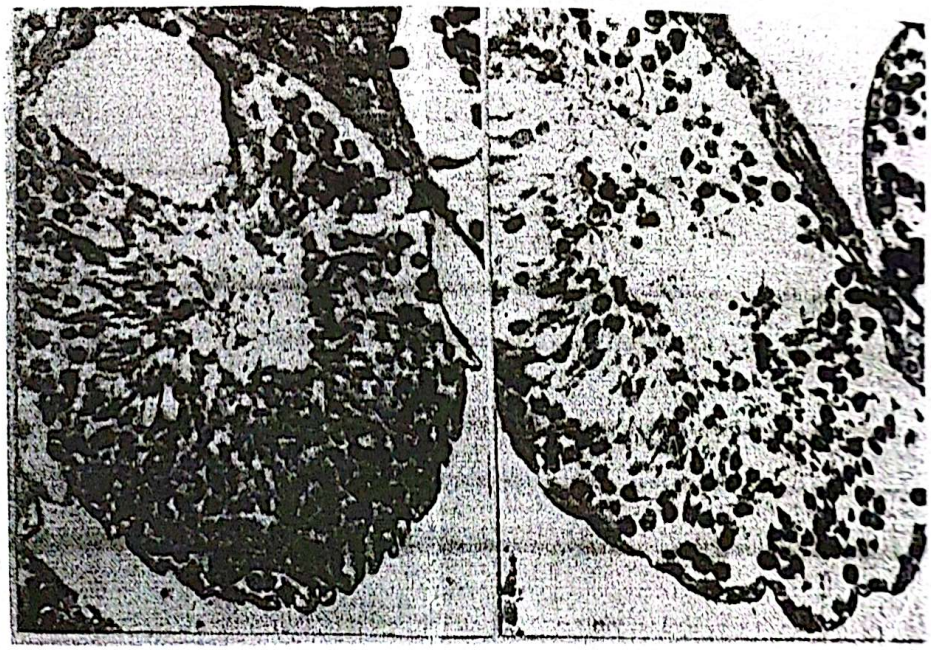

Fig (13): Testis of male rat intoxicated with 1/20 LD_{50} for two months revealing:

A- Large vacuoles retained deep in the spermategenic epithelium.

B- Decreased cellularity with total exfoliation of germ cells into the tubular lumena with hyaline thickening of the basale lamina.

(A and B H & E. X 400)

Fig (14): Testis of male rat intoxicated with 1/10 LD_{50} for two months revealing:
A- Spermiostasis in the lumen of the seminiferous tubules.
B- Spermiostasis in the intratesticular rete testes beneath the tunica albuginea with focal deposition of calcium salts (calcinosis).
(A and B H & E X 160)

