Effect of Bee Venom on Sexual Efficiency in Normal and Hydrogen Peroxide Treated Adult Male Rats

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Abstract :

This study designed to detect effects of honey bee (Apis mellifera) venom on sexual efficiency of normal and hydrogen peroxide treated adult albino male rats by evaluating male genital organs weights , total sperm count , dead and abnormal sperm ratios , gross and histopathological examination of testes . Twenty four rats used , divided into 4 groups : group 1: non treated group as control . group 2: hydrogen peroxide H_2O_2 1% as drinking water . group 3 : honey bee stings . group 4 : hydrogen peroxide with stings . the treatment persist for 49 days as experiment period according to(155 stings) program . The results showed a significant increase in testes weights at group 4 , A significant increase in the weights of head and decrease in the weight of body of epididymis at groups 3 and 4 comparing with H_2O_2 treated group . The results also demonstrated that bee stings at 4^{th} group significantly reduces ratios of abnormal and dead sperms and increases the total sperm count comparing with 2^{nd} group treated with H_2O_2 . histopathological examination of testes revealed hyperemia and interstitial edema at all treated groups more severely noticed at groups 3 and 4 with mild lymphocytic infiltrations and proliferation of lyedig cells . Different stages of coagulative degeneration in seminifrous epithelial cells were noticed at many sections of group 2 . An obvious increase of the density of sperm bundles at seminiferous tubular lumen in some animals at group 3 . It has been concluded that honey bee stings showed a role in protection and maintenance of some sexual efficiency parameters at hydrogen peroxide treated albino male rats with a mild tissue changes .

Introduction :

Since the old times , Man has been using honey as a medicine for treating diseases , But now modern research has come to conclusion that bee stings is a new and distinctive method for treatment , This type of alternative treatment became wide spread in east Asia countries , Middle east countries , Egypt and Sudan (1) .
Bee stings are used for treating rheumatic fever , arthritis , varicocele with an obvious therapeutic value in multiple sclerosis and migraines (2 ,3) . Male infertility as oligospermia and female weak ovulation were listed between the cases treated with a bee stings (1) . Some researchers concluded that melittin , the main polypeptide in the bee venom has an antioxidant properties (4) , and diminishes severity of acute inflammations by depressing cycloxygenase pathway of arachedonic acid through stimulating of pituitary gland to secret ACTH leading to liberation of cortisol from adrenal cortex (5,6) . Some researchers stated that mild cortisol elevation induced DNA replication in spermatogenesis and improves male fertility (7) , other researchers concluded that cortisol elevation decreases testesteron level and cause retardation of testicular development (8) . For detecting effects of bee venom on reproductive system and some fertility parameters in normal and hydrogen peroxide treated adult albino male rats , The present study designed .

Materials and methods :

Animals : twenty four albino male rats were used in the study at 4-5 months age and (200-230) gm weight , housing and breeding was previously performed in experemental animal house / college of veterinary medicine , animals were placed in standerd polypropylene cages (20 × 25 × 20) Cm W,L,H , and under standerd husbandary condition (12 hr light / dark cycle : 25±3 c°) . The rats were supplied diet and water *ad libitum* .

Honey bee and the stinging method :

Adult honey bee workers from active colonies were carefully picked with thumb forceps and collected with a plastic container with multiple ventlation holes for respiration . Back region of the animals were shaved , rats were stinged by placing the bee on the back region skin , the singing thorn were implanted with in the skin , the bee removed to leave the

stinging aparatus for 5 minutes to ejeculate the whole venom then removed .

Stinging program :

Stinging program according to (9) was applied which included 155 stings started with 5 days of treatment starting with 1 sting at 1st day adding one sting in each following days to reach 5 stings in the 5th day , Totally reaches 15 stings as first stinging period . Rest for 2 days were given before starting the 2nd stinging period that included 40 stings distributed on 8 days , as 5 stings daily followed by rest for 7 days . The 3rd and 4th stinging periods included 50 stings for 10 days for each period separated by rest period for 7 days . At the end of experement included 49 days each animal recieved (155) stings .

Experemental design :

The 24 animals were randomely divided into 4 groups of 6 rats each , received the follwing treatments :

Group 1 : rats served as non treated control group twinged with a pin at the stinging manipulation , frequency and time to avoid the stinging manipulation stress variances .

Group 2 : received hydrogen peroxide (H_2O_2) 1% as drinking water and twinged with a pin .

Group 3 : revieved bee stings as the program mentioned above .

