Original Article

Effect of Prenatal Exposure to Tartrazine on the Postnatal Development of Gonads in Albino Rats and Protective Role of Sesame Seed Oil

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ABSTRACT

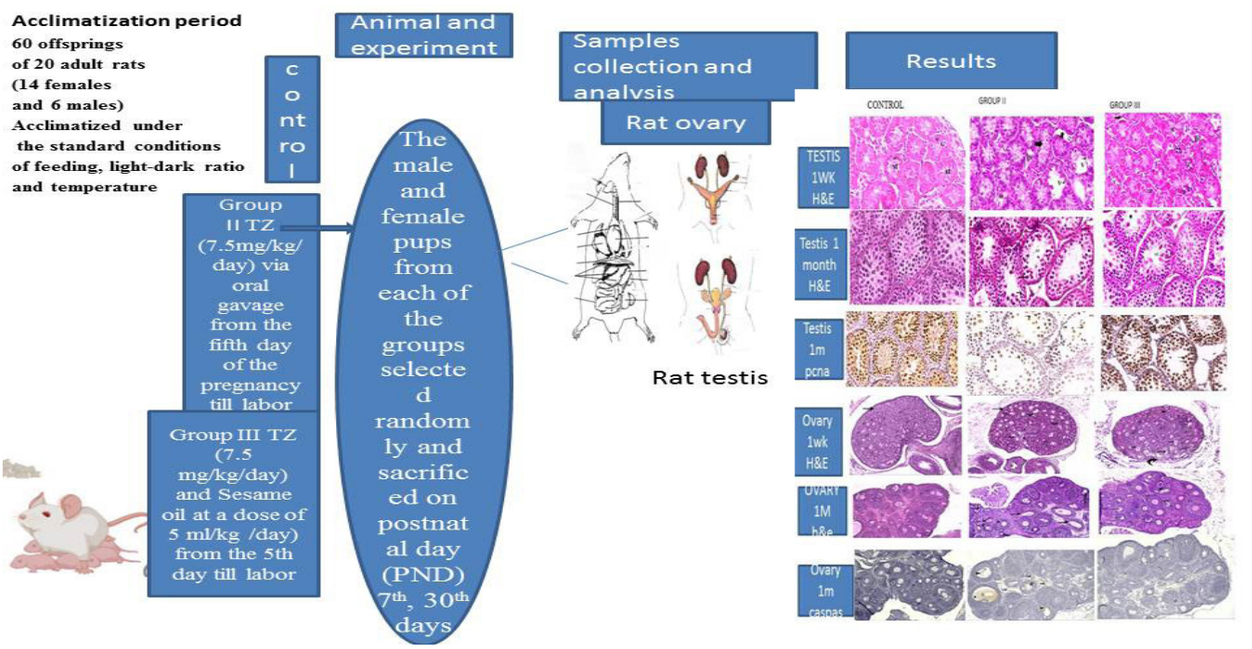
Introduction: Tartrazine is an artificial dye widely used in foods marketed especially towards children such as candy, ice cream, and soft drinks; it has harmful effects on different body organs and the maturation of male and female reproductive organs.

Aim of the Work: The present work aims to study the effect of tartrazine on the postnatal development of gonads and the protective effect of sesame oil.

Materials and Methods: This research was done on offspring of adult rats which were divided equally into 3 groups: Group I (Control Group): offspring of mother who received distilled water. Group II (Tartrazine group): offspring of mothers who received TZ (7.5mg/kg/day) via oral gavage from the fifth day of the pregnancy till labor. Group III (sesame oil group offspring of mothers who received TZ (7.5 mg/kg/day) via oral gavage and oral administration of Sesame oil at a dose of 5 ml/kg /day) from the 5th day of pregnancy till labor.

Results: In tartrazine treated group the ovary appeared reduced in size, the follicles appeared with destructed granulosa cells and empty oocytes while in the testis the seminiferous tubules showed disorganization of most germinal epithelial cells and vacuolations in Leydig cells. The sesame and tartrazine treated group has a weak role in protection where the ovary appeared with destructed granulosa cells and oocytes while in male rats there was a mild improvement. the immunohistochemical result there was a reaction to caspase -3 in ovarian tissue and to PCNA stain in the testicular tissue.

Conclusion: This study discovered that tartrazine leads to disruptions and distortions of ovarian and testicular cells and administration of sesame oil had a weak observable effect.



Graphical Abstract

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Key Words: Caspase, ovary, PCNA, tartrazine, testis.

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INTRODUCTION

Tartrazine is a member food additive; its substance is added to food to protect flavor or work on its taste[1].

It is an azo dye of a chemical substance obtained from coal tar, is rapidly soluble in water, and can be used in various ways such as in the food, cosmetic, and pharmaceutical industries[2].

Its toxicity is due to its transformation to azo bonds which lead to the production of toxic oxidative substances such as aryl amines, reactive amines, and free radicals[3].

Some research approved that tartrazine has mutagenic and teratogenic adverse effects on the brain, immune system, genetic materials, and cardiovascular and reproductive organs[4].

Other research approved that tartrazine affected the level of estradiol hormone which causes hormonal disturbance and apoptosis of ovarian cells[5].

The usage of tartrazine for a long time produces a reduction in motility and density of sperm through the reduction of tester one secretion[6].

Sesame seed oil (SO) is a palatable oil harvest, it has high lipid and protein content, and it is rich in bioactive compounds such as (phytosterol, fatty acid, tocopherol, sesame, and other multiple vitamins)[7].

(SO) has anti-inflammatory, antioxidant, and anti-cancer, and its activity through a decrease in lipid peroxidation by the collaborative effects between its components as lignans and tocopherols[8].

It has a protective effect by inhibiting the production of free radical reactive oxygen species (ROS) and decreasing lipid peroxidation to reduce damage to the organ caused by different endotoxins[9].

