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Effect of *Alpinia Officinarum* Rhizome Extract on Fertility and Sexual Behavior of Adult Male Albino Rats Treated with Sotalol

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ABSTRACT

Background: Infertility in cardiovascular patients is not only attributed to pathogenesis of the disease but, also to the used therapeutic drugs. Beta blockers which are corner stone in treatment of these patients are reported to affect fertility and sexual behavior in patients using them. *Alpinia officinarum* (*A. officinarum*) rhizome extracts are widely used for their antioxidant and anti-inflammatory effects to improve oxidative stress which affects organs due to using different drugs or pathogenesis of different diseases.

Objective: The current work aimed to study effect of *Alpinia officinarum* rhizome extract on improving the reproductive function and sexual behavior of adult male Albino rat treated with Sotalol.

Materials and Methods: Fifty adult male Albino rats were categorized into five groups. Group I control, group II (*A. officinarum* group) received *A. officinarum* extract at a dose of 200mg/kg/day in olive oil orally by gastric tube for 21 days, group III (Sotalol group) received Sotalol at a dose of 3mg/kg/day orally by gastric tube for 21 days, group IV (protected group) received the extract then Sotalol and group V (treated group) received Sotalol then the extract. Then fifty adult female rats were used for assessment of sexual behavior through observing mount latency and frequency.

Results: Sotalol induced testicular destruction in the form of shedding of germinal epithelium, hyaline deposition, decreased sperm count with decreased testosterone level, increased oxidative stress in the form of decreased Serum superoxide dismutase (SOD) level and increased Serum malondialdehyde (MDA) level. Deterioration of sexual behavior in the form of increased mount latency and decreased mount frequency was noticed in the Sotalol group. *A. officinarum* extract produced a great preservation of testicular structure and sexual behavior in the protected group (IV) together with improvement in the treated group (V).

Conclusion: Sotalol induced destructive effects on the testicular structure, function and sexual behavior which were greatly improved by using *A. officinarum* extract.

Keywords: Sotalol, Testis, Sexual behavior, *A. officinarum* extract.

INTRODUCTION

Infertility is a worldwide health problem affecting about 8–12% of couples all over the world, the male factor accounts for about 40–50% of its cases. Male infertility may be attributed to testicular dysfunction or sexual dysfunction (impaired sexual behavior) [(1)]. Male sexual behavior consists of a complex pattern of genital responses including pre-mating and mating behaviors [(2)]. Medications are a common exogenous factor that must be evaluated in infertile men, due to their impact on the different reproductive parameters including spermatogenesis, sperm parameters and sexual behavior [(3)]. Sotalol, a non-selective beta blocker is also classified as class III antiarrhythmic drug [(4)].

It is used for treatment of atrial fibrillation, ventricular tachycardia, premature ventricular contractions, and supraventricular tachycardia [(5)]. Sexual dysfunction is a distressing problem for many people. The available drugs for management of this problem have many side effects. Herbal medications recently provided the clue for safe management of the sexual dysfunction as they have no side effects [(2)].

Alpinia officinarum Hance (lesser galangal), an important member of family Zingiberaceae is widely present in the tropical and subtropical regions of Southeast Asia. Its rhizomes are used as an anti-inflammatory, analgesic, and antioxidant agent in conventional medicine [(6)].

Alpinia officinarum extract was reported to improve sperm parameters and histological damage in testis of the diabetic rats [(7)].

Alpinia officinarum has been also used for treatment of sexual dysfunction in the traditional Persian medicine [(8)].

MATERIAL AND METHODS

Chemicals:

a. Sotalol Hydrochloride-Sotalol hydrochloride (Betacor 80mg®) was obtained as tablets manufactured by Amoun Pharmaceutical Company, Cairo, Egypt. Each tablet contained 80 mg Sotalol hydrochloride. Each tablet was dissolved in 100 ml distilled water to obtain a concentration of 0.8mg/ml then it was given orally by modified plastic syringe to animals in a dose of 3mg/kg body weight [(9)].

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b. Ethinyl estradiol- Ethinyl estradiol (Ethinyl estradiol 50mcg®) was obtained as tablets manufactured by Kahira Pharmaceutical Company, Cairo, Egypt.

c. **Progesterone-**Progesterone (Progest 100mg®) was obtained as capsules manufactured by Pharco Pharmaceutical Company, Alexandria, Egypt.

d. **Olive oil and sesame oil:** were obtained from local market. The olive oil was used as a vehicle for the extract while sesame oil was used as a vehicle for progesterone.

e. N- hexane- N-hexane was obtained from Piochem Company,6 October city, Cairo, Egypt. It was used for *Alpinia officinarum* extract preparation.

***Alpinia officinarum* rhizome extract:**

Alpinia officinarum dried rhizomes were obtained from local market. The plant material was authenticated by Aromatic Plant Department, Faculty of Agriculture, Menoufia University.

Dried rhizomes of *A. officinarum* were ground then soaked in n-hexane for 3 days then filtered using gauze first then filter paper. The filtered extract was evaporated under reduced pressure in a rotary flash evaporator at Faculty of Pharmacy, Menoufia University to leave a viscous residue. In this study, the crude extract was used in a dose of 200 mg/kg [(10)].

Animals:

Fifty adult male Albino rats and fifty adult female rats with average weight of 200-250 gm were used in this study. They were obtained from Theodor Bilharz Research Institute Animal House, ElWarak, Cairo, Egypt. The rats were housed, at this animal house, maintained at constant temperature.

They were provided with free access to water and balanced diet. The procedure was approved by the ethics committee on animal experiment of the Faculty of Medicine, Menoufia University, Egypt in accordance with the international regulations on care and use of laboratory animals.

Experimental design:

The male animals were randomly categorized into five main groups: -

- **Group I (Control):** Composed of 10 rats which were further subdivided into 2 equal subgroups, each one was composed of 5 rats.
- **Subgroup Ia:**5 rats received distilled water (vehicle of Sotalol hydrochloride) orally by gastric tube for 21 days.
- **Subgroup Ib:** 5 rats received olive oil (vehicle of extract) orally by gastric tube for 21 days.
- **Group II (*A. officinarum* group)**: composed of 10 rats which received *Alpinia officinarum* rhizome extract at a dose of 200mg/kg/day in olive oil orally by gastric tube for 21 days [(10)].
- **Group III (Sotalol group)**: composed of 10 rats which received Sotalol at a dose of 3mg/kg/day orally by gastric tube for 21 days [(11)].
- **Group IV (Protected group**): composed of 10 rats which received the extract at a dose of 200mg/kg/day in olive oil for 21 days orally by gavage then received Sotalol at a dose of 3mg/kg/day for further 21 days orally by gavage [(12)].
- **Group V (Treated group):** composed of 10 rats which received Sotalol at a dose of 3mg/kg/day for 21 days orally by gavage then received the extract at a dose of 200mg/kg/day in olive oil for further 21 days by gavage.

Methods:

In this study fifty adult female rats with average weight of 200-250 gm were used for assessment of sexual behavior. Female rats allow mating only during the estrus phase, so they were given a suspension of ethinyl estradiol orally at the dose of 100 µg/animal 48 hours prior to pairing and progesterone 0.5mg/rat dissolved in 0.2ml sesame oil injected subcutaneously 6hours before pairing with adult male rats under the study [(13)].