Group 4 : received 1% (H_2O_2) as drinking water and stinged as mentioned above .

Sample collection and analysis :

At 49th day of experement , animals were sacrificed under miled either anaesthesia (10) . Immediat necropsy performed , testes and accessory sex organs (Epididymis , Seminal vesicles and prostate gland) were dissected out , cleared and weighed . Epididymis was sectioned immediately the content of the head of epididymis was sequeezed gently in a clear watch glass contained 9.8 ml buffer formalin with 10 ml eosin stain (5%) this was used for counting the sperm using hemocytometeric technique (11 , 12) . The percentage of live and morphologically abnormal sperms were counted in smear prepared from epididymal tail

content by using eosin-nigrosin stain diluted with 3% sodium citrate (13) .

Histological analysis :

Testes were fixed in Bouin`s fluid , passed through ascending series of ethanol and then through xylene and embedded in paraffin wax. Tissues were sectioned at the thickness of 2-3µm and stain with hematoxillin and eosin (14).

Statistical Analysis :

The results were expressed as mean ± SE . Our data were analyzed statistically using one and two_ways analysis of variance (Anova).Group differences were determined using Duncan multiple range test. Differences were considered significant when ($P \leq 0.05$) (15) .

Results :

1- Weight of testis and accessory sex organs :

The results described in table (1) showed no significant differences in the weight of accessory sex organs (prostate gland and seminal vesicle) in all treated groups comparing with control . A significant increase in the weight of testes also recorded at 4th group (sting+ H_2O_2) with no significant differences of other treated groups from control . A significant decrease from control was noticed in the weight of epidydimal head at 2nd group(H_2O_2) and in the weight of epidydimal body at both (sting) and (sting+ H_2O_2) groups . No significant change in the weight of epidydimal tail were noticed at all treated groups comparing with control .

Table (1) : effects of stings , Hydrogen peroxide and mixed treatment on testis and accessory sex organs weights in rats .

Organ groups	Epididymis weight (mg/100gB.W)			Testis weight (mg/100g B.W.)	Seminal vesicle (mg /100g B.W.)	Prostate gland (mg /100g B.W.)
	Head	Body	Tail			
Control	65.9 ± 1.3 a	13.9±0.3 a	62.6±3.8 a	397.6±11.9 b	74.2±3.7 a	439.8±30.6 a
H_2O_2	53.6 ± 6.1 b	15.8±0.9 a	56.3±6.9 a	395.9 ±44.7 b	72.8±8.5 a	417.8±63.6 a
Sting	68.7± 3.8 a	11.9±0.6 b	55.5±3.3 a	430.1 ±17.6 ab	91.6 ±4.6 a	506.2±43.5 a
Sting+ H_2O_2	75.8± 1.2 a	10.8±0.3 b	65.9±1.9 a	503.0±15.0 a	85.7±7.5 a	559.8±35.6 a

Number of animals 6 rats/ group.
Values were expressed as means ± SE.
Vertically :Values with different letters are significantly different (P≤0.05).

2- Characteristics of sperms :

Sperm count at group (H_2O_2) significantly decreased in comparing with control and other treated groups which does not recorded any differences from control .
The 2nd group treated with (H_2O_2) recorded a significant increase in ratios of dead sperms and abnormal sperms comparing with control and other treated groups , Also there was significant increase in abnormal and dead sperm ratio at group (sting + H_2O_2) comparing with control and significant increase of abnormal sperm ratio comparing with group treated with stings only . Table (2) .
Table (2) : Effects of stings , Hydrogen peroxide and mixed treatment on sperm characteristics in rats.

groups	Sperm counts sperm× 10^6/ml	Dead sperm%	Abnormal sperm %
Control	1.98 ± 0.17 a	25.2 ± 1.8 c	11.6± 0.9 c
H_2O_2	1.05 ± 9.35 b	56.2 ± 1.4 a	28.2± 1.1 a
Sting	1.80 ± 0.12 a	29.0 ± 3.2 bc	13.0± 1.2 c
Sting+H_2O_2	1.75± 0.22 a	36.8± 3.9 b	17.8± 1.3 b

Number of animals 6 rats/ group.
Values were expressed as means ± SE.
Vertically : Values with different letters are significantly different (P≤0.05)

3- Gross and histopathological examination :

Gross examination does not revealed specific ultrations in the testes between treated group comparing with control exept congestion of blood vessels specially at groups (sting) and (sting+ H_2O_2) .