It has a role in the improvement of gonad function as the production of sperm, oogenesis, increasing oocyte quality, and the testicular parameters due to its antioxidant effects[10,11].

AIM OF THE WORK

The present work aims to study the effect of tartrazine on the postnatal development of gonads and the protective effect of sesame oil on albino rats.

MATERIAL AND METHODS

Chemicals and preparations

Tartrazine was brought and it was given at a dose of (7.5 mg/kg/day) via oral gavage[12].

Sesame oil (SO) will be purchased from a local market in Sohag and orally given daily at a dose of 5 ml/kg body weight[13].

Animals and treatments

In this study a total of 20 adult albino rats; 14 females their weight range from 200-250g were used and 6 males. The animals were brought from the animal house of the Sohag faculty of science. They were reared under the standard conditions of feeding, light-dark ratio, and temperature, in the Sohag Faculty of Medicine animal house.

Rat was kept in polycarbonate cages in a ratio of 1:2 males and females. Females were examined for vaginal plugs (an indication of the presence of sperm in the vagina) and separated for the experimental protocol and their gestational days were recorded.

The animals will be subdivided into three groups:

Groups

Group I (Control Group): include (20) offspring of mothers who received distilled water.

Group II (Tartrazine group): Includes (20 (offspring of mothers who received TZ (7.5mg/kg/day) via oral gavage from the fifth day of the pregnancy till labor.

Group III (sesame oil group) Includes (20(offspring of mothers who received TZ (7.5 mg/kg/day) via oral gavage and oral administration of Sesame oil at a dose of 5 ml/kg / day) from the 5th day of pregnancy till labor.

Method

The male and female pups from each of the groups were selected randomly and sacrificed on postnatal day (PND) 7th, and 30th days, the animals were anesthetized by intramuscular IM injection of a mixture of Ketamine (90 mg/kg body weight) and Xylazine (10 mg/kg body weight) then their ovaries and testis were taken for light and immunohistochemical study.

Light Microscopic Study

The specimens were fixed by 10% formalin and stained by hematoxylin and eosin.

Immunohistochemical staining: sections were boiled in 10 mm citrate buffer (AP9003) at pH 6 for 10 minutes to retrieve antigen, then incubated for 1h with the following antibodies.

1. Proliferating cell nuclear antigen (PCNA) for testicular tissue[14].
2. caspase-3 (rabbit polyclonal antibody, ab13847) for apoptosis[15].

Ethical approval

The experiment was performed according to the guidelines of the Sohag University Committee for Animal Care with approval certificate number 5-12-2023-07. The experiment conformed to the "Guide for the Care and Use of Laboratory Animals" for the use and welfare of experimental animals.

Morphometric and statistical study

The following measures were taken:

A. Diameter of primary and secondary follicles at the age of 1weak

B. Diameter of secondary and tertiary follicles at the age of 1 month

C. Area percent of PCNA immunoreaction was measured using an objective lens of x40 magnification.

10 nonoverlapping fields for each section were taken. This was done using Image J software (version 1.51k, Wayne Rasband, National Institutes of Health, USA). From each variable, the mean± SD (Mean ± standard deviation of mean) was measured using SPSS program version 16. one-way analysis of variance and a post-hoc test was used to find the statistical difference between the groups when the *P value* was ≤ 0.05 it was considered statistically significant.

RESULT

Light microscopic and Histological examination results

At 1weak offspring rats (PND7)

Ovary

Examination of ovarian tissue of the control neonatal (PND7) offspring showed germ cells near the cortex, and several primordial follicles each primordial follicle was formed of a single layer of flattened follicular cells surrounding 1ry oocyte, primary follicles also formed of a single layer of cuboidal follicular cells and immature secondary follicles were formed of two or more layers of cuboidal follicular cells. (Figure 1)

While in tartrazine treated group the ovary appeared reduced in size with an area of destruction appearing in the cortex. Some primordial follicles appeared with vacuolated cytoplasm, several primary and secondary follicles had destructed granulosa cells and the oocyte appeared with vacuolated cytoplasm and shrunken darkly stained nucleus (Figure 2), in tartrazine and sesame-treated group ovary reduced in size with irregularity in the surface epithelium and destruction in the cortex, some primary and secondary follicles appeared normal with intact granulosa cells and oocyte while other appeared with destructed granulosa cells and oocyte also some atretic follicles appeared at medulla (Figure 3).

Testis

Testicular tissue examination of the control neonatal (PND7) offspring showed the tubules lined by two types of cells; gonocytes and mitotically active Sertoli cells). The Sertoli cells rested along the basement membrane and had distinctive oval to elongated nuclei. The gonocytes (fetal germ cells), were mitotically inactive and appeared as a large round cell with a centrally located nucleus with dispersed chromatin and a prominent nucleolus. (Figure 4)

In tartrazine-treated rats, the seminiferous tubules had some variations in the size of the tubules some enlarged and some of the tubules shrunken and showed marked cellular destruction with large vacuoles presented also the interstitial spaces showed fewer Leydig cells. (Figure 5)

In tartrazine and sesame-treated rats, most of the seminiferous tubules were nearly normal but a few tubules were shrunken and had some dark nuclei with small vacuolations were found; also, the interstitial spaces were nearly normal. (Figure 6)

At 1month offspring rats (30PND)

Ovary

In the control group, the ovarian cortex appeared normal with a presentation of primary, secondary, and preantral follicles near it, follicles appeared with intact granulosa cells and oocytes with a central rounded nucleus (Figure 7), in tartrazine treated group the cortex appeared with the vacuolated area, the follicles appeared with destructed granulosa cells with the damaged oocyte, some follicles presented near medulla and appeared dilated, some primordial follicles still presented (Figure 8), in sesame and tartrazine treated group degree of destruction reduced some follicles appeared normal with intact granulosa cells and oocyte with central rounded nucleus and other follicles had damaged oocyte and destructed granulosa cells (Figure 9).