Sexual behavior was assessed as follow:

Following 24 h of last treatment (on male rats), the female rats were paired (1:1) with the males. The female rats were tested by vaginal smear to confirm being estrus, then placed with males for testing sexual behavior. The observation was performed in quiet room from appropriate distance. After 10 min of the pre-copulatory period, the rats were observed for 20 min for sexual behavior parameters including mount latency (ML) and mount frequency (MF) [(14)].

Mount latency (ML) was considered as the time from the introduction of a female to the incidence of first mount. Mount frequency (MF) showed the number of mounts during the specified period of observation. Mount latency & frequency are important measures of both libido and potency [(2)].

At the end of determined period of the experiment of each group and after testing the sexual behavior, male rats were anaesthetized by diethyl ether inhalation, blood samples were collected from the retro-orbital venous plexus and then a median abdominal incision was performed to expose the testes. The two testes of each rat were carefully dissected out, fixed in 10% formol saline for light microscopic study.

Sperm count:

By excision of epididymis of each rat of all the groups, spermatozoa were collected. Using the haemocytometer, the collected spermatozoa were counted and expressed as number of sperm in million/milliliter of suspension [(15)].

Biochemical analysis:

The collected blood samples were used for measurement of:

1- Total serum level of testosterone hormone by ELISA method [16].
2- Serum superoxide dismutase (SOD) level through colorimetric assay [17].
3- Serum malondialdehyde (MDA) level by colorimetric assay [17].

Light microscopic study:

Histological study: tissue samples were fixed in 10% formol saline for 2 days then processed to prepare 5-µm-thickness paraffin sections for H& E and PAS stains.

Immunohistochemical study: poly-L-lysine coated slides were deparaffinized and rehydrated. They were inserted in 3%hydrogen peroxide to block the endogenous peroxidase. Microwave antigen retrieval procedure was used. The sections then were incubated in primary antibodies to PCNA (Clone PC 10, DAKO A/S Denmark) for detection of DNA damage" nuclear expression"& primary antibody to Bax (rabbit polyclonal antibody, Dako, Carpinteria California, USA) for detection of apoptosis" cytoplasmic expression".

Morphometric study:

The morphometric study was performed by using five non-overlapping fields, randomly captured by a Leica Microscope DML B2/11888111 equipped with a Leica camera DFC450.

The examined parameters were assessed using image J software (Maryland, USA) for at least five sections /animal and averaged for animal. The following parameters were measured:

From H & E sections of the different experimental groups, germinal epithelial height and seminiferous tubular diameter were measured.

From PAS sections of the different experimental groups, color intensity of PAS +ve materials was measured.

From immunohistochemical sections (PCNA) of the different experimental groups, counting of PCNA positive cells in each seminiferous tubule was done. Only the basal germ cells of these tubules were counted.

From immunohistochemical sections (Bax) of the different experimental groups the area percentage of Bax +ve cells was detected.

Ethical approval:

This study was conducted in accordance with ethical procedures and policies approved by Animal Care and Use Committee of Faculty of Medicine, Menoufia University, Cairo, Egypt.

Statistical analysis

The collected data were analyzed by SPSS (statistical package for social science) version 23.0 on IBM compatible computer using Mann Whitney U test. The data were expressed as mean (X^2) ± standard deviation (SD).

The significance of data obtained from the previous test was determined by the P value (probability of chance): P value >0.05 was considered statistically non-significant. P value <0.05 was considered statistically significant.

RESULTS

Statistically, there was no significant difference between the results of subgroups Ia and Ib, so both were represented as control group.

Biochemical results:

Total serum testosterone level:

There was a significant decrease in testosterone level in Sotalol group (P <0.001) as compared to the control group, on the other hand, there was no significant difference in its level in *A. Officinarum* group (P >0.05) as compared to the control group. Also, there was a significant increase in its level in both the protected group (P <0.001) and the treated group (P <0.05) as compared to Sotalol group. There was no significant difference in testosterone level in the protected group (P >0.05) compared to the treated group **(Table1)**.

Serum SOD level:

There was a significant decrease in serum SOD level in Sotalol group (P <0.001) as compared to the control group, but there was no significant difference between *A. Officinarum* group (P >0.05) as compared to the control group. Also, there was a significant increase in its level in both the protected group (P <0.001) and treated group (P <0.001) as compared to Sotalol group. There was no significant difference in serum SOD level in the protected group (P >0.05) compared to the treated group **(Table 1)**.

Serum MDA level:

There was a significant increase in serum MDA level in Sotalol group (P <0.001) as compared to the control group, but there was no significant difference between *A. officinarum* group (P >0.05) as compared to the control group.

Also, there was a significant decrease in its level in both the protected group (P <0.001) and the treated group (P <0.001) as compared to Sotalol group. There was no significant difference in serum MDA level in the protected group (P >0.05) compared to the treated group **(Table 1)**.

Sperm count:

There was a significant decrease in sperm count in Sotalol group (P <0.001) as compared to *officinarum* group (P > 0.05) as compared to the control group. Also, there was a significant increase in sperm count in both the protected group (P <0.05) and the treated group (P <0.05) as compared to Sotalol group.
There was no significant difference in sperm count in the protected group (P >0.05) compared to the treated group **(Table2)**.

Sexual behavior assessment:
Mount latency:
There was a significant increase in mount latency in Sotalol group (P <0.001) as compared to the control group, while there was no significant difference in it in *A. officinarum* group (P >0.05) as compared to the control group.
Also, there was a significant decrease in mount latency in both the protected group (P <0.05) and the treated group (P <0.05) as compared to Sotalol group. There was no significant difference in mount latency in the protected group (P >0.05) compared to the treated group. There was no significant difference in mount latency in the protected group (P >0.05) compared to the treated group **(Table2)**.

-Mount frequency:
There was a significant decrease in mount frequency in Sotalol group (P <0.001) as compared to the control group, while there was no significant difference in it in *A. officinarum* group (P > 0.05) as compared to the control group.
Also, there was a significant increase in mount frequency in both the protected group (P <0.001) and the treated group (P <0.05) as compared to Sotalol group. There was no significant difference in mount frequency in the protected group (P >0.05) compared to the treated group **(Table2)**.