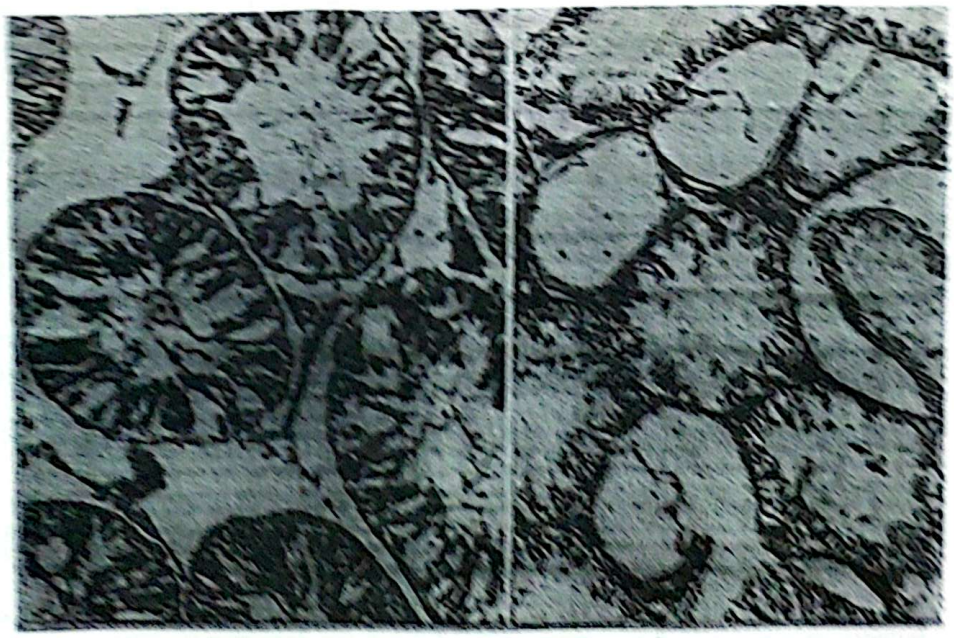

Fig (15): Testis of male rat intoxicated with 1/10 LD_{50} for two months illustrating:
A- Atrophy of the seminiferous tubules where they were lined only by Sertoli cells and few germ cells.
B- Most of the seminiferous epithelium disappeared and the seminiferous tubules were lined by remnants of germinal epithelium.
(A and B H & E X 400)

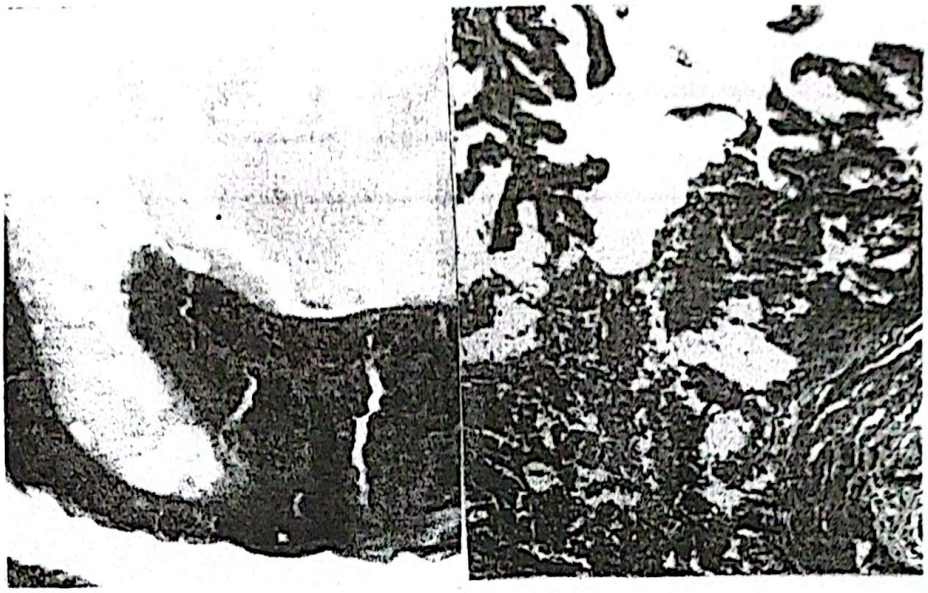

Fig (16):

A- Prostate gland of male rat intoxicated with 1/10 LD_{50} for two month showing papillary hyperplasia of the epithelial linings the prostatic acinus.

B- Seminal vesicle of male rat intoxicated with 1/10 LD_{50} for two months demonstrating extensive haemorrhage in the lumen of seminal vesicle lobules and alveoli.

(A and B H & E. X 160)

