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Assessment of Fertility and Mating Capabilities in Adult Male Rats after Single Intra-Testicular Injection of Formalin Solution

[1]Al-Omari Osama, [2]Khamas Wael and [1]El-Beteiha Ahmed
[1]Department of Applied Biological Sciences, Faculty of Science and Arts,
Jordan University of Science and Technology, P.O.Box 3030, Irbid, 22110, Jordan
[2]Department of Basic Medical Sciences, School of Veterinary Medicine,
Purdue University, West Lafayette, IN 47907, USA

Abstract: Eighty adult male Sprage-Dawley rats, assigned into groups (5 in each) and given one dose (50 μL) of different concentrations of formalin solution (2.5, 5 and 10%) intratestically. Weight, food and water consumptions were monitered. Animals were maintained in a controlled atmosphere 21°C±1°C under 12 hrs light: 12 hrs darkness schedule. Paraformeldehyde, prilled 95% obtained from Aldrich Chemical Company (Milwaukee, WI 53233, USA) was dissolved in water and different concentrations were prepared. One and two months post injection animals were kept for ten days for mating with virgin untreated females to evaluate mating capability of treated rats. Animals were sacrificed and the following organs were collected for evaluation: testes, epididimidis, seminal vesicles, preputial glands, liver and kidneys. Testes and epididymidis were cut in halves, one half was placed in 10% neutral buffered formalin and processed following standard histological procedures. Formalin injection intratestically did not result in any apparent changes on condition or behavior of the treated rats. However, reduction in size and weights of the testes, epididymidis and seminal vesicles were obvious. Also, bilateral injection resulted in impairment of mating capabilities for all concentration used. These changes were less pronounced in unilateral groups. Sperm counts decreased in testes and epididymidis in all treated groups. Histological changes appeared to be more severe close to the locus of injection with the seminiferous tubules filled with connective tissue and sperm debris. The degree of changes beyond the central lesion showed poor structural status having one or two cell layers with no sperm inside the lumen or very few depending on the concentration of the formalin used. Intact and normal histological architecture were observed at the periphery of the testes and much larger number in the 2.5% group. Intertubular connective tissue thickened and cells were replaced with collagenous tissue especially in testes injected with 10% formalin solution. Epididymidis were devoid of sperms in their lumen. Besides, vacuoles were observed within the epithelium of the epididymis. No histological changes were observed within the liver and the kidneys of all groups. This study showed that a single dose intratesticular injection of formalin solution has as adverse effect on the reproductive system of the male rats. This experiment could be modified and used as a new method of chemical castration especially if it is used in young animals (before puberty).

Key words: Fertility, male rats, intra-testicular, formalin

Introduction

Formalin is one of the most widely used and produced as a byproduct in industry. Millions of pounds are produced and consumed in the industrialized world annually. It was first described by early researchers while attempting to prepare methylene glycol. Monographs succeeded to further describe its chemical nature and recruitment in vast industrial applications. Formaldehyde is the smallest member of the aldehyde series (HCOH), in which it contains only one carbon atom, described as colorless flammable gas with a strong pungent suffocating odor. It is the most reactive compound of those containing one carbon atom and of the aldehydes as well, polymerizes with itself spontaneously and undergo many reactions like condensation, oxidation and reduction. It is known by other names like formalin liquid and paraformaldehyde, which is the solid polymer of the compound (Gerberich *et al*,, 1980).

Formaldehyde is used to produce synthetic resins and adhesives by reaction with phenols, urea and melamine. It is also used in textiles, dyes, drugs, paper, leather, photographic materials, embalming agents, disinfectants and insecticides (Madestau, 1987). It is used as both preservative and fixative of anatomical, histological and pathological specimens. General population is also subject to several commercial products that contain formaldehyde in alarming quantities like cosmetics, cigarettes, fertilizers and food preservatives (Feinman, 1988a).

Formaldehyde ingestion or inhalation resembles systemic exposure and intoxication, which have been extensively studied through extensive research and case studies. A topic or local exposures were mostly prone to skin, where formaldehyde is well known to cause allergic contact dermatitis and manifest unpleasant symptoms and pathologic lesions (Imbus, 1985, Feinman, 1988b and Restani *et al*., 1992).

This study was designed to investigate the effect of single intra-testicular injection of formalin solution on the adult male rat mating capabilty and fertility.

The following parameters were monitered recorded:

- General health and condition.
- Body weights, water and food consumption.

- Mating capability and fertility.
- Testicular and epididymal sperm counts.
- Male reproductive organs weights including; testes, epididymidis, seminal vesicles (stripped of seminal fluid) and preputial glands and
- Histological evaluation of the testes, epididymidis, liver and kidneys.