Table (1): Biochemical results expressed as mean ± standard deviation

Groups	Total serum testosterone level in (ng/ml)	Serum SOD level (u/l)	Serum MDA level (umol/l)
Control	4.43±0.38	4.70±0.20	1.48±0.22
A. officinarum	4.73±0.66	4.87±0.25	1.38±0.16
Sotalol	3.08±0.43	1.52±0.22	4.50±0.22
protected	4.12±0.16	3.92±0.40	2.06±0.23
Treated	3.92±0.19	3.58±0.45	2.30±0.41

Table (2): Reproductive function parameters expressed as mean ± standard deviation

Groups	Sperm count x10^6/ml.	Mount latency in seconds	Mount frequency
Control	66.40±1.67	52.60±2.41	8.20±1.30
A. officinarum	67.76±2.44	49.20±5.63	9.40±1.14
Sotalol	47.80±3.27	64.60±4.62	4.00±1.58
Protected	55.60±3.36	55.80±5.89	7.40±1.14
Treated	55.20±5.26	57.20±3.96	6.80±0.84

Histological results:
(a)Hematoxylin and Eosin (H. &E.) stain:
The control group and *A. officinarum* group showed that the testis is composed of seminiferous tubules which are rounded or oval in shape and characterized by being well arranged and regular.Each tubule is surrounded by a basement menbrane and lined with spermatogenic cells and Sertoli cells which are present between the spermatogenic cells.
The spermatogenic cells were composed of spermatogonia that appeared as small rounded cells resting on the basement membrane, primary spermatocytes which were the largest in size with rounded dark nuclei and spermatids whether rounded or elongated appeared close to the lumen. Sertoli cells with triangle nuclei were seen between the germ cells. The sperms appeared filling the lumen of the tubules **(Figs.1a,1b,2a &2b)**. In between the tubules, there was interstitial tissue containing Leydig cells and normal blood vessels. Myoid cells with flat nuclei were seen around the basement membrane **(Fig.2a)**.
Sotalol group showed distorted architecture of the testicular tissue in the form of irregular seminiferous tubules with widening of interstitium areas and hyaline deposition. Dilated and congested blood vessels can be observed in the interstitium. The was degeneration and shedding of the lining spermatogenic cells, so some seminiferous tubules were lined with single layer of cells while, others showed shedding of the whole thickness of spermatogenic cells. Lumens of some seminiferous tubules were devoid of sperms others showed degenerated amalgamated sperms in their lumens **(Figs.1c&2c).**
Protected group showed preserved architecture of testes in most of seminiferous tubules being regular with intact basement membranes and normal spermatogenic cells; the interstitial tissue contained normal blood vessels and Leydig cells between the tubules **(Figs.1d&2d).**
Treated group showed restoration of normal germinal epithelium lining most of the tubules. Congested blood vessels were observed in the interstitial tissue with hyaline deposition in the interstitial tissue. Sperms were seen in the lumens of the tubules **(Figs.1e&2e).**

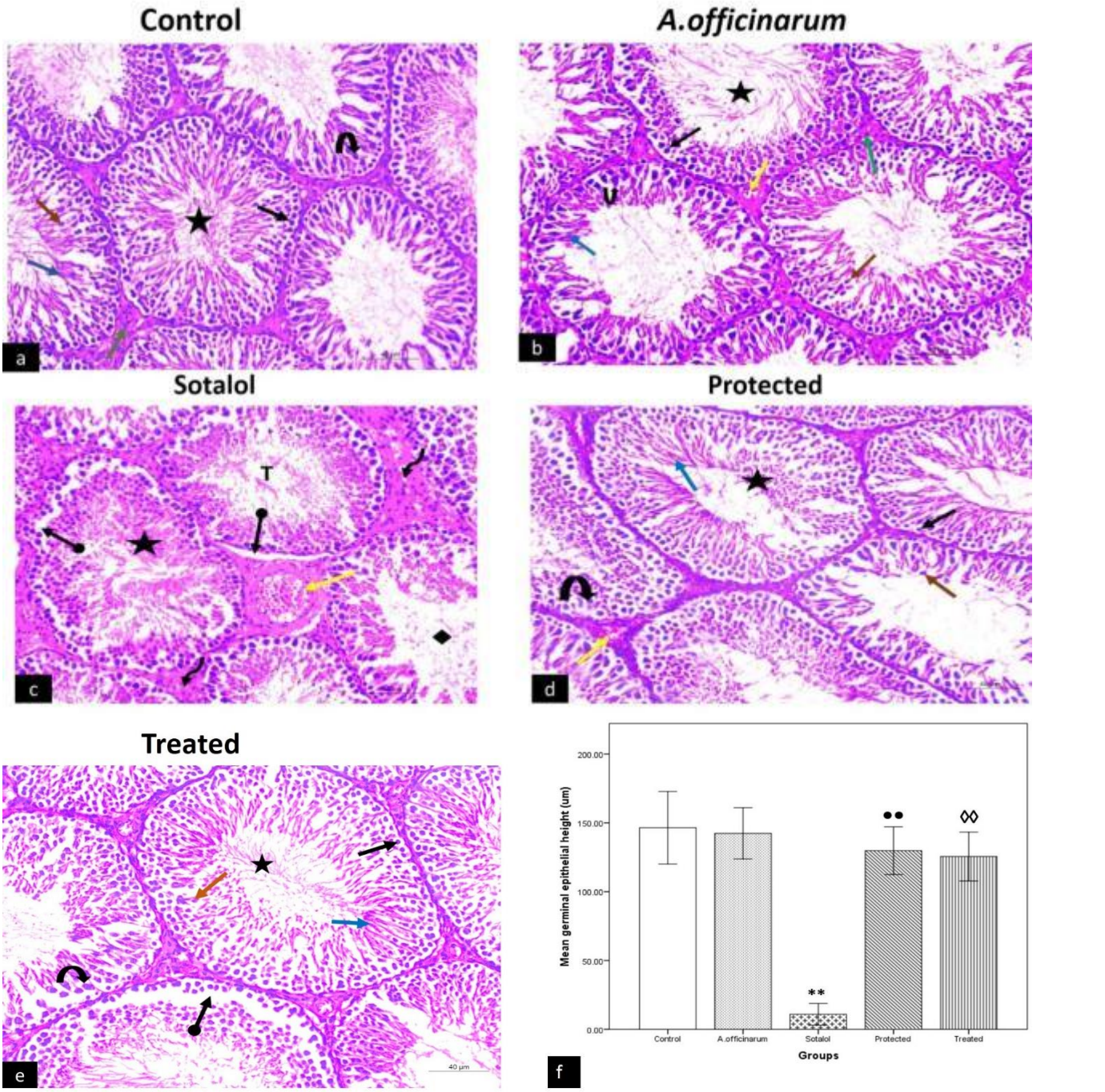

Figure 1: photomicrographs of sections of testis a) Control group b) *A. officinarum* group d) the protected group showing the seminiferous tubules lined with spermatogenic cells; spermatogonium (black arrow), primary spermatocytes (curved arrow), rounded spermatid (brown arrow), elongated spermatid (blue arrow) and mature sperms (star). The interstitial cells of Leydig (green arrow) and blood vessels (yellow arrow) located in between the seminiferous tubules. C) Sotalol group showing degenerated irregular seminiferous tubules with shedding of the spermatogenic cells (dot ended arrow). The lumens of some tubules are devoid of sperms (black rhomboid shape) with degenerated sperms (star) in other tubules and hyaline deposition in the interstitium (bent black arrow). Dilated, congested and thickened wall of the blood vessel (yellow arrow) can also be seen in the interstitium) Treated group showing a seminiferous tubule with shedded spermatogenic cells (dot ended arrow), others resemble the control. (H&Ex200) f) Histogram 1: mean germinal epithelial height.** Significant decrease in germinal epithelial height in Sotalol group compared to the control group. ●●Significant increase in germinal epithelial height in the protected group compared to Sotalol group.◊◊ Significant increase in germinal epithelial height in treated group compared to Sotalol group.