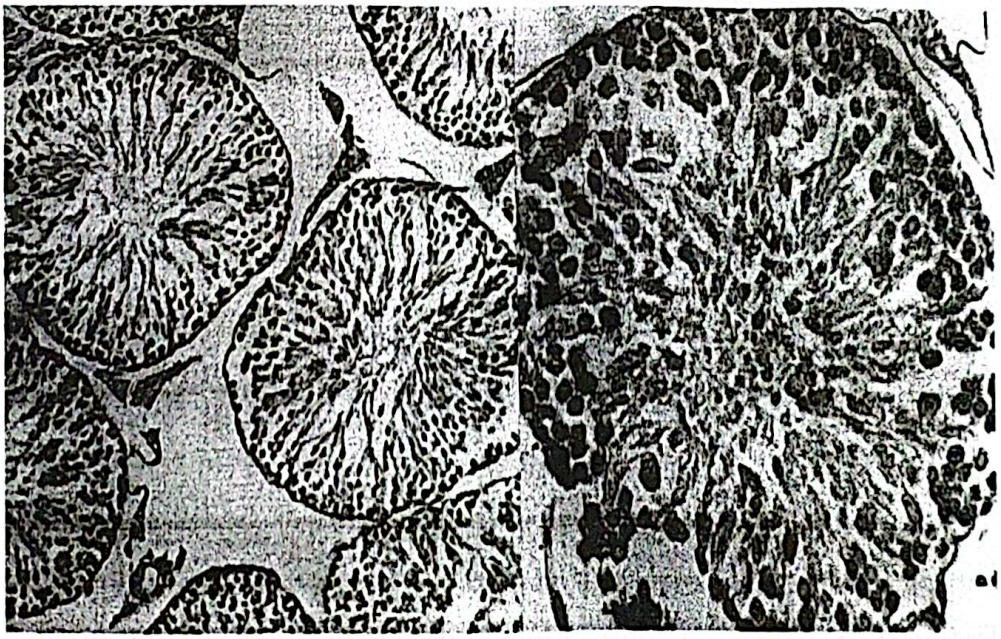

Fig. (17): A- Testis of normal male rat revealing lack of P_{53} protein expression.

B- Testis of male rat intoxicated with 1/10 LD_{50} for two months revealing mild to moderate cytoplasmic and nuclear immune-reactivity for P_{53} protein expressions in the degenerated spermatocytes and round spermatids. (Avidin biotin complex method counterstained hematoxylin A x 160. B x 400)

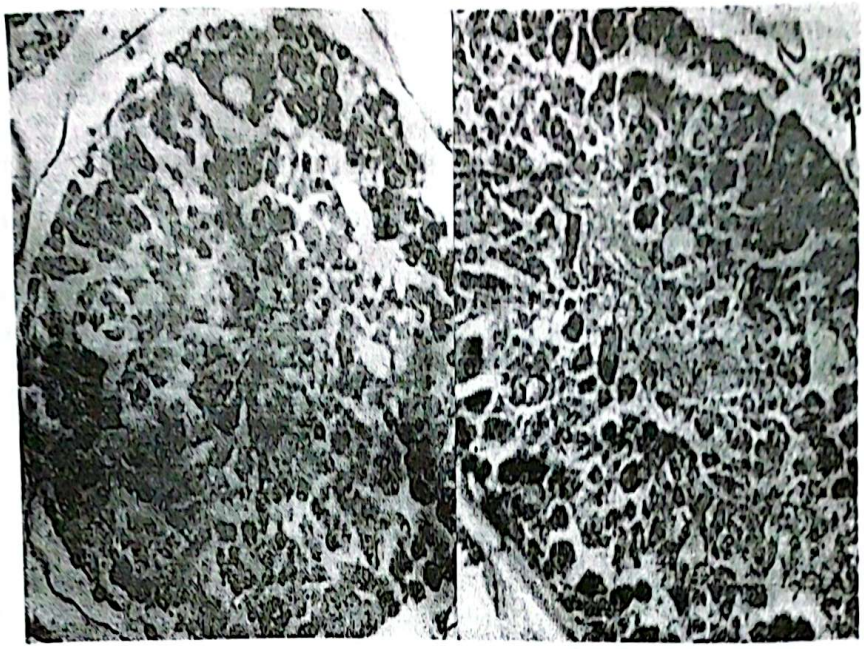

Fig. (18): Testis of male rat intoxicated with 1/10 LD_{50} for two months illustrating:
A- Cytoplamic immunoreactivity for P_{53} protein for Sertoli cells and other degenerated germ cells.
B- Diffuse cytoplasmic immunoreactivity for P_{53} protein in exfoliated necrotic germ cells.
(A & B-Avidin biotin complex method counterstained hematoxylin X400).