Testis

In the control group of one-month rats, testis showed well-organized, round Seminiferous tubules characterized by many layers of primary spermatocytes, secondary spermatocytes, primary spermatids, separated by Sertoli cells that had large vesicular nuclei and pale staining cytoplasm rested on the basement membrane. The interstitial spaces contained clusters of Leydig cells (Figure 10), in tartrazine treated group the seminiferous tubules appeared with a marked reduction of the number, disorganization, and vacuolation of most germinal epithelial cells, and most of the cells that line the tubules had pyknotic nuclei also congested blood vessel was present, with some area of hemorrhage, the interstitial space appeared with numerous vacuolation in Leydig cells (Figure 11), in sesame and tartrazine treated group showed nearly normal germinal epithelial lining cells which rest on the basement membrane, some tubules had some pyknotic cells, and some cells appeared vacuolated, and also less than normal Leydig cells with area empty interstitial space (Figure 12).

Immune histochemical stain in 1month (30PND) offspring rats

Ovary

Sections from the control show negative immunoreactivity to caspase-3 (Figure 13) while the tartrazine-treated group and in sesame and tartrazine-treated group show a mild positive reaction to caspase 3 in

the cytoplasm of the oocyte and a mild reaction appeared in the medulla (Figures 14,15).

Testis

Sections from the control showed a PCNA immune positive reaction in germinal epithelial cells in the seminiferous tubules (Figure 16), in tartrazine treated group had a mild PCNA immune reaction in the cells of seminiferous tubules (Figure 17) while in tartrazine and sesame oil showed moderate PCNA immune positive reaction cells in the seminiferous tubules (Figure 18).

Morphometric result

Ovary

In 1-week tartrazine-treated rats and tartrazine & sesame-treated rats there was a highly significant decrease in the diameter of the primary follicle compared to the control group.

Also, there mild significant decrease in the diameter of secondary follicles in both groups compared to the control one while no significant difference between tartrazine treated group and tartrazine and sesame-treated group which indicates no protective effect on sesame (Table 1, Histogram 1).

In the 1month tartrazine group, there was significant dilation in secondary follicles compared to no significant change in the diameter of tertiary follicles compared to the control group.

While tartrazine and sea seme treated group showed significant dilatation in the diameter of secondary follicles while no significant change in the diameter of tertiary follicles compared to the control group. also, there is no significant difference between the two treated groups (Table 2, Histogram 2).

Testis

In one month, tartrazine treated there was a negative reaction to PCNA compared to the control group and tartrazine and sesame treated group, while there was a moderate reaction to the PCNA in the tartrazine and sesame treated group compared to normal. (Table 3, Histogram 3).

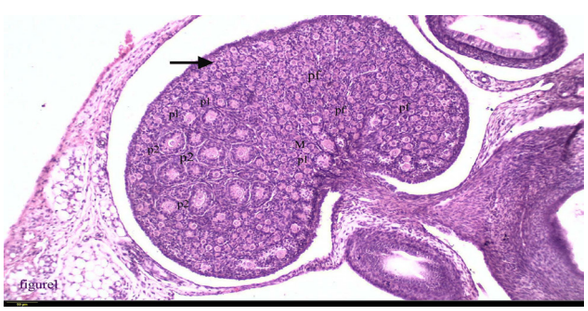


Fig. 1: section in the Ovary of 1weak control group showing the normal structure of surface epithelium with primordial germ cell near it (arrow) and its medulla (M) crowded with Primordial follicles (pf), Primary follicles (P1), and secondary follicle(p2) which appear clearly in the center.H&EX100.

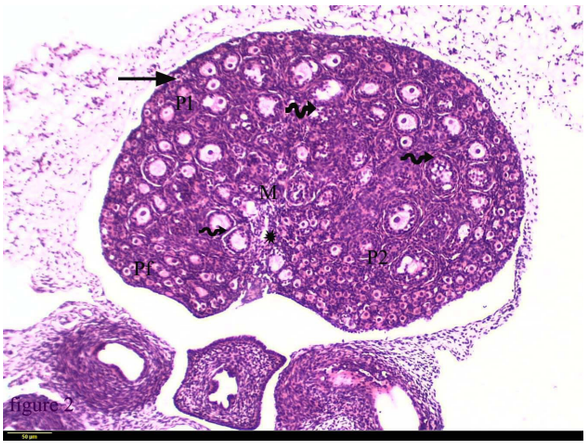


Fig. 2: section in the ovary of 1 weak tartrazine-treated rat shows a reduction in the size of the ovary, the surface epithelium is intact with Primordial germ cells present near it (arrow), primordial follicles(pf) appear near the cortex with vacuolated cytoplasm, several follicles show destruction in their granulosa cell and their oocyte (irregular arrow). (p2) Secondary follicles appear with destructed granulosa cells and empty oocytes.H&Ex100.

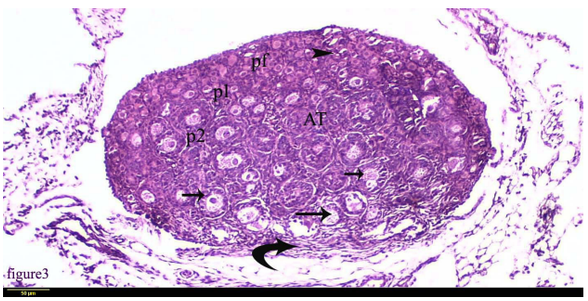


Fig. 3: section in the ovary of 1 weak tartrazine and sesame-treated rats shows a reduction in size of the ovary, cortex show area of destruction ( ), (pf)present near the cortex with area of destruction(arrowhead),(p1) primary follicle some appears normal and some appear destructed,(p2) (arrow)appear with destructed granulosa cell and damaged oocyte, several atretic follicles appear in the center(AT).H&EX100.