Materials and Methods

Experimental animals: Sprague-Dawley male and female rats age 4 to 5 months were chosen to serve as animal models for the context of this experiment. Rats were provided by the animal house unit, Faculty of Medicine, Jordan University of Science and Technology. They were maintained in a controlled atmosphere 21°C±1°C under 12 hrs light: 12 hrs darkness schedule. Food and tap water were obtained from the facility and provided *ad libitum*. Animals appeared to be healthy and normal with regard to condition and behavior. Before the beginning of experimentation, three rats were selected randomly and killed for gross examination of internal organs. No abnormalities or disorders of any kind were detected.

Test substance: Paraformaldehyde, prilled 95% was obtained from Aldrich Chemical Company (Milwaukee, WI 53233, USA). Paraformaldehyde was dissolved in normal saline (0.9% Nacl) by continuous stirring and heating at 80°C until the solution was clear. Different dilutions were then prepared, 2.5, 5 and 10% w/v, for intra-testicular injection.

Number and grouping of animals: Eighty male rats were assigned for the experiment. They were caged five animals per cage. For each concentration, animals were injected with formalin solution inside the testes unilaterally (right testis only) or bilaterally (right and left). Animals were kept for one and two months under observation (Table 1). Additional 80 healthy virgin untreated females were used for evaluation of fertility and mating capabilities of the treated males.

Table 1: Formalin concentrations used for intra-testicular injection and the observation periods

Formalin concentration (w/v)	Observation period: 40 days		70 days	
	R	R and L	R	R and L
0%	5*	5	5	5
2.5%	5	5	5	5
5%	5	5	5	5
10%	5	5	5	5

R; Right, L; Left, * Number of animals in the group

Table 1: Effect of intra-testicular injection of formalin on fertility of adult male rats. Each male was mated with untreated healthy female

Formalin concentration (w/v)	Bilateral injection		Unilateral injection	
	40 days	70 days	40 days	70 days
0%	4/5[†]	4/4	4/4	5/5
2.5%	1/5*	0/5**	4/4	3/5
5%	0/5*	0/5**	2/5	2/4
10%	1/5*	0/5**	3/5	2/5

[†]Data expressed as number of pregnant females/ number of mated females, *P<0.05, **P<0.01 (Fishers exact test)

Injection protocol: Fifty µl formalin solutions of 2.5, 5 and 10% were injected in the rats right and / or left testes once at the beginning of the experiment, using insulin syringes (Gauge 27). Control groups were injected with the same volume of sterile normal saline. Injection was performed under light ether anesthesia. Skin, at the site of injection, was thoroughly and gently cleaned with 70% alcohol before injection. All solutions were delivered to one locus in the center of the testes.

Observation period: Animals were kept under observation for general condition and behavior after injection for a maximum period of two months. They were weighed throughout the experiment on a weekly basis. Food and water consumption were calculated every day for the first week and then three random days per week for the remaining period.

Evaluation of fertility and mating capability of treated rats: After one and two months post-injection, animals were kept for ten more days for mating. Each animal was placed in an individual cage with a virgin untreated healthy female of the same strain. They were left together for ten days during which, two estrus cycles should have elapsed. The day in which male and female rats were placed together was considered day zero.
Males were removed and sacrificed by cervical dislocation under light ether anesthesia for further analysis at day ten. Females were sacrificed at day 20 using the same mentioned procedure. The uteri were examined and number of pregnant females were recorded.

Organ weights and sperm counts of treated male rats: At the end of the mating experiments (day 40 and 70 post-injection) male rats were sacrificed for autopsy as mentioned above. The following organs were excised and weighed: right and left testes, right and left epididymidis, seminal vesicles (stripped of fluid) and preputial glands.
Excised left and right testes and epididymidis were cut in halves. One half was immediately placed in 10% neutral buffered formalin solution for histological examination and the other half was placed in normal saline (0.9% Nacl) and was used for sperm counts.
Sperm counts procedure was performed according to Amann and Lambiase (1969) using a hematocytometer chamber. The homogenate was diluted with normal saline, placed in a screw-cap tube, mixed using a vortex and aliquot was placed in a hematocyometer chamber.
Testicular sperm counts and epididymal sperm counts were expressed as counted number per gram of weighed organ. The estimates of daily sperm production (DSP) per testis and per gram of testis per day (Efficiency) were calculated based on a factor of 6.1 (F_d) which is the duration of a seminiferous cycle during which developing spermatozoa are in the spermatid stage (Amann *et al*, 1976).

Histological evaluation of the testis, epididymis, kidney and liver: The excised testis, epididymidis, liver and kidney were prepared for histological analysis using standard techniques and all tissues were stained with hematoxylin and eosin. Further, testicular tissues were stained with and Van Gieson stain (Bancroft and Stevens, 1990).