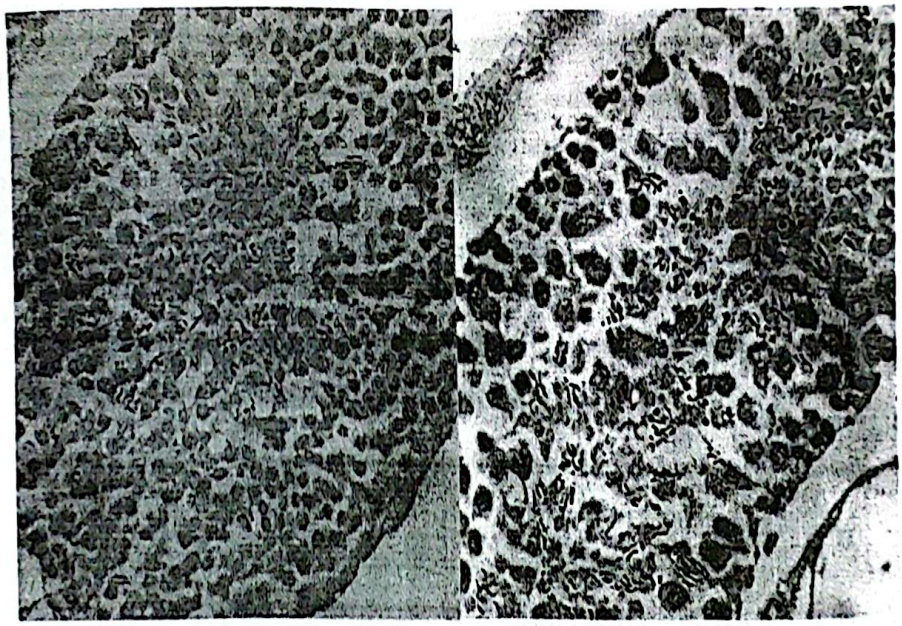

Fig. (19): Testis of male rat intoxicated with 1/10 LD_{50} for two months revealing intense immunoreactivity of denuded necrotic rounded and elongated spermatids.
(A& B- Avidin biotin complex method counterstained hematoxylin x 400).

DISCUSSION

Thallium is considered one of the most important environmental pollutants in industrial areas as it has adverse effects on human, animal and plants (WHO, 1996 and Schäfer et al., 1999).

Thallium is known to be responsible for severe neurological manifestations in human (Arzate et al., 2000) and affects all organs as it has direct cytotoxic effects as well as indirect effects through damage of the nervous system (WHO, 1996). It distributes in all organs in a time dependent manner where maximal concentrations occurs in kidneys with equal levels in brain and testes (Talas and Wellhner, 1983 and Arzate et al., 2005).

On the other side, Aoyama (1989) pointed out that repeated treatment with thallium salts resulted in similar or even higher concentrations of thallium being found in testes compared to other organs.

Thallium toxicity is usually related to alterations in glutathione- dependent antioxidant system where it leads to generation of reactive species and to oxidative stress in the central nervous system (Arzate et al., 2005). Moreover, Majno and Joris (1996) and Jones et al. (1997), attributed damage of toxic agents to free radicals liberations such as O_2 which is capable of peroxidation of cells and organelle membranes, inactivation of cellular enzymes and damage to DNA.

This hypothesis is supported by Schäfer et al. (1999), who assumed that thallium ions oxidizes glutathione enzymes and the reaction takes place in mitochondrial compartments and cause mitochondrial damage.

Halestrap et al. (2000) related damage of mitochondria under the effect of many insults to the fact that mitochondria posses a latent pore which produce non- specific increase in permeability known as mitochondrial permeability transition pore (MPTP) and it thought to play a critical role in both necrotic and a poptotic cell death.

The present study exported out that intoxication of male albino rats with thallium salts induced dose and time dependent tissue alterations in male reproductive system since thallium ions tends to accumulate in mammalian tissues after continuous exposure (Schäfer et al., 1999).

In the current work, the acute oral LD_{50} of thallium calculated as 15.49 mg/kg B.wt. of thallium nitrate in male albino rats. Similar result was reported by Dieke and Richter (1946), who found that the oral LD_{50} of thallium sulfate to rats is 15.8 mg/kg.

The clinical symptoms observed were alopecia, weakness and gastrointestinal disturbance. These results were in agreement with Formigli et al.

(1986), Feldman and Levisohn (1993), Mulkey and Oehme (1993), Hoffman (2003) and Jha et al. (2006).

Also, in the present work, male fertility was studied in albino rats intoxicated with thallium (0.78 and 1.55 mg/kg b.wt.) alone or in combination with potassium containing diet (0.36 %) for 2 months in comparison to -ve control as well as +ve control (potassium chloride treated group).

The results revealed that thallium induced adverse effects in male reproduction in all thallium treated rats with or without potassium, whereas animals received thallium with potassium showed lesser trend. The effect was dose and time dependent and the most affection appear in group (B) which given 1/10 LD_{50} thallium.

These adverse effects (Table, 2) were in the form of a significant decrease in testicular weights; with an increase in the weights of seminal vesicles and prostate glands compared to control groups. The decrease in testes' weights may be due to direct destructive effect of thallium on testes.

Thallium poisoning has been reported to produce testicular atrophy in laboratory animals (Truhaut, 1958 and Jones et al., 1997). Thallium has been shown to accumulate in rat testis (Sabbioni et al., 1980 and Aoyama, 1989), and rat epididymis (Lameijer and Van Zwieten, 1977).