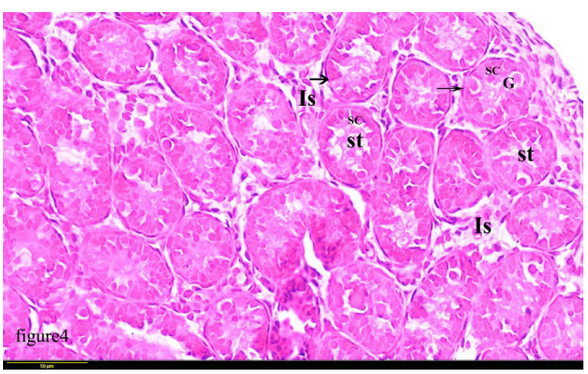


Fig. 4: section in the testis of 1weak control rat, showing the seminiferous tubules (st) lined by abundant mitotically active Sertoli cells (SC) that rest on the basement membrane. Gonocytes (G) are numerous, mitotically inactive, and have a large round nucleus centrally placed within the tubules. Intact Interstitial cells (IS) between the tubules. H&E 200.

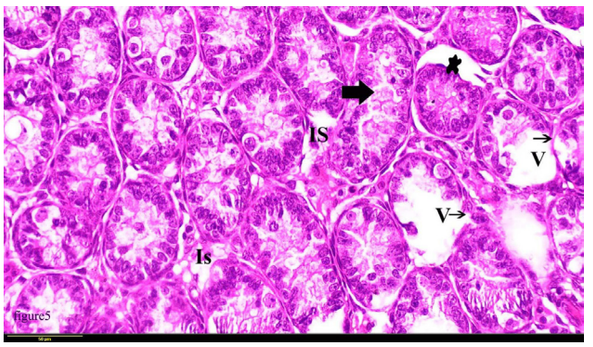


Fig. 5: section in the testis of 1weak tartrazine treated rat, showing some variations in the size of the seminiferous tubules some enlarged in size (arrow), some of the tubules are shrunken (star) and other tubules showing marked cellular destruction and large vacuoles are present (v), interstitial spaces show numerous vacuolation in Leydig cells (Is). H&E X200.

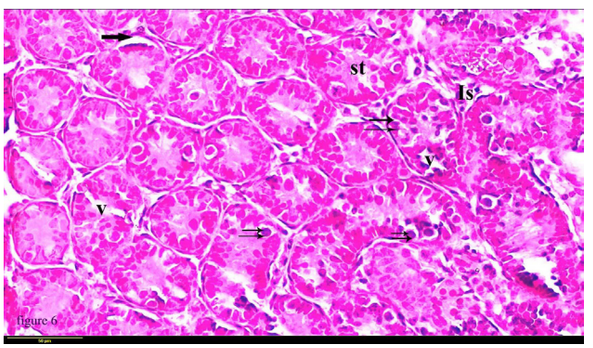


Fig. 6: section in the testis of 1weak tartrazine and sesame treated rat showing most of the seminiferous tubule is nearly normal (st), some of the tubules are shrunken (thick arrow) and some tubules show some dark nuclei (thin arrows) and small vacuolations are present (v), intestinal spaces have nearly normal Leydig cells (Is). H&E x200.

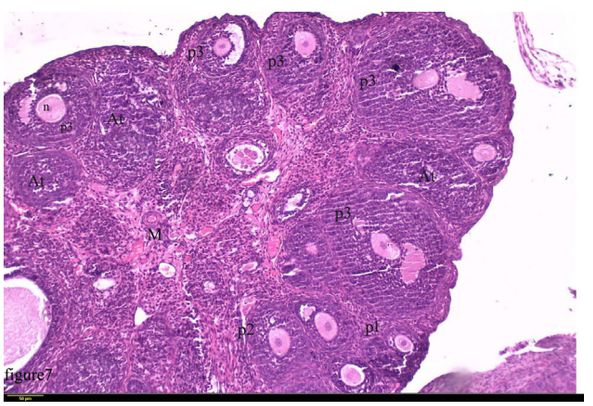


Fig. 7: section in the ovary of 1month control rats shows intact cortex with, numerous (p1) primary, (p2) secondary, (p3) preantral follicles appear with intact granulosa cell and oocyte with central rounded nucleus (n)and At atretic follicle present near cortex .H&EX100

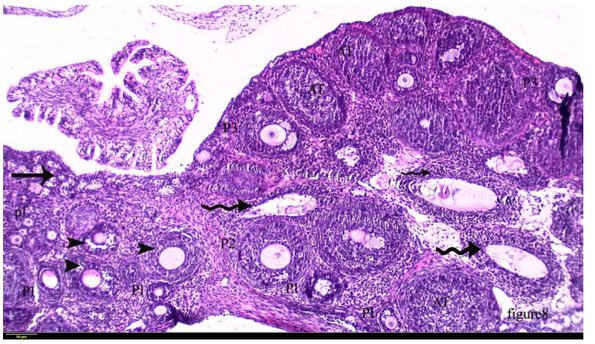


Fig. 8: section in the ovary of 1month tartrazine treated rats shows numerous types of follicles present in the cortex and medulla, (arrow) area of destructed follicles in the cortex,(pf)numerous primordial follicle present, (p1)primary follicles show destruction in the granulosa cell with the damaged oocyte,(p2)secondary follicle with granulosa cell near normal while oocyte with vacuolated cytoplasm,(arrowhead)secondary follicles with destructed granulosa cell and empty oocyte, medulla show numerous dilated and distorted follicles (irregular arrow), several atretic follicles also present (AT). H&EX100