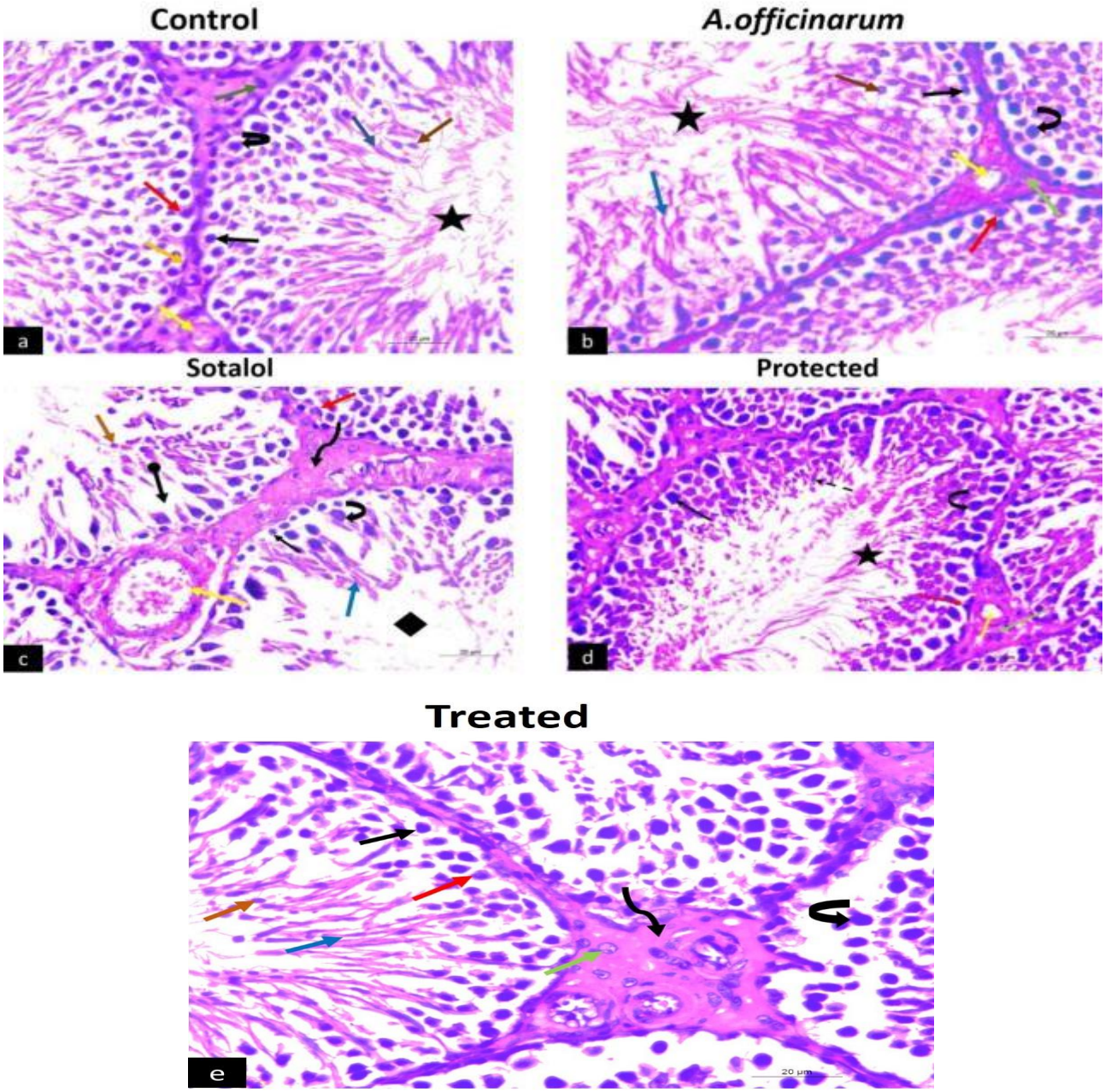

Figure 2: photomicrographs of sections of testis a) Control group b) *A. officinarum* group d) protected group showing the seminiferous tubules lined with spermatogenic cells; Spermatogonium (black arrow), primary spermatocytes (curved arrow), rounded spermatid (brown arrow), elongated spermatid (blue arrow), mature sperms (star) and Sertoli cell (red arrow). The interstitial cells of Leydig (green arrow) & blood vessels (yellow arrow) located in between the seminiferous tubules. Myoid cells with flat nuclei (orange arrow) can be noted in control group around seminiferous tubules. c) Sotalol group showing degenerated seminiferous tubules with shedding of the spermatogenic cells (dot ended arrow). Lumens of some tubules are devoid of sperms (black rhomboid shape). Congested blood vessel (yellow arrow) and hyaline deposition (bent black arrow) can be noted in the interstitium e) Treated group showing restoration of spermatogenic cells. Hyaline deposition (bent black arrow) can be noted in the interstitium (H&Ex400).

(b)PAS stain:

The control group and *A. officinarum* group showed strong positive reaction in the basement membrane surrounding the seminiferous tubules, in interstitium and also in the spermatogenic cells **(Fig.3a&b)**.

Sotalol group showed moderate PAS reaction in basement membranes of the seminiferous tubules and in the spermatogenic cells. They also showed deposition of strong PAS positive materials in-between the seminiferous tubules **(Fig.3c)**

Protected group showed strong PAS reaction in basement membrane of seminiferous tubule, interstitium and in spermatogenic cells **(Fig.3d)** and also **Treated group** showed strong PAS reaction in basement membranes of the seminiferous tubule, interstitium and in spermatogenic cells **(Fig.3e).**