Spermatozoal examination (Table, 3) revealed a significant decrease in sperm cell count, percentage of motile and live sperms with increases in sperm cell abnormalities in comparison to controls. These observations could be regarded to the destructive effect of thallium on testes which confirmed the histopathological findings.

Significant decrease in sperm motility was observed in all thallium treated rats after 60 days of thallium exposure, this may reflect a direct action of thallium on the testis and/or post-testicular changes related to the accumulation of spermiotoxic thallium levels in the excretory pathways of the reproductive system (Formigli et al., 1986).

The obtained results were confirmed by the histopathological studies which revealed that thallium inhibits spermatogenesis and testicular function due to degeneration and desquamation of germinal layers of seminiferous tubules and degenerative changes of the Sertoli cells. While the observed increase in seminal vesicles and prostate glands weight could be attributed to oedema and hyperplasia.

The result of poisoning of thallium treated male albino rats alone or with potassium in serial mating experiment (Table, 4) revealed various effects on maternal and fetal values such as decreased number of implantation sites /litter, alive and dead feti percent, fetal body weight and lengths, while displayed a significant increase in

number of resorption, percent of mal formed feti, meanwhile, placental weights showed non significant variations.

These results could be attributed to sperm cell abnormalities which are usually taken as characteristic criteria that can be applied for monitoring the mutagenic potential for many chemicals (Brusick, 1980).

Also, the present study showed a marked decline in fertility index in the form of decreased pregnancy percent, high incidence of abortion percent occurred in thallium treated animals in respective to controls. The depressing action of the tested compound on fertility index may be due to reduction of sperm motility and lowered sperm cell concentration, whereas the increased sperm cell abnormalities may have left them incapable for fertilization in addition to the weakness state which was observed on animals.

Thallium and its salts were considered as potential testicular toxicants for animals where it brings about release of immature sperm cells in the semen and reduced life spane of offsprings after sublethal thallium poisoning of the fathers (Formigli et al., 1986; Gregotti et al., 1992 and WHO, 1996).

In histopathological findings in the group of rats intoxicated with 1/20 LD_{50} for 1 months, the most pronounced changes encomposed oedema, cytoplasmic vacuolation of the sertoli cells in some tubules with degenerative changes in the spermatogenic epithelium particularly spermatocytes and round spermatids.

The appearance of faintly eosinophilic oedema fluid in the interstitial tissue and in the lumen of some tubules is considered a common toxic response due to alterations in vascular permeability and Sertoli cell barrier (blood-testis barriers) as those described by Ladds, (1993), Haschek and Rousseux, (1998), Acland, (2001) and Troedsson and Madill (2004).

Moreover, Arbiser et al. (1997) pointed out that thallium may act on endothelial cells, where it exertes pleiotrophic effects on proliferation and cell shape. The detection of degenerative changes in the spermatocytes and round spermatids in association with sertoli cell vacuolation was in close resemblance to the results obtained by Formigli et al. (1986) and Gregotti et al. (1992) in male albino rats intoxicated with thallium. Since, the process of spermatogenesis is a dynamic process, it depends primarily on the specialized functions of the sertoli cells, which are central to the integrity of the seminiferous epithelium and the shape and profiles of its plasma membrane were important aspects of Sertoli cell interactions with germ cells as theorized by Haschek and Rousseux, (1998).

Moreover, Troedsson and Madill (2004) explored

out that Sertoli cells secret controlling factors that regulate germ cell differentiations and maturation.

Cytoplasmic vacuolation of the Sertoli cells is a primary morphologic event in thallium intoxicated male rats since it targets primarily the sertoli cells in the mechanism underlying testicular damage as described by Formigli et al. (1986), Gregotti et al. (1992) and WHO (1996).

There were degenerative changes of the spermatogenic epithelium (spermatocytes and round spermatids) with release of immature germ cells in association with cytoplasmic vacuolation of sertoli cells. These findings coincided with those reported by many authors (McEntee, 1990, Boorman et al., 1990; Ladds, 1993; Osweiler, 1996; Haschek and Rousseux, 1998 and Troedsson and Madill, 2004). They mentioned that impairment of the functions of the Sertoli cells may provide less nutrition, degeneration and less developmental support of the developing germ cells. The loss and exfoliation of immature, germ cells into the tubular lumina seems principally in attribute to retraction of the Sertoli cell lateral processes and loss of tenacious contact between Sertoli cells and germ cells (Haschek and Rousseux, 1998). Sertoli cells are commonly thought of as supportive cells within the seminiferous tubules provided a multitude factor required for spermatogenesis (Troedsson and Madill, 2004). Moreover, Ladds (1993) cleared out those degenerative changes of the Sertoli cells (cytoplasmic vacuolations) are usually preceed germ cell degeneration.