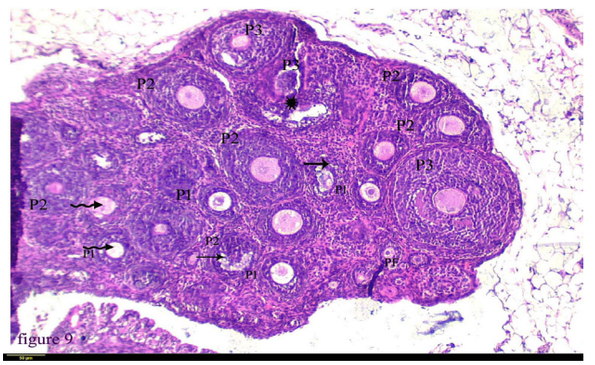


Fig. 9: section in the ovary of one-month tartrazine and sesame treated rat shows numerous types of follicles (pf)few primordial follicles still present, (p1) primary follicles. (p2) secondary, (p3)tertiary (preantral) follicles appear normal while other follicles show irregularity and destruction in the granulosa cell (arrow), and oocyte with vacuolated cytoplasm(irregular arrow).also destructed tertiary follicles present( ) .H&Ex100.

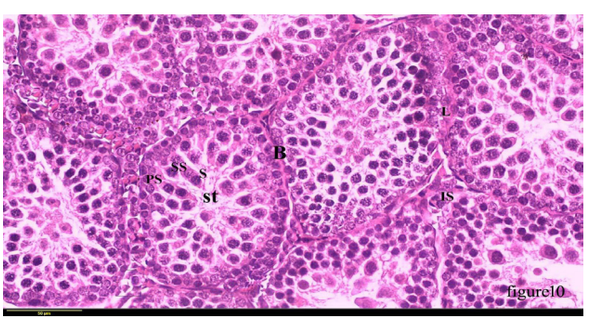


Fig. 10: section in the testis of one month control rat, showed seminiferous tubule (st) lines by many layers of primary spermatocytes (ps), secondary spermatocytes (ss) primary spermatids (S) separated by Sertoli cells (SE) that have large vesicular nuclei and pale staining cytoplasm resting on the basement membrane (B). The interstitial spaces (IS) contain clusters of Leydig cells (L). H&E x200.

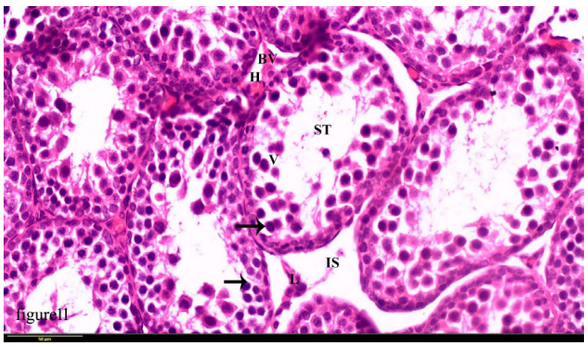


Fig. 11: section in the testis of one-month tartrazine-treated rats, showing seminiferous tubules (ST) with marked reduction of the number, disorganization, and vacuolation (V) of most germinal epithelial cells. A lot of cells that line the tubules show pyknotic nuclei (arrow). Congested blood vessel is presented (BV), with some area of hemorrhage (H). Interstitial tissue (IS) displays few Leydig cells (L). (H&E200)

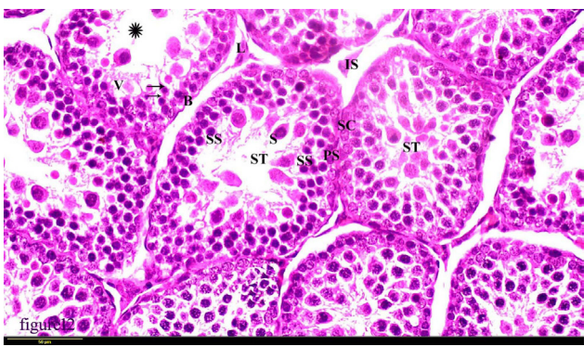


Fig. 12: section in the testis of one month rat treated with tartrazine and sesame oil showing speciously normal seminiferous tubules (ST) with nearly normal lining of primary spermatocytes (PS), secondary spermatocytes(SS), spermatids (S) and Sertoli cells (SC), which rest on basement membrane (B), some tubule(star) showing some pyknotic cells(arrow), and some vacuolated cells(v) Leydig cells appears (L) less than normal in interstitial space (IS) (H&E 200)

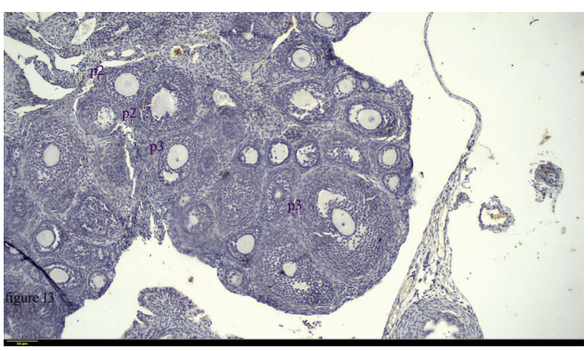


Fig. 13: immunohistochemical section in the ovary of 1month control rats showing negative immunoreaction for caspase 3 in the follicular cells of the primary (p1), the secondary (p2) and tertiary (preantral) follicles. (Caspase 3 x100)

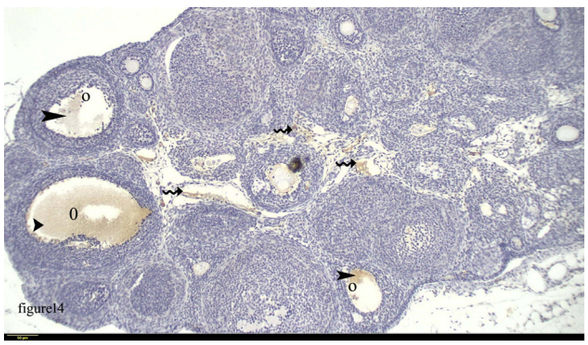


Fig. 14: The immunohistochemical section in the ovary of 1month tartrazine treated rats shows a mild positive immune reaction to caspase 3 in the cytoplasm of the oocyte (arrowhead)and some areas of the medulla (irregular arrow) (caspas-3x 100)