Histopathological examination revealed presence of hyperemia in blood vessels of testes at the 3 treated groups , most severely noticed at group (sting+H_2O_2) and ranged from sever to moderate at group (sting) and mostly mild at group (H_2O_2) . Figures (1,2) . The examination also demonstrated the presence of edema between seminiferous tubules and

under testicular capsule variated from sever to moderate at group (sting + H_2O_2) and (sting) to mild at group (H_2O_2) . Figures (3,4) .
Miled infiltration of lymphocytes between tubules at all treated groups with miled proliferation of leydig cells also appeared at the sections of groups (sting) and (sting+H_2O_2) . Different stages of coagulative degenerations or acute cell swelling was obvious at seminiferous epithelial cells in many tubules at testicular the sections of groups (H_2O_2) and (sting+ H_2O_2) seminiferous epithelial hyperplasia were noticed in some tubules at groups (sting) and (sting+ H_2O_2) . Figure (5) , also there was noticable increase in the density of sperms bundles in the lumen of many tubules at the sections of group (sting) . Figure (6) .

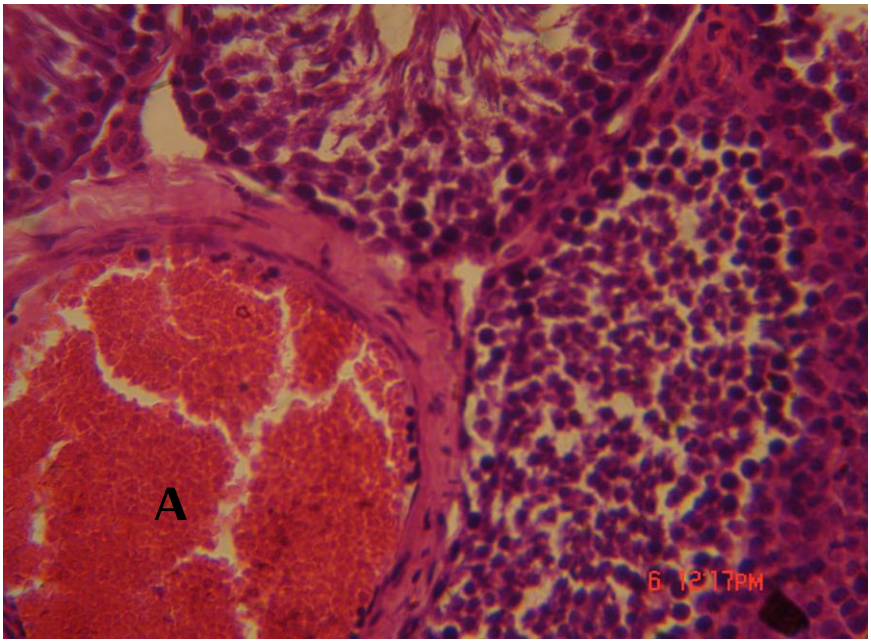

Figure (1) : Histological section of the testis from group (sting+ H_2O_2) showing sever hypermia in one of the testicular arterioles (A) . H&E . Mag 370 X .

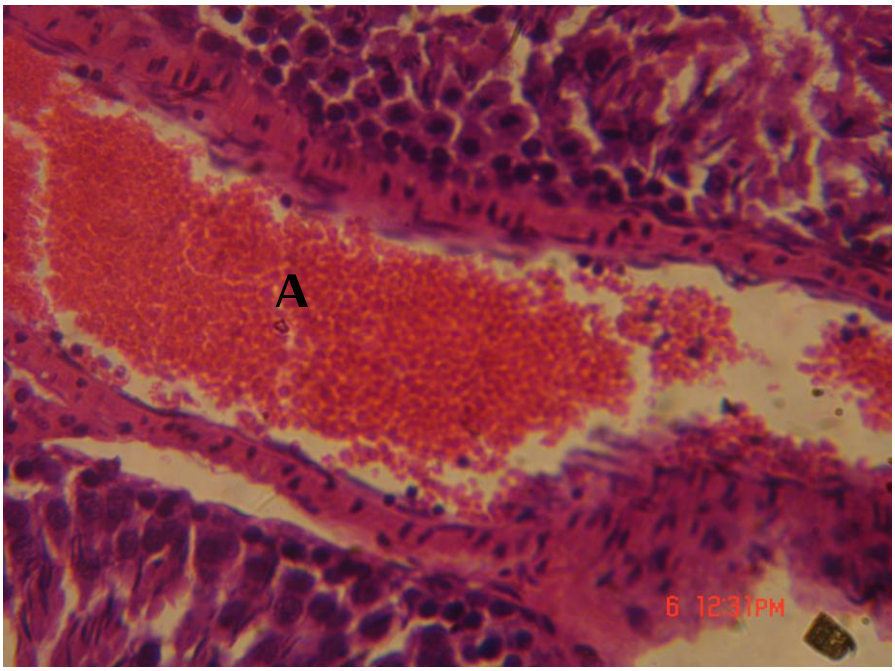

Figure (2) : Histological section of testis from group (sting) showing longtudinal section of hypremic blood vessle (A) . H&E . Mag 560 X .