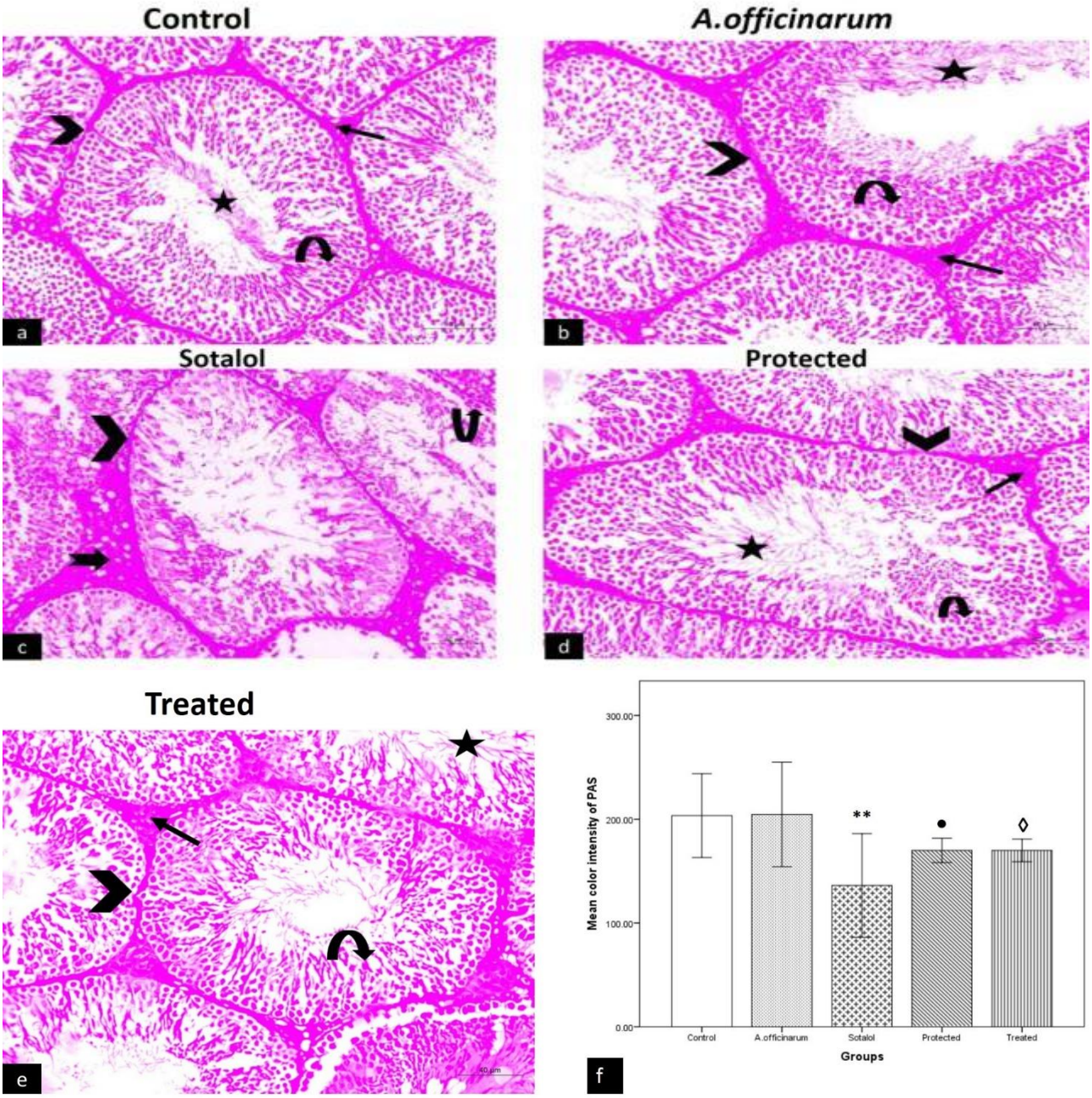

Figure 3: photomicrographs of sections of testis a) Control group b) *A. officinarum* group d) protected group showing strong positive PAS reaction in well demarcated intact basement membranes of the seminiferous tubules (arrow head). The strong reaction can be seen also in the interstitium (black arrow), spermatogenic cells (curved arrow) and sperms (star). C) Sotalol group showing moderate PAS reaction in basement membranes of the seminiferous tubules (arrow head) and in the spermatogenic cells (curved arrow). Strong PAS positive materials deposition is realized in between the seminiferous tubules (notched arrow). e) Treated group showing also strong PAS reaction in well demarcated intact basement membranes of the seminiferous tubules (arrow head). A strong reaction can be seen also in the interstitium (black arrow), spermatogenic cells (curved arrow) and sperms (star). (PAS x 200). f) Histogram (2) showing mean color intensity of PAS +ve materials.** Significant decrease in color intensity of PAS +ve materials is observed in testis of Sotalol group compared to the control group. ●Significant increase in color intensity of PAS +ve materials can be noticed in testis of the protected group compared to Sotalol group. ◊ Significant increase in color intensity of PAS +ve materials is demonstrated in testis of the treated group compared to Sotalol group.

(c)PCNA stain:

Control group and *A. officinarum* group showed positive PCNA immunostaining (deep brown nuclear reaction) in all nuclei of the basal germ cells mainly the spermatogonia and primary spermatocytes **(Fig.4a&b).**

Sotalol group showed few positive PCNA immunostaining germ cells in basal germ cells **(Fig.4c).**

Protected group showed positive PCNA immunostaining (deep brown nuclear reaction) in all nuclei of the basal germ cells mainly the spermatogonia and primary spermatocytes similar to the control group **(Fig.4d).**

Treated group showed positive PCNA immunostaining (deep brown nuclear reaction) in all nuclei of the basal germ cells mainly the spermatogonia and primary spermatocytes similar to the control group **(Fig.4e).**