Statistical analysis: Data were analyzed on IBM compatible PC computer using Microsoft Excel 97, STATMOST and SPSS programs. Statistical differences were determined between control and treatment groups using t-test. Fisher exact test was used to determine statistical significance in number of pregnant females.

Results
Single intra-testicular injection of formalin solution: Formalin injection did not result in any apparent changes on condition, or behavior of the treated rats. Body weights, water and food consumption of experimental rats did not differ from rats injected with normal saline. Upon gross examination of the internal organs, no changes were seen except for the testes and epididymidis in which they showed reduction in size in different concentrations used (Fig. 1). Injected testes showed dominating white areas as well as congested blood vessels at the locus of injection or around it. In few cases, a swelling of the scrotum caused by edema was also observed.

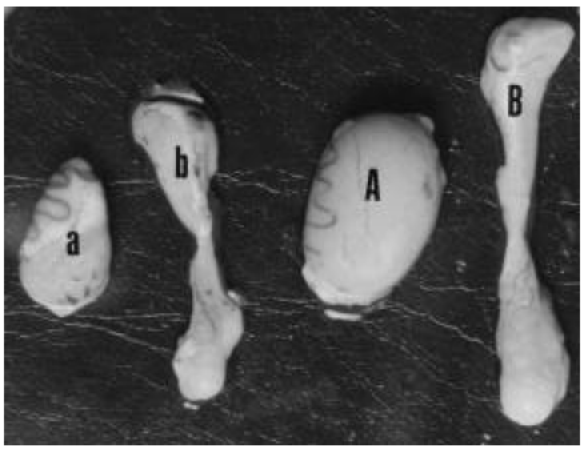

Fig. 1: Testes and epididymidis of rats injected intratestically with 50 µL of 10% formalin solution compared with the control injected with normal saline solution. Notice the differences in size of organs and also the white coloration on the treated testis due to the effect of formalin injection. (a) Treated testis. (b) Treated epididymis. (A) Control testis. (B) Control epididymis

Table 2: Sperm counts in the right testis after 40 and 70 days of 50 µL formalin injection unilaterally inside the right testes

Formalin Conc.(w/v)	Days after injection	Testis weight (g)	Total sperm /testis (x10^6)	Sperm/g testis (x10^6)	Sperm/testis/Fd (x10^6, DSP)	Sperm/gtestis/Fd (x10^6,Efficiency)
0%	40	1.61±0.21 [a]	103.09±14.99	64.07±5.89	16.90±2.46	10.50±0.96
	70	1.58±0.13	67.13±28.03	41.51±14.42	11.01±4.60	6.81±2.36
2.50%	40	0.80±0.25***	41.50±11.16****	55.01±16.09	6.80±1.83****	9.02±2.64
	70	0.97±0.18***	59.42±16.72	61.38±14.83*	9.74±2.74	10.06±2.43*
5%	40	0.65±0.25***	33.41±33.67**	45.70±28.50	5.48±5.52**	7.49±4.67
	70	0.70±0.30***	58.88±47.03	78.78±42.58	9.65±7.71	12.92±6.98
10%	40	0.72±0.07****	75.78±29.38	105.56±41.66*	12.42±4.82	17.31±6.83*
	70	0.53±0.15****	60.23±19.57	171.95±36.84****	14.64±3.21	28.19±6.04****

DSP Daily Sperm Prodcution, [a] Results are expressed as mean±standard deviation, *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001(student t-test), Fd = 6.1 in rats, The duration of a seminiferous cycle during which developing spermatozoa are in the spermatid stage

Table 3: Right epididymal sperm counts after 40 and 70 days of unilateral injection of 50 µL formalin solution inside the right testes

Formalin Conc. (w/v)	Days after injection	Epididymal weight (g)	Total sperm/ Epididymis	Sperm/ g Epididymis
0%	40	0.59±0.07[a]	228.44±51.56	393.56±90.47
	70	0.54±0.04	188.11±54.98	352.27±121.60
2.50%	40	0.35±0.05***	31.57±22.69****	83.42±57.91***
	70	0.42±0.09**	78.22±41.22**	179.52±82.00*
5%	40	0.27±0.10***	10.01±21.81****	30.09±64.74***
	70	0.25±0.07****	0.99±1.42***	4.42±6.64***
10%	40	0.31±0.03****	4.19±7.12****	13.04±21.98****
	70	0.32±0.02****	0.27±0.24****	0.86±0.80****

[a]Results are expressed as mean±standard deviation, *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001 (student t-test)

Weights of reproductive organs: Formalin injections have resulted in a significant decrease in testicular and epididymal weights, with respect to testes injected with normal saline or the untreated contralateral testes.
Rats injected unilaterally and bilaterally examined after forty days showed decrease in weights of testes and epididymidis (Table 2 and 3). Absolute and relative weights of the seminal vesicles were slighty decreased in rats injected with formalin; while preputial glands relative weights flactuate in rats injected with different concentrations of formalin.
Rats injected bilaterally and examined after seventy days showed a significant decrease in seminal vesicle glands weights with respect to the formalin concentrations used (2.5, 5, 10% formalin).
In general, unilateral injection of formalin did not cause a significant change in the contralateral left testes (the untreated testes) and epididymidis.