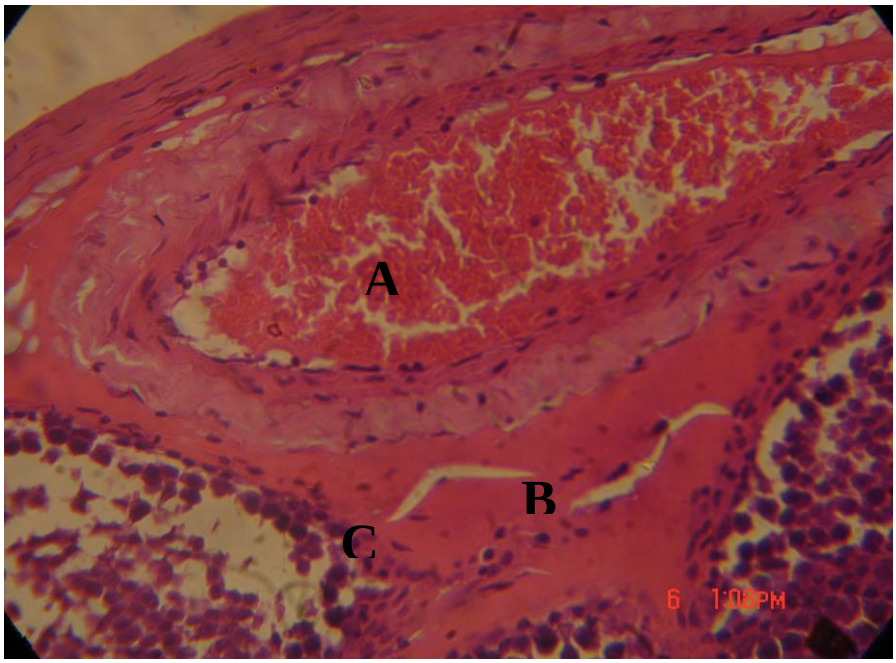

Figure (3) : Histological section of testis from group (sting+ H_2O_2) demonstrating hyperemia (A) , interstetial and subcapsular edema (B) , miled lymphocytic infiltration between seminiferous tubules (C) . H&E . Mag 450 .