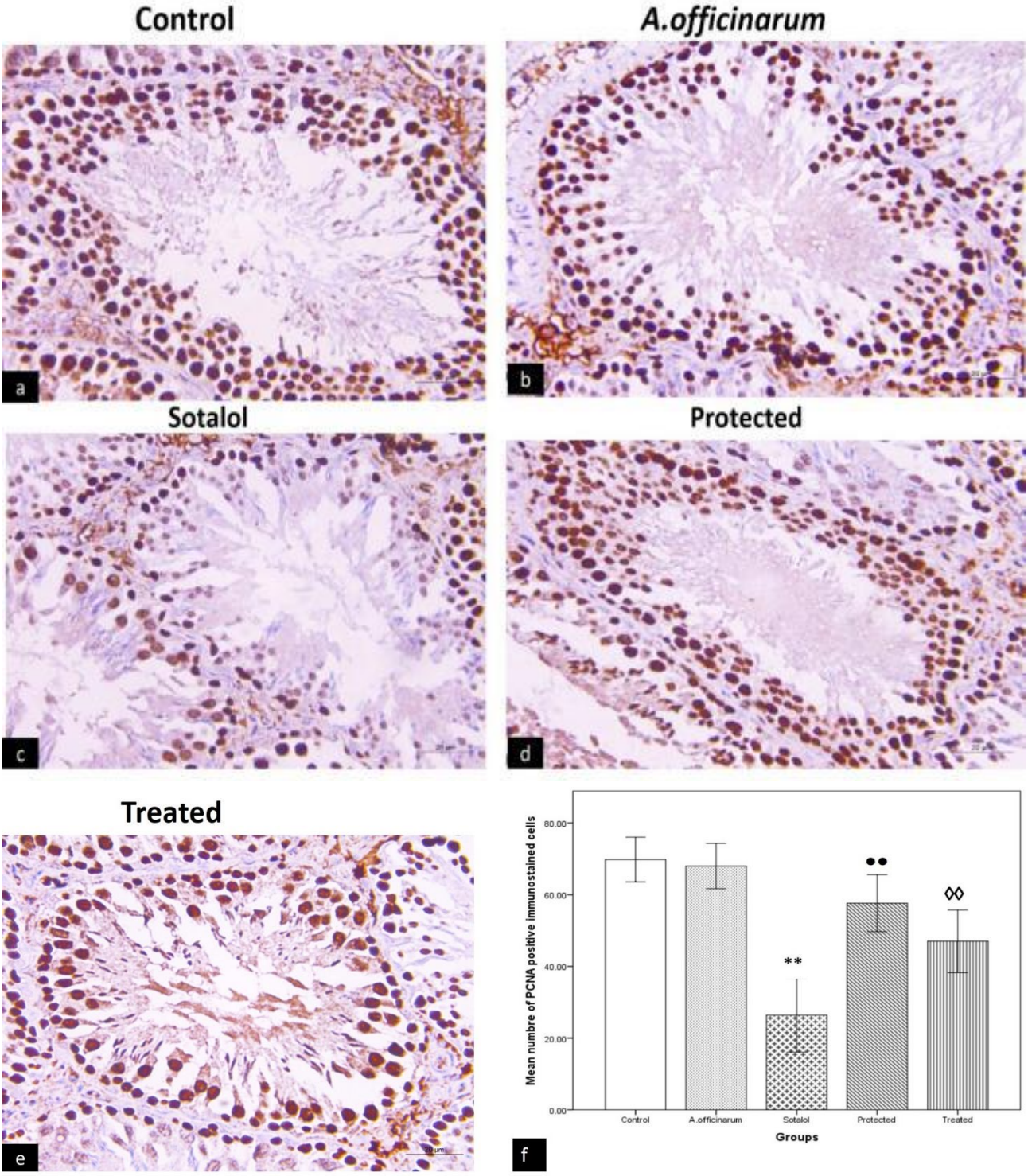

Figure 4: photomicrographs of sections of testis a) Control group b) *A. officinarum* group d) protected group showing positive PCNA immunostaining (deep brown nuclear reaction) in most nuclei of the basal germ cells inside the seminiferous tubules. C) Sotalol group showing few positive PCNA immunostaining in basal germ cells). e) Treated group showing positive PCNA immunostaining (deep brown nuclear reaction) in most nuclei of the basal germ cells inside the seminiferous tubules (PCNA X400). f) Histogram 3 showing mean number of PCNA positive cells.** Significant decrease in number of PCNA positive cells in Sotalol group compared to the control group.●● Significant increase in number of PCNA positive cells in protected group compared to Sotalol group.◊◊ Significant increase in number of PCNA positive cells in the treated group compared to Sotalol group.

(d)Bax marker:

Control group and *A. officinarum* group showed negative reaction for Bax antibody (a marker for apoptosis) (**Fig.5a&b).**
Sotalol group showed increased area percentage of Bax immunostaining (cytoplasmic expression) **(Fig.5c).**
Protected group showed decreased area percentage of Bax immunostaining (cytoplasmic expression) **(Fig.5d).**
Treated group showed decreased area percentage of Bax immunostaining **(Fig.5e).**

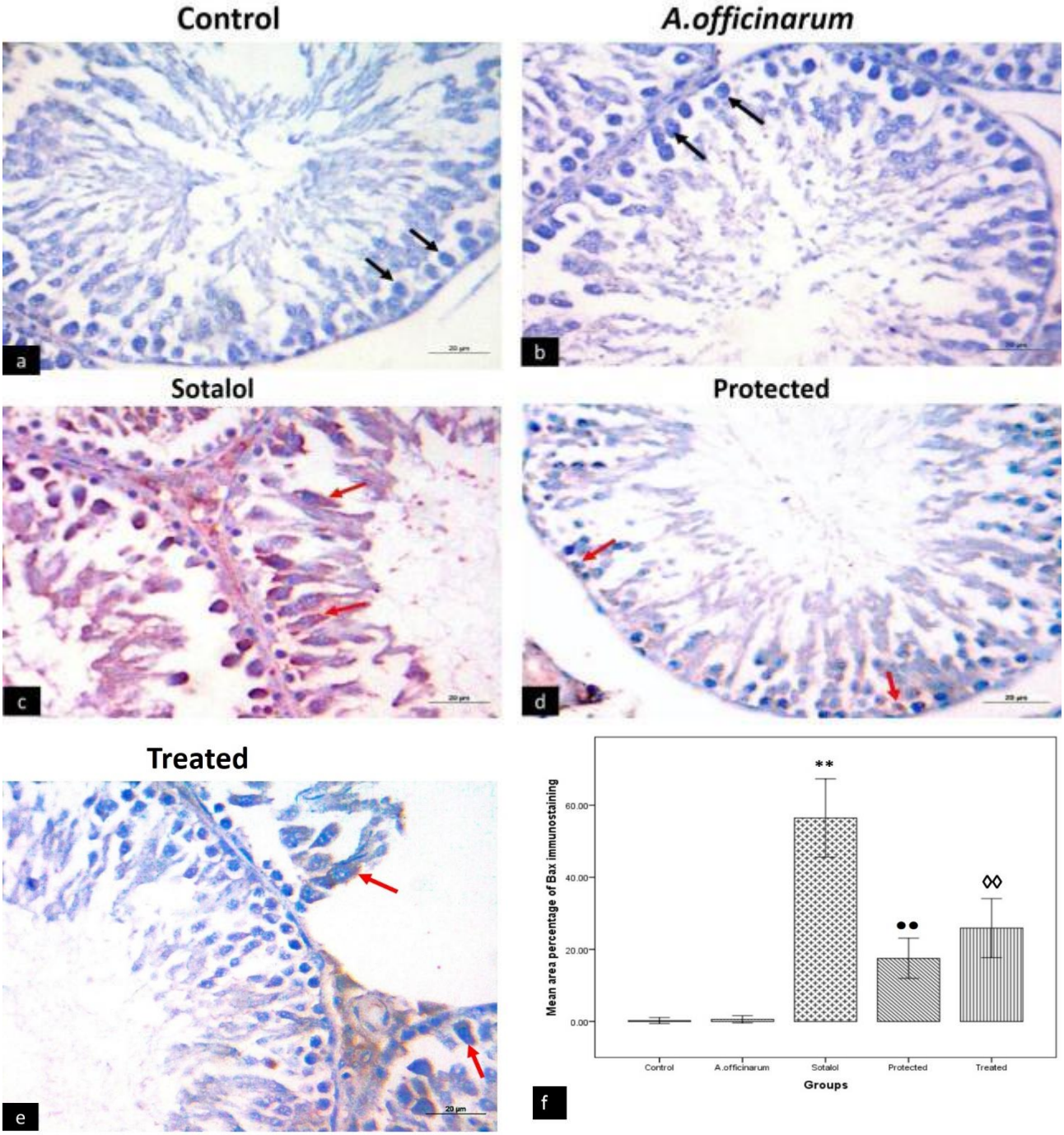

Figure 5: photomicrographs of sections of testis a) Control group b) *A. officinarum* group showing negative Bax immunoreaction (black arrows). c) Sotalol group showing many immunoreactive positive cells for Bax protein (cytoplasmic expression) (red arrows). d) Protected group showing some immunoreactive positive cells for Bax protein (cytoplasmic expression) (red arrows). e) Treated group showing decreased number immunoreactive positive cells for Bax protein (cytoplasmic expression) (red arrows) (Bax immunoreactivity X400). f) Histogram 4 showing mean area percentage of Bax immunostaining.** Significant increase of area percentage of Bax immunostainingin Sotalol group compared to the control group.●● Significant decrease of area percentage of Bax immunostaining in the protected group compared to Sotalol group.◊◊ Significant decrease of area percentage of Bax immunostainingin the treated group compared to Sotalol group.