Mating capability of treated rats: Bilateral injections of formalin solution have resulted in impairment of the mating capability or dcreased fertilization rate of the treated rats for all concentrations after one and two months of injection. These changes were less pronounced in unilateral injection groups examined after one month of injection of 5 and 10% concentrations, while no statistical difference was observed in the 2.5% concentration group (Table 1).

Unilateral formalin injection
Sperm counts after forty day observation period: Data obtained from sperm counts of the right testis showed a significant decrease in sperm counts per whole testis and in daily sperm production (DSP) in testes injected with different concentrations of formalin (Table 2), however, testes injected with 10% formalin showed a significant increase in sperm counts per gram of testis which is reflected on a significant increase in the efficiency.
Sperm counts of the right epididymidis showed a sharp dose-related decrease in the sperm counts per epididymis and counts per gram of epididymis (Table 3). There were no changes in the sperm counts in the left (untreated) epididymis.

Sperm counts after seventy days observation period: Right testes injected with formalin resulted in a significant decrease in the sperm counts per gram of testis as well as total sperm/testis (efficiency). In testes injected with 5% formalin, spermatid counts per testis and DSP where significantly decreased. However, there were increase in sperm/g testis/Fd efficiency in most concentrations used (Table 2). For the left testes no significant changes on any tested parameter for all groups were detected.

Epididymal sperm counts performed on the right epididymidis showed a significant decrease in sperm counts per epididymis and sperm counts per gram epididymis for all groups in a dose related manner (Table 3).

Bilateral formalin injection

Forty days observation period: Injection with formalin of different concentrations resulted in a decrease in the total sperm counts/testis and in the DSP in the same testes (Table 4). While increase sperm counts/ testis as well as efficiency increased in most of the cases.

Right and left epididymidis showed a significant decrease in sperm counts/ epididymis and sperm count/g epididymis for all groups in a dose related manner (Table 5 and 6) respectively.

Seventy days observation period: Sperm counts in the right testes showed a significant decrease in the sperm count/ testis and DSP for rats injected with formalin for all concentrations. Rats injected with 10% formalin showed a significant increase in the sperm count/g testis and efficiency (Table 4).

Right and left epididymidis showed a significant decrease in sperm counts/ epididymis and sperm count/g epididymis for all groups in a dose related manner (Table 5 and 6) respectively.

Histological examination: The magnitude of the histological changes were related to formalin concentration used. Changes were observed within unilateral and bilateral injected testes with a higher magnitude appeared in the bilaterally injected testes. Rats testes injected with formalin showed a complete replacement of the cellular components with connective tissue in large number of the seminiferous tubules in and around the injection site (Fig. 2). Variable quantities of sperms and debris were detected within the tubules. These lesions either filled the entire section or dominated over large area surrounding the injection site in both 5 and 10% groups. Seminiferous tubules beyond these lesions showed poor structural status having only one or two cell layers and their lumen contain no sperms (Fig. 3). Intact seminiferous tubules with normal histological architecture were observed at the periphery of the testes and much larger number observed in the 2.5% group. These tubules appeared to be normal and similar to control testes histology (Fig. 4).

Table 4: Spermatid counts in the right testis after 40 and 70 days of 50 µL formalin injection inside the testes bilaterally

Formalin Conc. (w/v)	Days after injection weight	Testis testis ($x10^6$)	Total sperm/ testis ($x10^6$)	Sperm/ g ($x10^6$, Efficiency) (g)	Sperm/ testis/Fd ($x10^6$, DSP)	Sperm/g testis/Fd
0%	40	1.55±0.09 [a]	112.87±20.74	72.87±14.07	18.50±3.40	11.95±2.31
	70	1.53±0.25	110.15±29.37	71.67±13.30	18.06±4.87	11.75±2.18
2.50%	40	0.50±0.34****	62.48±56.68*	105.72±39.87	10.24±9.29*	17.33±6.54
	70	0.68±0.19***	51.72±12.84**	77.50±14.55	8.48±2.10**	12.70±2.39
5%	40	0.62±0.21****	35.23±12.10***	57.35±12.16	5.78±1.98***	9.40±1.99
	70	0.76±0.29**	64.59±1.78**	95.04±35.47	10.59±0.29**	15.58±5.82
10%	40	0.76±0.16****	81.74±17.57*	108.49±19.76**	13.40±2.88*	17.79±3.24**
	70	0.73±0.11****	82.34±29.49	112.38±35.97*	13.50±4.83	18.42±5.90*