The degenerative changes of the spermatocytes characterized by deeply eosinophilic cytoplasm, karyopyknosis of the nuclei. Meanwhile, degeneration of round spermatids manifested by peripheral condensation of the nuclear chromatin giving the picture of round ring nuclei.

These results are in accordance to the features of testicular degeneration described by Formigli et al. (1986); Boorman et al. (1990); McEntee (1990); Ladds (1993); Acland (2001) and Troedsson and Madill (2004).

Moreover, in the group of rats intoxicated with 1/10 LD_{50} for one month. The same histological changes were observed but in a more severe manner. These findings are in agreement to those reported by Talas and Wellhner (1983) and Aoyama (1989) who mentioned that thallium intoxication is a dose dependent process.

One of the most important findings in this group was the detection of multinucleated syncytial giant cells in some seminiferous tubules which can be regarded as a pathognomic entity of testicular degeneration by McEntee (1990) and Haschek and Rousseux (1998).

The occurrence of multinucleated giant cells is usually as a result of coalscence of groups of

degenerated round spermatids and occasionally spermatocytes as described by Haschek and Rousseux, (1998).

Moreover, Boorman et al. (1990) thought that giant cells resulted from inability of the spermatocytes or spermatids to maintain the constriction of the intercellular bridges that connect them. Furthermore, these findings are supported by Formigli et al. (1986) and WHO (1996) who stated that thallium posses antimitotic properties. There were various pathologic entities of the basale lamina of the seminiferous tubules such as irregular contorus, undulation or bucklings which represented prominent features of testicular damage (Ladds, 1993). They occur mainly due to disruption of Sertoli cell functions which provide structural support of the germ cells as well as to germ cell affections where the affected tubules reach its full size then it frequently collapse as described by Acland (2001). The recognition of misshaped necrotic spermatids retained deep in spermatogenic epithelium adjacent to the periphery of the tubules was supported by the explanation of Boorman et al. (1990) and Haschek and Rousseux (1998) who stated that these necrotic spermatides may be phagositized by the Sertoli cells. Moreover, a true pathologic entity in this group is moderate stasis of sperms in the rete testis beneath the tunica albuginea admixed with immature germ cells which regarded as an important feature of testicular affections by Mc Entee (1990); Ladds (1993) and Troedsson and Madill (2004).

In the group of rats intoxicated with 1/20 LD_{50} for 2 months, the most pronounced changes were maturation arrest of the spermatogonia at the stage of spermatocytes, since the process of spermatogenesis encomposing a series of successive mitotic divisions of spermatogonial cells (Haschek and Rousseux, 1998) and thallium posses antimitotic properties (Formigli et al., 1986) therefore, maturation arrest of spermatogenia developed where it failed to progress to full maturation.

One of the most important findings in this group was the appearance of circumscribed large vacuoles and /or spaces in the spermatogenic epithelial cells (focal germ cell depletion).

These vacuoles and/ or large spaces represented populations of germ cells underwent necrotic changes and phagositized by the Sertoli cells with no evidence of cell death other than absence of affected germ cell populations as suggested by Haschek and Rousseux, (1998). Moreover, Mc Entee, (1990) and Boorman et al. (1990) considered these pictures as an important signs of advanced testicular degeneration.

In the group of rats treated with 1/10 LD_{50} for two months, the testes exhibited severe changes manifested by extensive necrosis and total exfoliation of germ cells in some seminiferous tubules

spermiostasis in other seminiferous tubules and the testes with focal calcification in one case and atrophied tubules with severe loss of germ cells. These findings are remarkable lesions of advanced testicular damage as those mentioned by Jones et al. (1997 and Acland, (2001) and focal calcification is usually seen as sequence to spermiostasis and necrosis of spermatids.

The epididymis in all groups exhibited necrobiotic changes in the epithelial linings of the epididymal ducts, a finding which observed in association with chemical toxicants as mentioned by Haschek and Rousseux, (1998).