Morphometric study and statistical analysis:

-Germinal epithelial height:

There was a significant decrease in the germinal epithelial height in Sotalol group (P <0.001) with a mean value of 10.92±3.96 compared to the control group with a mean value of 146.41±13.18, but there was no significant difference between *A. officinarum* group (P > 0.05)with a mean value of 142.37±9.30 compared to the control group. Also, there was a significant increase in both the protected group (P <0.001) with a mean value of 129.78±8.68& treated group (P <0.001) with a mean value of 125.54±8.88 as compared to Sotalol group. There was no significant difference in the germinal epithelial height in the protected group (P >0.05) compared to the treated group **(Fig.1f-histogram1).**

-Color intensity of PAS:

There was a significant decrease in the color intensity of PAS in Sotalol group (P <0.001) a mean value 136.17±24.95 compared to the control group with a mean value of 203.50±20.21, on the other hand, there was no significant difference in *A. officinarum* group (P >0.05) with a mean value of 204.64±25.19 compared with control group. Also, there was significant increase in the intensity in both protected group (P <0.05) with a mean value of 169.96±5.88 & treated group (P <0.05) with a mean value of 169.98±5.44 as compared to Sotalol group. There was no significant difference in the color intensity of PAS in the protected group (P >0.05) compared to the treated group **(Fig.3f-histogram 2).**

-Number of PCNA positive cells:

There was a significant decrease in the number of PCNA positive cells in Sotalol group (P <0.001) with a mean value 26.40±5.18 compared to the control group with a mean value of 69.80±3.11, on the other hand, there was no significant difference between *A. officinarum* group (P >0.05) with a mean value of 68.00±3.16as compared to the control group. Also, there was a significant increase in both the protected group (P <0.001) 57.60±3.97 and treated group (P <0.001) with a mean value of 47.00±4.36 as compared to Sotalol group. There was no significant difference in the number of PCNA positive cells in the protected group (P >0.05) compared to the treated group **(Fig.4f-histogram 3).**

- The area percentage of Bax +ve cells:

There was a significant increase in the area percentage of Bax positive cells in Sotalol group (P <0.001) with a mean value 56.44±5.43 compared to the control group with a mean value of 0.26±0.42, on the other hand, there was no significant difference between *A. officinarum* group (P >0.05) with a mean value of 0.25±0.37 as compared to the control group. Also, there was a significant decrease in both the protected (P <0.001) with a mean value of 17.52±2.79 and treated groups (P <0.001) with a mean value of 25.90±4.09 as compared to Sotalol group. There was no significant difference the area percentage of Bax positive cells in the protected group (P >0.05) compared with the treated group **(Fig.5f-histogram 4).**

DISCUSSION

Infertility in patients with chronic disease not only caused by pathogenesis of the disease, but also due to the medications that the patients receive [(18)]. Beta-blockers are widely used in treatment of cardiovascular diseases, but they were proved to cause many sexual adverse effects. Moreover, they have harmful effects on the process of spermatogenesis in the form of destruction of the germinal cells and deformities in sperm (head and tail) [(11)]. **Mohammadi *et al.*** [(19)] stated also that beta blockers as propranolol decrease sperm count and quality with decrease of testosterone level. Testicular atrophy, loss of libido, decrease in sperm motility, viability and count were reported after unilateral lumbar sympathectomy. Increased sexual adverse effects similar to lumbar sympathectomy were reported after the use of beta-blockers [(11)]. *A. officinarum* was found to improve reproductive function of male albino rat in the form of increase in the testosterone hormone level and increase sperm count. These effects were attributed to direct action of the plant extract on the testes [(20)].

Kolangi *et al.* [(8)] also stated that *A. officinarum* improved sperm quality and sperm count without causing adverse effects. These effects were explained to be due to the antioxidant and scavenging activity against the reactive oxygen species through plant components as galangin. Moreover, it improves the semen quality as reported by **Akbar** [(21)]**.** Our study showed that there was improvement in sexual behavior of both (protected and treated) groups in the form of significant decrease in mount latency and significant increase in mount frequency when compared with Sotalol group. *A. officinarum* was found to improve sexual desire and erection. Holding a piece of *A. officinarum* in the mouth caused erections of the penis as reported by **Akbar** [(21)]**. Oktay *et al.*** [(22)] postulated that amiodarone which belongs to group III antiarrhythmic drugs as Sotalol hydrochloride caused lipid peroxidation and increased MDA level. **Negm and Ragheb** [(20)] reported that *A. officinarum* improved oxidative stress in the form of increased in serum SOD and decreased serum MDA which indicates protection against lipid peroxidation. These findings can be attributed to the antioxidant properties of *A. officinarum* rhizome as a result of the presence of the compounds that scavenge reactive oxygen species as diarylheptanoid compounds, polyphenols, and flavonoids.

The testes of the rats received Sotalol showed a decrease in the germinal epithelial height. Also, the spermatogenic cells were degenerated and shed in lumens of the seminiferous tubules. The interstitium showed dilated congested blood vessels with deposition of homogenous acidophilic hyaline material resulting in widening of the interstitium. These findings are in agreement with **Mohammadi *et al.*** [(19)]**.** Sloughing of germ cells may be attributed to

decrease in the seminiferous tubules fluid secretion as a result of affection of Sertoli cells, which further resulted in sloughing or shedding of germ cell and their death [23]. **Duncker and Bache** [24] stated that dilated blood vessels can be attributed to the disturbed ratio of oxygen supply to oxygen needs leading to increasing adenosine production which in turn causes the dilatation of the vessels and the increase in testicular blood flow to restore that ratio to the equilibrium.

El-Ghawet [25] explained appearance of homogenous acidophilic exudates in the interstitium by lipid peroxidation and increased testicular levels of nitric oxide. Amiodarone which belongs to class III antiarrhythmic drugs as Sotalol was reported to cause an imbalance in reactive oxygen species and antioxidant defense mechanisms resulting in endothelial injury, which leading to edema, worsening inflammation, and severe lung injury [26]. In our study, the Sotalol group showed a significant decrease in PAS reaction in the basement membranes and in spermatogenic cells which indicated affection of structure and function of cells. This may be due to glycogen depletion as reported by **Matus *et al.*** [27] who stated that propranolol caused a decrease in the intensity of PAS reaction in hepatocytes with glycogen depletion. While, there was a strong positive PAS reaction in the interstitium which may be attributed to deposition of hyaline material in it. In the present study, PCNA immunostaining of Sotalol-treated testes showed few positive basal spermatogenic cells with a highly significant decrease when compared to the control group. These findings indicated DNA damage according to **Badr El-Din and Abd-El Aty** [28]**.**

This may confirm the oxidative stress and reactive oxygen species overproduction induced by Sotalol. **Wandrer *et al.*** [29] reported that amiodarone caused apoptosis in hepatocytes and attributed the apoptosis to mitochondrial dysfunction leading to an increase in the production of reactive oxygen species (ROS) which causes the peroxidation of fatty acids and thus the generation of pro-inflammatory cytokines. In the protected group, there was preserved architecture of testis with regular seminiferous tubules and significant increase in the germinal epithelial height. There was also strong PAS positive reaction. Moreover, the treated group showed restoration of germinal epithelium in most of the seminiferous tubules with significant increase in germinal epithelial height. There was a strong PAS positive reaction compared to Sotalol group.