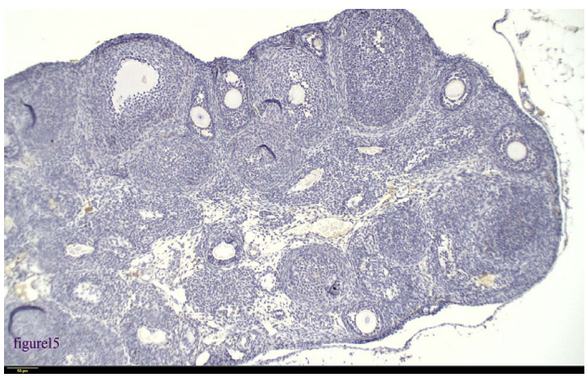


Fig. 15: immunohistochemical section in the ovary of 1 month tartrazine and sesame-treated rats show a mild positive immune reaction to caspase 3 in the cytoplasm of the oocyte and in some areas of the medulla. (Caspas-3x 100)

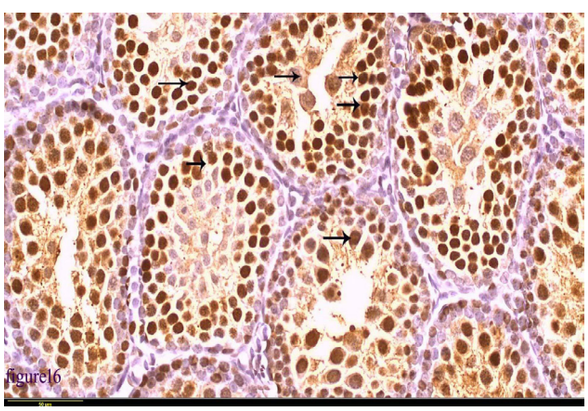


Fig. 16: immunohistochemical section in the testis of one month control rat presenting abundant PCNA immune positive reaction in germinal epithelial cells of the seminiferous tubules (arrow). (PCNA ×200).

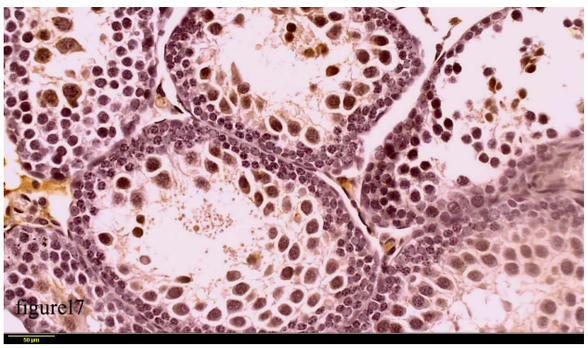


Fig. 17: immunohistochemical section in the testis of 1one month treated rat with tartrazine showing little PCNA immune positive reaction cells of the seminiferous tubules (PCNA ×200)

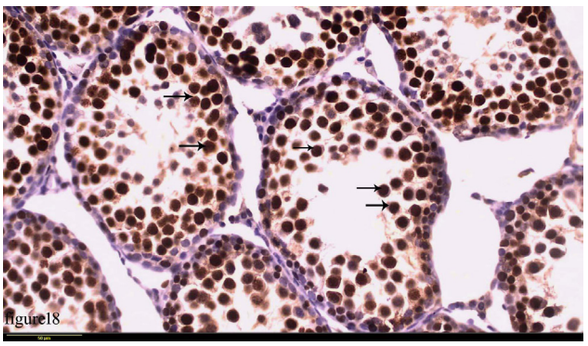


Fig. 18: photomicrograph of a section in the testis of one month rat treated with tartrazine and sesame oil, presenting numerous PCNA immune positive reactions in cells of the seminiferous tubules (arrow) (PCNA ×200)

Table 1: statistics for mean ±SD of the diameter of primary and secondary follicles in 1weak rats in different groups. (p1) difference between control and tartrazine treated group, (p2) difference between tartrazine treated group and tartrazine and sesame treated group, (p3) differences between control and tartrazine end sesame treated group .

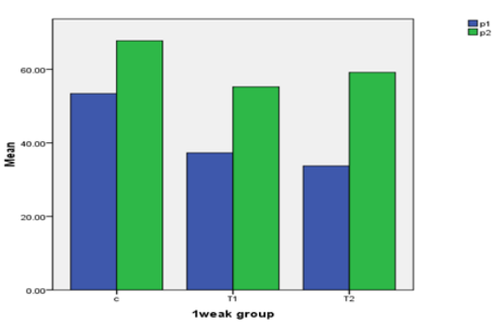
Group	Control	Tartrazine treated group	Tartrazine and sesame treated group	P1	P2	P3
Primary follicles	53.42±4.96	37.3±7.1	33.75±7.82	0.000	0.141	0.000
Secondary follicles	67.77±11.8	55.24±12.8	59.18±5.62	0.003	0.216	0.006

Table 2: statistics for mean ±SD of the diameter of secondary and tertiary follicles in 1month rats in different groups.