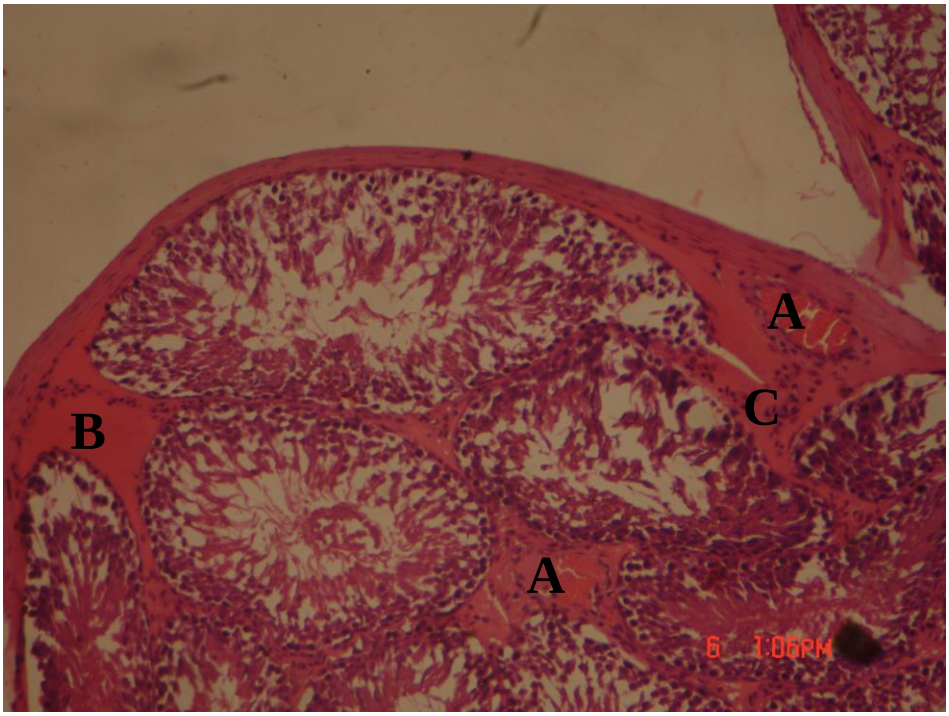

Figure (4) : Histological section of testis from group (sting+ H_2O_2) showing hyperemia (A) . Sever and diffused edema (B) and miled lymphocytic infiltration (C) . H&E . Mag 165 X .

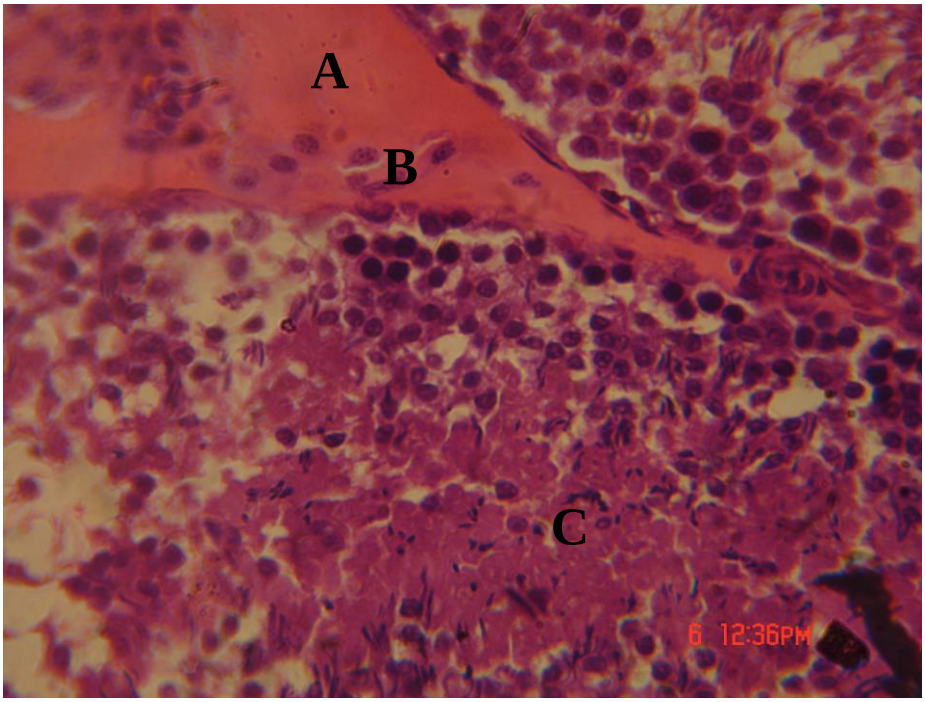

Figure (5) : Histological section of testis from group (sting+ H_2O_2) showing interstetial edema (A) , proliferation of leydig cells (B) and claudy swelling of seminiferous epithelial cells (C) . H&E . Mag 450 X .