[a]Results are expressed as mean±standard deviation, *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001 (student t-test)

Table 5: Right epididymal sperm count after 40 and 70 days of 50 µL bilateral formalin injection inside the testes

Formalin Conc. (w/v)	Days after injection	Epididymal weight (g)	Total sperm/ epididymis ($x10^6$)	Sperm/g epididymis ($x10^6$)
0%	40	0.50±0.05 [a]	201.70±47.85	399.03±75.56
	70	0.51±0.04	203.98±50.75	399.21±89.37
2.50%	40	0.23±0.15**	36.13±25.02****	154.71±5.01****
	70	0.26±0.05****	23.34±4.12****	95.08±28.08****
5%	40	0.33±0.07**	5.87±10.10****	14.74±24.77****
	70	0.31±0.12**	2.52±1.97****	6.01±6.40****
10%	40	0.35±0.03***	5.06±10.49****	14.48±27.01****
	70	0.37±0.10**	2.19±4.40****	7.83±16.41****

[a]Results are expressed as mean±standard deviation, *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001 (student t-test)

Table 6: Left epididymal sperm count after 40 and 70 days of 50 µL bilateral formalin injection.inside the testes

Formalin Conc. (w/v)	Days after injection	Epididymal weight (g)	Total sperm/ epididymis ($x10^6$)	Sperm/g epididymis ($x10^6$)
0%	40	0.53±0.06 [a]	167.38±34.58	317.49±68.08
	70	0.57±0.08	183.16±56.14	320.76±75.78
2.50%	40	0.25±0.12***	17.10±12.20****	72.67±34.85****
	70	0.29±0.05****	16.25±13.24****	52.48±41.45****
5%	40	0.36±0.08**	6.39±9.30****	15.64±19.84****
	70	0.34±0.06***	3.21±3.89***	9.89±12.48****
10%	40	0.36±0.01***	4.61±9.97****	12.17±26.29****
	70	0.34±0.07***	0.16±0.16****	0.45±0.44****

[a]Results are expressed as mean±standard deviation, *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001 (student t-test)

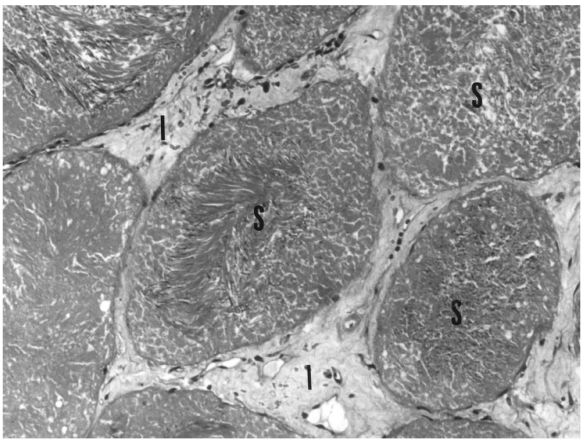

Fig. 2: Damages inflicted on the seminiferous tubules through the injection of 50 µL formalin solution (10%) inside the testis of a rat. Notice the amount of connective tissue between the tubules. (I) Interstitial connective tissue. (S) Seminiferous tubules with cells replaced by collagenous fibers. H and E stain. Mag. 205 X

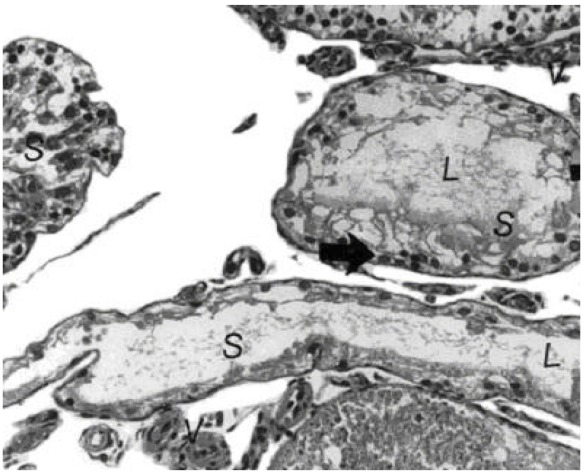

Fig. 3: Cross section of the seminiferous tubules (S) of injected testis with formalin solution (5%) showing variable levels of degeneration with congestion of the blood vessels (V) around the tubules. (L) Lumen. (Arrows) Basal cells. H and E stain. Mag. 205 X