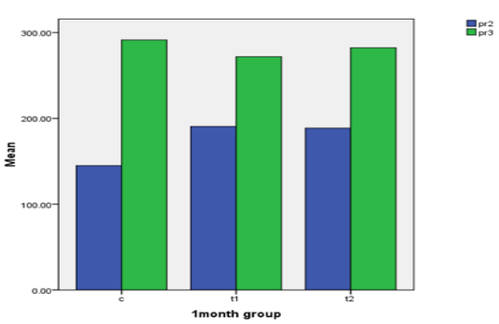
Group	Control	Tartrazine treated group	Tartrazine and sesame treated group	P1	P2	P3
secondary follicles	144.92±33.89	190.58±38.87	188.64±39.48	0.000	0.876	.001
Tertiary follicles	291.37 ±50.93	271.86±47.8	282.22±56.79	.219	0.553	.609

Table 3 : statistics for mean ±SD of the area percentage of PCNA in seminiferous tubules of 1month rats in different groups:

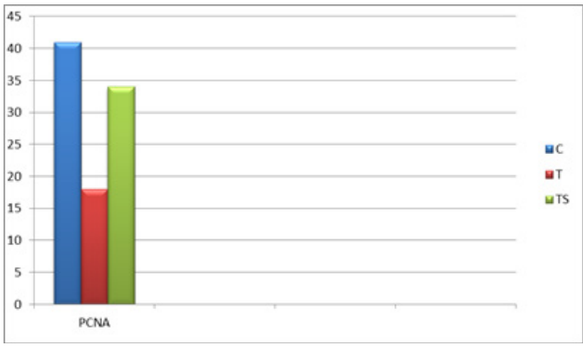
	Control	group II	Group III	P1	P2	P3
Area percentage of PCNA	2.1 41.78±	18.77± 8.07	34.43± 4.27	.000	.01	.01



Histogram1: difference in the diameter of primary and secondary follicle in 1weak rats in different group.



Histogram2: difference in the diameter of secondary and teritary follicle in 1month rats in different group.



Histogram3: difference in the PCNA immunoreaction area percentage in 1month rats in different groups.

DISCUSSION

The use of food dyes is widely distributed but the extent of their effect on the organs is still scientifically controversial, especially at the accepted daily dose intake[16].

This work aimed to assess the effects of tartrazine on the development of the testis and ovary and the possible protective effect of sesame oil.

In our study, the histological examination of the ovary in offspring of tartrazine treated group showed a reduction in the size of the ovary with an area of destruction appearing in the cortex. primordial follicle appeared with vacuolated cytoplasm, several primary and secondary follicles with destructed granulosa cells and the oocyte appeared with vacuolated cytoplasm and shrunken darkly stained nucleus, there was a decrease in the diameter of primary &secondary follicles in 1weak treated rats. While 1-month offspring of treated rats' degree of destruction in the granulosa cell and oocyte reduced and some secondary follicles showed dilatation in the diameter.

The damaging effect of tartrazine on ovarian tissue resulted from its oxidative damage due to its effect on the production of reactive oxygen species (ROS) which induced biochemical and physiological lesions that may lead to the distortion of cells and damage their structure[17].

This comes with Elekima *et al*[13] who observed that the use of tartrazine over 60 days affected the integrity of the ovary but did not have an observable effect on the hormonal parameter,

And with Sharma[18]. who saw spaces between ovarian cells due to apoptosis of cells in rats treated with Krisi powder (mixture of tartrazine and sunset yellow 6).

Mindang *et al*[19] approved that administration of tartrazine affected on the production of hormones of the hypothalamic-pituitary-ovarian axis (LH and Estradiol). That leads to an increase in the number of ovarian follicles.

Also, Elekima & Nwachuku[20] approved that after acute treatment with azo dye (after 24 h of intraperitoneal injection) there was disorganization of follicular cells due to vacuolation between cells with normal theca interna and corpus luteum, while in chronic use of tartrazine (over 30,60.90 days), non-significant differences seen between the treated and control rats.

Ara C *et al*[21] found that the use of tartrazine causes disorganizations in follicular cells of the ovary and atresia of the follicle with mild vacuolations can also be seen within its cell.

In this study the histological examination of the testis in offspring of tartrazine treated showed some variations in the size of the tubules some enlarged and some of them were shrunken and showed marked cellular destruction with large vacuoles presented also the interstitial spaces showed decreased Leydig cells in 1wk rats, the destructive effect of tartrazine on the seminiferous tubules was extended up to the age of one month treated rats the seminiferous tubules appeared with marked reduction of the number with disorganization and vacuolation of most germinal epithelial cells, most of the cells that line the tubules had pyknotic nuclei also congested blood vessel was presented, with some area of hemorrhage, the interstitial tissue displayed few Leydig cells.

Tartrazine produces its effect on the testis by interrupting its normal functional genes and manipulating the receptor genes of fertility hormone that affect the process of spermatogenesis, consequently causing infertility[22].

In agreement with the previous results Wopara *et al*., 2021[22] and Boussada *et al*., 2017[23] proved that tartrazine caused damage to the germ cells of rat testis through increased production of ROS (reactive oxygen species) and oxidative stress that led to damage to their plasma membrane.

Dos Santos *et al*., 2022[24] proved that tartrazine's cytotoxic effect is not due to its oxidizing effects, but it has mutagenic and genotoxic hazards due to continuous DNA damage and increased vulnerabilities of cancers caused by steady intake of food containing TRZ.

Fijak *et al*., 2015[25] stated that the combined influence of tartrazine and erythrosine leads to reproductive hormone deficiency and excessive formation of cytokines such as TNF-α, IL-1α, and IL-1β that lead to inflammation in testicular cells testicular cell death.

Meena& Meena[26] approved that intake of tartrazine leads to reduced space between seminiferous tubules and limited Leydig cell, separation of their spermatogenic cells, some of the tubules had oval prolongation.

In this study, the use of sesame oil as an antioxidant where the balance between ROS produced by tartrazine and antioxidant enzymes to avoid cellular damage where it prevents overproduction of ROS and free radicals[10].

Tartrazine and sesame treated group at 1weak offspring ovary reduced in size with irregularity in the surface epithelium and area of destruction in the cortex, some primary and secondary follicles appeared with destructed granulosa cells and oocyte also some atretic follicles

appeared at medulla while in 1month offspring less degree of destruction compare to tartrazine treated group and in male rats the seminiferous tubules showed near normal germinal epithelial lining cells which rest on the basement membrane but some tubules had some pyknotic cells, and some cells appeared vacuolated, Leydig cells was nearly normal.