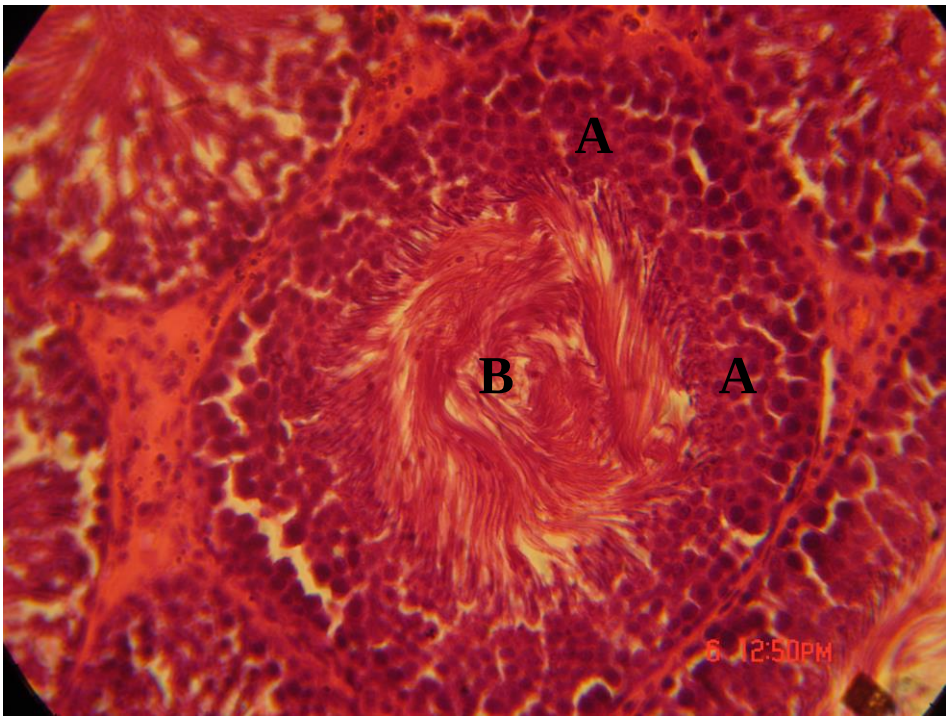

Figure (6) : Histological section of testis from group (sting) showing seminiferous epithelial hyperplasia (A) and increase in the density of sperm bundles in the lumen of seminiferous tubules (B) . H&E . Mag 280 X .

Discussion :

The results showed a significant decrease in the weight of epididymal head , Total sperm count with elevation percentage of dead and abnormal sperms at H_2O_2 treated group , comparing with control , Those results are directly connected to the adverse effects of H_2O_2 on spermatogenesis and sperm functions in dose and time dependent manor with a deleterious effects on spermatides at high concentrations (16) . The oxidative stress by excessive reactive oxygen species can initiate changes in lipid or protein components of sperm plasma membrane , Additionally changes in DNA can be induced (17) . A positive correlation was revealed between H_2O_2 treatment and DNA strand breaks in human , bull and mouse spermatozoa , the damage can drive from aberrant chromatin packaging during spermatogenesis leading to defective apoptosis (18) . The same oxidative stress induced by H_2O_2 can express cloudy swelling of seminiferous epithelium (spermatogonia) , congestion , Edema noticed at testicle histological sections of H_2O_2 treated groups as an inflammatory response (19) .

At the sting group there was a significant decrease in epididymal body weight with slight non significant increase in dead sperm percentage comparing with control , Because the precise biological effects of bee venom on the reproductive system and spermatogenesis did not studied before , The results will be discussed on the basis of systemic effects of one of the venom components called Milittin which is a polypeptide composing 52% of the weight of the dry component of the bee venom (4) . The injection of milittin stimulates pituitary gland to release Adrenocorticotrophic hormone ACTH causing liberation of glucocorticoids , mainly cortisol from the adrenal cortex (5) , this property of milittin enabled the bee venom to have a thraputic value in cases with arthritis and rheumatoid diseases (6) . The release of excess cortisol to circulation can hypothesized to have an adverse effects on spermatogenesis and fertility , The researchers (20) stated that cortisol treatment caused retardation of pubertal testes development and reduced the LH pituitary content , and reduced synthesis of androgen at male common carp fish . Its hypothesized that excess cortisole inhibits sertoli cells from releasing activin-B that normally stimulates spermatogonia to

induce mitosis to form spermatocytes in mammalian testes (21) . This hypothesis is supported by the fact that sertoli cells responds to glucocorticoids through presence of glucocorticoids receptors on these cells (22 , 23) . in addition glucocorticoids have been shown interference with cell cycle proteins by inhibiting the cell cycle progression (24) . The significant increase in the weight of testes at both sting and sting + H_2O_2 groups can be explained due to congestion and edema demonstrated at histopathological examination which makes the organ heaver then normal (25) . The noticed increase in the intense of spermatide bundles at testicular sections of sting group did not accompanied with an significant increase in total sperm count above control , so those accumulations in seminiferous tubules may be resulted from decrease of libido and ejaculation due to depressing testosterone levels under influence of excess glucocorticoids (26) , also stings significantly increased sperm count and decreased abnormal sperm ratio at 4^{th} group comparing with 2^{nd} group treated with H_2O_2 only . the reduction of testosterone production and secretion can stimulate proliferation of leydig cells as feed back mechanism that noticed at the both sting and sting+ H_2O_2 groups . As a final conclusion , It was obvious that bee stings treatment did not improve any of the reproductive parameters comparing with control , But it significantly diminished the harmful effects of H_2O_2 on some parameters of the reproductive system of adult male rats , Although bee venom it self induced a mild tissue changes .

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