Table 7: Sex organs weights after 40 days of 50 µL formalin injection in the right and left testes of male rats

Formalin conc. (w/v)	No. of males	Body weight(g)	Relative testis weight (g/ kg BW[†]) Absolute weight (g)[b] Right	Left	Relative epididymal weight (g/ kg BW) Absolute weight (g)[b] Right	Left	Relative and absolute seminal vesicles weight	Relative and absolute preputial glands weight
0%	5	378.2±30.5[a]	4.115±0.24 1.552± 0.09[b]	3.904±0.35 1.469±0.07[b]	1.336± 0.1 0.504±0.05[b]	1.403±0.16 0.529±0.06[b]	1.607±0.1 0.605±0.02[b]	0.448±0.08 0.169±0.03[b]
2.50%	5	295.4±60.5*	1.610±0.8**** 0.505±0.34****	2.538±1.03* 0.751±0.36***	0.731±0.38** 0.230±0.15***	0.818±0.28** 0.249±0.12***	1.511±0.2 0.439±0.06***	0.673±0.27 0.193±0.07
5%	4	407.8±20.4	1.512±0.44**** 0.333±0.07**	1.638±0.57**** 0.359±0.08**	0.811±0.14**** 0.600±0.12	0.882±0.2** 0.154±0.03	1.594±0.25 0.623±0.21****	0.378±0.07 0.2** 0.667±0.24****
10%	5	424.6±61.2	1.799±0.3**** 0.761±0.17****	1.924±0.22**** 0.808±0.06****	0.825±0.13**** 0.346±0.04****	0.865±0.14**** 0.360±0.01****	1.431±0.33 0.595±0.09	0.437±0.12 0.186±0.06

Results are expressed as mean±standard deviation, [a]Relative weights, [b]Absolute weights are expressed as mean±standard deviation, [†]Body weight, *P< 0.05. **P< 0.01. ***P< 0.001. ****P< 0.0001 (student t-test)

The intertubular (interstitial) tissue of injected testes was thickened and cells were replaced with collagenous tissue particularly in testes injected with 10% formalin and examined 70 days post injection.

All histological sections of the epididymis in rats injected with 5 and 10% formalin solution showed ductus epididymidis devoided of sperm reserves or extremely few within their lumen. This was observed, less severely, in rats injected with 2.5% formalin solution when compared to the control. Besides, apparent vacuoles in relatively high numbers were observed dwelling the epithelial lining of the epididymis (Fig. 5).

Table 8: Sex organs weights after 70 days of 50 µL formalin injection in the right and left testes of male rats

Formalin conc. (w/v)	No. of males	Body weight(g)	Relative testis weight (g/ kg BW[†]) Absolute weight (g) [b] Right	Left	Relative epididymal weight (g/ kg BW) Absolute weight (g) [b] Right	Left	Relative and absolute seminal vesisles weight	Relative and absolute preputial glands weight
0%	5	356.0±30.8[a]	4.263±0.34 1.525±0.25 [b]	4.791±0.82 1.698±0.27 [b]	1.433±0.3 0.510±0.04 [b]	1.602±0.28 0.566±	1.997±0.17 0.710±0.08 [b]	0.582±0.12 0.207±0.04 [b]
2.50%	5	332.2±54.9	2.026±0.29**** 0.680±0.19****	2.177±0.5**** 0.725±0.19****	0.766±0.05**** 0.255±0.05****	0.876±0.12**** 0.289±0.05****	1.480±0.03**** 0.492±0.09**	0.524±0.11 0.176±0.05
5%	4	378.3±51.2	1.965±0.54**** 0.760±0.29**	1.813±0.47**** 0.694±0.24****	0.818±0.28*** 0.312±0.12**	0.894±0.05*** 0.339±0.06***	1.644±0.15** 0.621±0.1	0.421±0.07* 0.158±0.02*
10%	5	374.2±41.0	1.962±0.37**** 0.727±0.11****	1.778±0.42**** 0.662±0.17****	0.973±0.18*** 0.366±0.1**	0.921±0.15*** 0.345±0.07***	1.472±0.33** 0.552±0.14*	0.361±0.10** 0.133±0.04**

[a]Results are expressed as mean±standard deviation, [b]Absolute weights are expressed as mean±standard deviation and shown in brackets, [†]Body weight, *P< 0.05, **P< 0.01, ***P< 0.001, ****P< 0.0001 (student t-test)