Moreover, the appearance of immature germ cells in the epididymal ducts is usually related to disturbance in the process of spermatogenesis (Boorman et al., 1990).

The epididymis of male rats intoxicated with 1/10 LD_{50} for 1 month revealed focal mononuclear cell infiltration in the interstitial tissue of the epididymis as well as vacuolation of the epithelial linings of the epididymal ducts. These results are in accordance to Acland (2001) who mentioned that degenerative changes of the epididymis may be associated with inflammatory response.

The detection of degenerative changes in the epithelial linings of the prostate and seminal vesicles in male rats intoxicated with thallium salts for 1 month were in accordance to Schäfer et al. (1999) who showed that thallium and its salts targets primarily the epithelial structures.

The histopathological findings in the prostate in the group of rats intoxicated for two months with 1/10 LD_{50} were papillary hyperplasia of the epithelial linings of the socretory acini. Ladds, 1993 stated that acinar hyperplasia is usually occurring due to alterations in the androgen estrogen ratio. Furthermore. Jones et al. (1997) believed that estrogen secreted in the testes has syngergestic effect with 5α dihydrotestesterone in indudtion of prostatic acinar hyperplasia.

Acland (2001) attributed prostatic hyperplasia to inflammation that is located primarily in the interstitial tissue. These findings augmented our results where mild inflammatory reaction was noticed in the prostatic interstitial tissue in most intoxicated rats.

The appearance of extensive haemorrhage in the seminal vesicle lobules and alveoli in the group of rats intoxicated with 1/10 for two months are parallel to the results of Arbiser et al. (1997) who mentioned that thallium may affect endothelial cells.

In the group of rats treated with thallium and potassium with 1/20 and 1/10 LD_{50} for 1 month showed relatively reduction in the testicular lesions in comparison to the group of rats intoxicated with thallium only.

These findings accentuate the hypothesis of many authors (Sabbioni et al., 1982; Leloux et al., 1990; Rios and Noyola, 1992; Barroso- Moguel et al., 1994; Meggs et al., 1997; Mulkey and Oehme, 1998 and Rusyniak et al., 2003) who showed that potassium and its salts decrease thallium concentration in different organs and beneficial in the treatment of thalltoxicosis.

However, Schäfer et al. (1999) assumed that potassium prevents the intestinal absorption of orally administrated thallium where it binds thallium ions after intestinal secretion.

Immunohistochemical demonstration of P_{53} protein in both normal and intoxicated rat testes were performed since P_{53}, protein mediates apoptosis during spermatogenesis by controlling germ cell numbers and eliminates defective germ cells to facilitate testicular homeostasis (Abou Donia et al., 2003). P_{53} protein under normal condition exists in largely inactive state that is inefficient to bind DNA (Offer et al., 2002). The present study cleared out that there were detectable expressions of P_{53} protein in the cytoplasm and nuclei of degenerated germ cells (spermatocytes and round spermatids) and Sertoli cells in the testes of intoxicated rats in comparison to normal rats' testes, which showed lack of expressions of P_{53} protein.

These results were in agreement with Lakin and Jackson (1999); Offer et al. (2002) and Abou Donia et al. (2003), who explored out that activation of P_{53} protein occur in response to a number of cellular stresses like DNA damage generating by many insults such as chemical toxicity. Abou Donia et al. (2003) showed that the reactive oxygen species generated by chemical toxicants is a powerful inducer of P_{53} protein. Moreover, the intense immunoreactivity in the denuded necrotic germ cells of the seminiferous tubules are in correlation with those mentioned by Siliciano et al., (1997) and Offer et al. (2002) who concluded that P_{53} protein were activated following genotoxic stress which stabilize and activate P_{53} protein by phosphorylation of its amino terminus and P_{53} protein level is determined by the level of accumulated DNA damage.

In conclusion, the present study indicates that thallium has a direct effect on male fertility as well as reproduction, therefore exposure of farm animals to such element through living in industrial places must be minimized, potassium chloride as antidote reduce the deleterious effects of thallium, but it couldn't give complete protection in animals.

Due to the fascinating chemistry and high toxicity potential that make thallium and its compounds of particular scientific interest and enviromental concern, it is recommended to minimize the use of thallium and using various treatment options and removal technologies to protect the environment from thallium toxicity and avoid its toxicity to animals and humans.

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