There was a significant increase in PCNA positive cells and decrease the area percentage of Bax positive cells in both protected and treated groups when compared with Sotalol group. These effects can be attributed to the antioxidative properties of the rhizomes of this plant which prevent the inflammatory cascade and prevent the damage of DNA as reported by **Alasmary *et al.*** [30]**.** Galangin which is main constituent of *A. officinarum* was reported to have the ability to reduce the production of reactive oxygen species and decrease apoptosis by inhibiting caspase, down-regulation of Bax and up-regulation of Bcl-2 expression [31]**.**

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REFERENCES

1. **Fahim A, El-Fattah A, Sadik N *et al.* (2019):** Resveratrol and dimethyl fumarate ameliorate testicular dysfunction caused by chronic unpredictable mild stress-induced depression in rats. Archives of Biochemistry and Biophysics, 665(1): 152-165.
2. **Atoigwe-Ogeyemhe B, Achukwu P, Odigie E (2019)**: Assessment of mating profile of male Wistar rats administered single and pooled extracts of Phoenix dactylifera and Cocosnucifera. Journal of Veterinary Sciences, 17(1): 38- 48.
3. **Semet M, Paci M, Saïas-Magnan J *et al.* (2017):** The impact of drugs on male fertility: a review. Andrology, 5(4): 640-663.
4. **Barton A, McGowan M, Smyth A *et al.* (2020):** Classification and choice of antiarrhythmic therapies. Prescriber, 31(3), 11-17.
5. **Kpaeyeh J,Wharton J (2016):** Sotalol**.** Cardiology and Electrophysiology Clinic, 8(2):437-452.
6. **Honmore V, Kandhare A, Kadam P *et al.* (2019):** Diarylheptanoid, a constituent isolated from methanol extract of *Alpinia officinarum* attenuates TNF-α level in Freund's complete adjuvant-induced arthritis in rats. Journal of Ethnopharmacology, 229: 233-245.
7. **Heidari H, Abdollahi M, Khani S *et al.* (2021):** Effect of *Alpinia officinarum* extract on reproductive damages in streptozotocin induced diabetic male rats. Journal of Diabetes and Metabolic Disorders, 21: 1-9.
8. **Kolangi F, Shafi H, Memariani Z *et al.* (2019):** Effect of *Alpinia officinarum Hance* rhizome extract on spermatogram factors in men with idiopathic infertility: A prospective double-blinded randomized clinical trial. Andrologia, 51(1): 13172-13182.
9. **Katagi J, Nakamura Y, Cao X *et al.* (2016):** Why can dl-sotalol prolong the QT interval in vivo despite its weak inhibitory effect on hERG K+ channels *in vitro*? Electrophysiological and pharmacokinetic analysis with the halothane-anesthetized guinea pig model. Cardiovascular Toxicology, 16(2): 138-146.
10. **Rajendiran V, Natarajan V, Devaraj S (2018):** Anti-inflammatory activity of *Alpinia officinarum Hance* on rat colon inflammation and tissue damage in DSS induced acute and chronic colitis models. Food Science and Human Wellness, 7(4): 273-281.
11. **Eren H, Sivrikaya A, Cobanoglu U *et al.* (2018):** Investigation the effect of propranolol, metoprolol and carvedilol on spermatogenesis in rat testis. International Journal of Research in Medical Sciences, 6(4): 1082-1091.
12. **Abass S, Abdel-Hamid N, Abouzed T *et al.* (2018):** Chemosensitizing effect of *Alpinia officinarum* rhizome extract in cisplatin-treated rats with hepatocellular carcinoma. Biomedicine and Pharmacotherapy, 101: 710-718.

13. **Zade V, Dabhadkar D, Thakare V *et al.* (2013):** Effect of aqueous extract of Moringaoleifera seed on sexual activity of male albino rats. Internet Journal of Biochemical Forum, 5: 129-40.
14. **Saheed S, Oladipipo A, Temitope B *et al.* (2016):** Aqueous extract of Cyperusesculentus L. restores and boosts sexual competence in paroxetine-dysfunctioned male Wistar rats. Journal of Experimental and Integrative Medicine, 6(1): 12-20.
15. **Sarkar R, Ghosh P, Tripathy A *et al.* (2019):** Correction of diabetes-induced testicular dysfunction by a hydro-methanol (60: 40) extract of Curcuma amada rhizomes: A dose-dependent study. Journal of Food Biochemistry, 43(5):12829-12838.
16. **Ashidi J, Owagboriaye F, Yaya F *et al.* (2019):** Assessment of reproductive function in male albino rat fed dietary meal supplemented with Mucunapruriens seed powder. Heliyon, 5(10): 2716- 2729.
17. **Shakeri F, Soukhtanloo M, Boskabady M (2017):** The effect of hydro-ethanolic extract of Curcuma longa rhizome and curcumin on total and differential WBC and serum oxidant, antioxidant biomarkers in rat model of asthma. Iranian Journal of Basic Medical Sciences, 20(2): 155-163.
18. **Drobnis E, Nangia A (2017):** Cardiovascular/ pulmonary medications and male reproduction. Adv. Exp. Med. Biol., 1034:103-130.
19. **Mohammadi S, Beheshti F, Elyasi S *et al.* (2020):** The effect of propranolol on sperm parameters. CatSper 2 gene and protein expression, and oxidative stress in adult Mice. Journal of Pharmaceutical Research International, 20: 54-63.
20. **Negm S, Ragheb E (2019):** Effect of *Alpinia officinarum Hance* on sex hormones and certain Biochemical Parameters of Adult Male Experimental Rats. Journal of Food and Dairy Sciences, 10(9): 315-322.
21. **Akbar S (2020):** *Alpinia officinarum Hance*. (Zingiberaceae). I Handbook of 200 Medicinal Plants. Springer, Cham., pp. 217-224. https://link.springer. com/chapter/ 10.1007%2F978-3-030-16807-0_20
22. **Oktay S, Turkyilmaz I, Kaya S *et al.* (2018):** Effects of combined treatment of amiodarone and vitamin U on rat gingiva. Indian Journal of Experimental Biology, 56: 759-763.
23. **Abdellatief R, Elgamal D, Mohamed E (2015):** Effects of chronic tramadol administration on testicular tissue in rats: an experimental study. Andrologia, 47(6): 674-679.
24. **Duncker D, Bache R (2008):** Regulation of coronary blood flow during exercise. Physiological Reviews, 88: 1009-1015.
25. **El-Ghawet H (2015):** Effects of tramadol on the reproductive function of Wistar Albino rats. Eur. J. Exp. Biol., 5: 56-64.
26. **Feduska E, Thoma B, Torjman M *et al.* (2021):** Acute Amiodarone Pulmonary Toxicity. Journal of Cardiothoracic and Vascular Anesthesia, 35(5):1485-1494.
27. **Matus G, Pereira B, Silva-Zacarin E *et al.* (2018):** Behavior and histopathology as biomarkers for evaluation of the effects of paracetamol and propranolol in the neotropical fish species Phallocerosharpagos. Environmental Science and Pollution Research, 25(28): 28601-28618.
28. **Bader El-Din S, Abd-El Aty O (2012):** Biochemical and immunocytochemical studies of the testicular changes after treatment with duloxetine hydrochloride and the possible protective effects of omega 3 in adult rat model of depression. AAMJ, 10(3): 264-300.
29. **Wandrer F, Frangež Ž, Liebig S *et al.* (2020):** Autophagy alleviates amiodarone-induced hepatotoxicity. Archives of Toxicology, 94(10): 3527-3539.
30. **Alasmary F, Assirey E, El-Meligy R *et al.* (2019):** Analysis of *Alpina officinarum Hance*, chemically and biologically. Saudi Pharmaceutical Journal, 27(8): 1107-1112.

    Li S, Wu C, Zhu L *et al.* (2012): By improving regional cortical blood flow, attenuating mitochondrial dysfunction and sequential apoptosis galangin acts as a potential neuroprotective agent after acute ischemic stroke. Molecules, 17(11): 13403-13423.