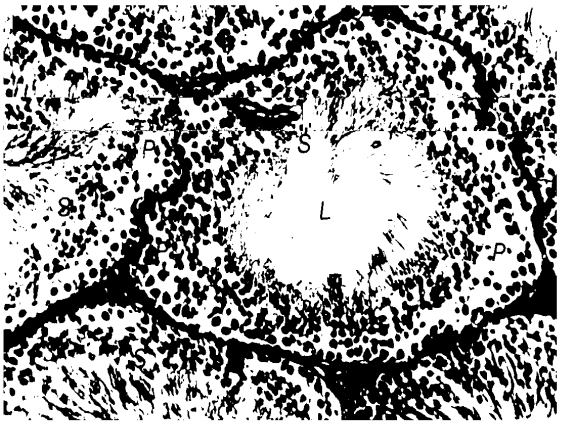

Fig. 4: Control rat testis injected with normal saline solution showing normal distribution of cellular elements with little interstitial connective tissue (I). The lumen is full of spermatids (T). (L) Lumen. (P) Spermatocytes. (S) seminiferous tubule. H and E stain. Mag. 205 X

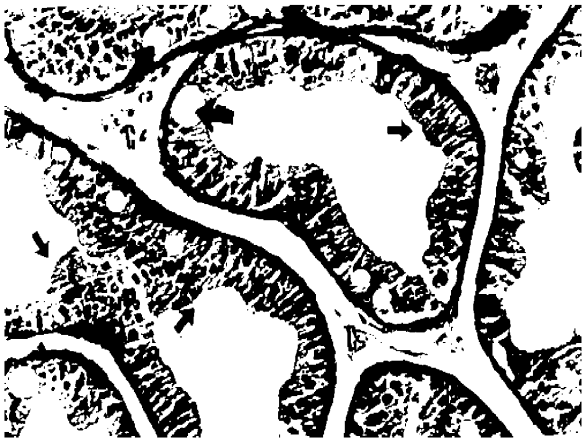

Fig. 5: A cross section of the epididymis of a rat injected with formalin solution (5%) intratestically. Notice the absence of sperms inside the lumen and the vacuolation (bold arrows) within the epithelium (E) due to the degenerative changes in its wall. (Arrows) Stereo-cilia. (C) Connective tissue. H and E stain. Mag. 410 X

No histological changes were observed in the liver and kidney for all injected animals when compared to the control groups.

Discussion

The present study demonstrated that intratesticular injection of formalin solution is an applicable method for evaluating the cytotoxic potentials of the compound without the induction of any systemic manifestations. This was evident by the lack of any apparent changes in body weight gain, food and water consumption compared to rats

injected with the normal saline solution alone. Russell *et al.*, (1987) studied the effect of using several vehicles with or without toxins including normal saline, to assess the function of several materials avoiding any systemic challenges that would exceed the aim of such studies. The findings of the present study are further supported by the histological evaluation of liver and kidneys in which they showed no pathological changes of any kind.

The results represented in this study showed that single intratesticular injection of formalin have an adverse effect on the reproductive system of adult male rats. The mating capability of rats injected bilaterally and allowed to mate after two months from injection was impaired. This was less pronounced in males after one month of injection of 5 and 10% concentrations, while no effect was observed in 2.5% concentration group. This is quite logical as the mating capability depends on sex hormones produced by the cells within the left untreated testes.

Epididymal sperm counts of treated rats further support the allegation as injection of formalin solution at all doses has caused a significant decrease in sperm counts when examined after 40 and 70 days post injection. This decrease was dose related, suggesting a direct effect of formalin on the ability of the tes tis to produce sperms. Moreover, histological analysis of the epididymis in rats injected with formalin solution have demonstrated, in most cases, complete absence of sperms from the lumen. Slott *et al.*, (1989) obtained similar results when they injected a mixture of diethylmaleate and buthionine sulfoximine (GSH-antagonists) intratestically. They observed a significant decrease in testis and epididymal weights and decrease of the cauda epididymal reserves. However, Das *et al.* (1982) observed atrophy of the seminiferous tubules in the mouse testes after intratesticular injection of BCG.

Histological changes, demonstrated by degeneration of the vast majority of the tubules and the presence of degenerated tubules (one to two cell layer) explain the failure of the testes to produce sperms. The degeneration of tubules is probably due to the cytotoxic potential of formalin and deprivation of blood/nerve supply to the region. However, some intact tubules are still evident in different regions of the testis, which are responsible for the appearance of decreased number of sperms in the epididymis. Even in most severe cases of testicular degeneration, normal areas in the polar remote sides of the testis were detected.

Slott *et al.*, (1989) described degenerated and atrophic tubules covering 10% of the testicular cross sections of rats injected with GSH-antagonists. The affected areas were surrounded by abnormal tubules but less severely affected. In the present study, the degeneration was more severe along the injection site and around it which covered large number of tubules. The difference in severity of lesions in the two experiments could be addressed to the different potentials of the used substances. Furthermore, the extact site of injection may paly a role in determining the end results as it might affect the testicular artery or nerves. It may also affect the rete testes which, in turn, block the passage of the sperms from the seminiferous tubules to the efferent ductules therefore a retrograde changes may follow.

In another study, adult rats received a single intratesticular injection of either 400 µL distilled water or five different doses of glycerol, ranging from 25 to 400 µL and were killed after 1 or 4 weeks respectively. Injection of glycerol caused focal destruction, so that the same testes contained intact tubules, tubules with spermatogonia and Sertoli cells and tubules devoid of cellular material. There was a close correlation between the frequency of intact tubules and the dose of glycerol and a similarly strong correlation between acellular tubules and the dose of glycerol (Weinbauer *et al.*, 1987). The effect of glycerol is probably due to the hyperosmolarity of the injected solutions rather than a specific action of the compounds to cause the testicular damage (Weinbauer *et al.*, 1985). In the present study, in addition to the cytotoxic effect it is well known that the formalin as a fixative has a shrinkage effect on tissues. Besides, damages to the blood vessels and or nerve supply resulted in cellular and tubular atrophy which,in turn, results in reduction of the size of the target organs. No signs of irritation in the testis were observed judged by the fluid accumulation in few cases around the scrotum only which could be due to the irritation effect of the fromalin solution dripped during injection.

Testicular sperm counts of injected rats dectreased in almost all cases with different concentrations used with few exceptions in 5 and 10% groups where increase number of sperms observed. These changes could be due to regenerative capablity of the destructted tubules in these specific tubules and the hypertrophy of the rest to compensate for the damage inflicted by the cytotoxic effect of the formalin solution. The damages in these case could be more localized due to the localized site of injection or bypassing the rete testes and or blood and nerve supply. It is always difficult to inject exactly at the same site inside the testes because of the small size of the rat testis in general.

Unilateral injection of formalin solution in all cases did not induce any significant changes to the contralateral testes. Degenerative changes of the seminiferous tubules and the presence of one or two cell layer explain the failure of the testes to produce regular number of sperms. However, intact tubules as a normal histological response will usualy hypertrophied and increase sperm production. In more severe cases of destruction and degeneration of the tubules, the only regions with normal histological tubules epithelial lining are at both poles of the testes.

Epididymal sperm counts further support the allegation of damaged inflicted by the formalin solution in all concentrations used unilaterally and bilaterally. These changes were dose related, suggesting a direct effect of formalin on the ability of the testes to produce sperms and the epididymis to store. Histological evaluation of the epididymis in rats injected with formalin in most cases demonstrated absence of sperms from their lumen. Slott *et al*, (1989) obtained similar results when they injected a mixture of diethylmaleate and buthionine sulfoximine (GSH-antagonist) intratestically. They observed a significant decrease in testes and epididymal weights and a decrease of cauda epididymal reserves.

Decrease in testicular weights demonstrated by this experiment, further describes the apparent adverse effects of formalin on the testicular function. While the epididymal reduction in weight is probably due to the decreased sperm reserves which might result in disuse atrophy.

In rats injected with formalin of all concentration goups, there were decreased in seminal vesicles weights, which reflects the effect on sex hormones. The reduction in seminal vesicles weights might be due to alteration in the pattern of testosterone secretion. However, the preputial glands showed flactuation in weights. The size and activity of the preputial gland in rodents are clearly influenced by a variety of steroid hormones and not only sex hormone. Therefore, flactuation in preputial glands weights can be attributed to their links to other steriod hormones and not only sex hormone. However, the decrease in weights after 70 days of injection might be due to disuse atrophy which followed the decrease in mating capability and sperm production in treated rats. This gland produces behavior modulating pheromones that alter fighting and other behavior (Ebling, 1963).

Libido demonstrated by mating attempts was not completely abolished but remarkably decreased especially in rats injected with 10% formalin solution bilaterally. Histological examination of the testes demonstrated a destructive effect on the interstitial tissue as well which was highly pronounced in examined sections of rats injected with 10% formalin and examined after 70 days. Leydig cells which are responsible for the production of testosterone seem to be largely affected if not destroyed by formalin. The presence of increased collagenous interstitial tissue and the absence of vegetative cells might explain the reduction in seminal vesicles weights. Mild degenerative changes in the epithelial linning of the epididymis reflects the effect of formalin on the blood and nerve supply of the organ.

This experiment could be modified and used as a new method of chemical castration especially if it is used in young animals (before puberty)

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