This is as Al-kadhi *et al*.[27] who Approved that sesame oil has modulator effects on the toxicity of the anticancer drug tamoxifen where the number of ovarian follicles and hormonal parameters had non-significant differences in the sesame and tamoxifen-treated group when compared to the control group.

In agreement with Marwa *et al*., 2022[28] proved that Sesame oil has good antioxidant, antiapoptotic, and anti-inflammatory effects on testicular damage, apoptotic enhancement, and induction of interstitial inflammation caused by acute kidney injury.

Also, Khaneshi *et al*., 2013[29] stated that sesame oil has an antioxidant effect that leads to a reduction of lipid peroxidation and oxidative DNA damage also shared in the reduction of mutagenic hazards resulting from any cellular oxidative stress.

Sesame was reported by Yusuf *et al*., 2018[30] to improve the histopathological disturbance of testicular tissue and disturbed sperm parameters in rats who received streptozotocin-induced diabetes and ketoconazole.

Elhamalawy *et al*[31] illustrated that sesame seed oil had antioxidant properties that attenuate the hepatotoxicity of Bisphenol-A. so it can categorize as edible oil with a high possible antioxidant activity.

The result of the immunohistochemical study with caspase -3 in ovarian tissue there was a mild positive reaction in both tartrazine treated group which indicate decrease destructive effect of tartrazine at age of 1month while the sesame and tartrazine treated group also showed a mild positive reaction to caspase -3.

Caspase 3 was used as an apoptotic marker to detect the increased activity of apoptosis, its pro-apoptotic protein which has an important task in the process of programmed cell death. Due to its important function in chromatin clumping, breaking down of DNA, and damage to cell membrane's integrity finally leads to the production of apoptotic bodies[32].

This agrees with Abdel-Aziz *et al*[33] who approved that use of tartrazine produced a high expression of caspase-3 protein in the cytoplasm of most follicular cells of the thyroid gland.

Also, abd - Elhakim *et al*.2019[34] found high immunoexpression of caspase-3 in the hepatic and renal tissue tartrazine-treated rats, where Caspase-3 is considered a main molecule in the apoptosis process.

Hassanin and Shenouda 2022[35] approved that tartrazine induced a significant increase in immune expression of caspase 3 at the tubular and acinar lining cells of the parotid gland.

PCNA consider as regulatory protein marker which related to DNA synthesis and initiation proliferation of the cell. So, the PCNA demonstration are used as a cell proliferation marker[36].

The testis is the site of spermatogenesis and spermatogenic cells have numerous types of proliferation in the testis. In this study we found control group showed PCNA immuno positive cells highly expressed in the seminiferous tubules which indicate spermatogenic cell proliferation, but tartrazine treated group show mild reaction to PCNA while tartrazine and sesame oil showed moderate PCNA immunopositive cells in the seminiferous tubules.

In agreement with Sakr *et al*., 2017[37] who stated that the PCNA index is a useful marker for assessing germ cell differentiation and spermatogenesis, as it decreases in the testis of rats exposed to tentative compounds that resulted in a reduction of proliferation and increase in apoptosis in the germinal epithelial cells of the rat seminiferous tubules.

El-Azab and El-Mahalaway, 2019[38] also reported a significant decrease in PCNA immunostaining with AgNPs. This was clarified by increased apoptosis and depletion of the active DNA contents in the dividing spermatogenic cells.

Tousson *et al*., 2011[39] evidenced a significant decrease in the PCNA index up to 25% of spermatogonia in testes of rats had reduced spermatogenesis due to prepubertal hypothyroidism whereas, in the control group, the PCNA index was positive in more than 96% of spermatogonia.

CONCLUSION

The results obtained in this study revealed that tartrazine causes disruptions and distortions of ovarian and testicular cells and administration of sesame oil had weak observable effect.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

تأثير التعرض قبل الولادة لماده الترترازين على تطور الغدد التناسلية بعد الولادة في الجرذان البيضاء والدور الوقائي لزيت بذور السمسم

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المقدمة: يعتبر التارترازين هو نوع من الصبغة الصناعية التي تحتوي على مجموعة الآزو وتستخدم على نطاق واسع في الأطعمة التي يتم تسويقها خصيصا للأطفال مثل الحلوى والآيس كريم والمشروبات الغازية وله آثار ضارة على أعضاء الجسم المختلفة، وعلى نضوج الأعضاء التناسلية لدى الذكر والأنثى.

الغرض من البحث: الهدف من هذا العمل هو دراسة تأثير التارترازين على تطور الغدد التناسلية بعد الولادة والتأثير الوقائي لزيت السمسم على الجرذان البيضاء من خلال الدراسة النسيجية والمناعية.

المواد المستخدمة: تم إجراء هذا البحث على عدد ٦٠ ذرية لـ ٢٠ فأراً بالغاً (١٤ أنثى و٦ ذكور) تم تقسيمهم بالتساوي إلى ٣ مجموعات: المجموعة الأولى (المجموعة الضابطة): وتضم (٢٠) ذرية لأمهات تلقت الماء المقطر. المجموعة الثانية (مجموعة التارترازين): تشمل (٢٠) ذرية لأمهات تلقت (٧,٥ ملغم/كغم/يوم) عن طريق التطعيم بالفم من اليوم الخامس للحمل حتى المخاض. المجموعة الثالثة (مجموعة التارترازين والسمسم) وتضم (٢٠) ذرية لأمهات تلقت تارترازين (٧,٥ ملغم/كغم/يوم) عن طريق التطعيم بالفم وإعطاء زيت السمسم عن طريق الفم بجرعة (٥ مل/كغم/يوم) من اليوم الخامس من الحمل حتى الولادة، ثم تم ذبح الفئران وأخذ العينات للدراسه الهستولوجيه والمناعية.

النتائج: في المجموعة المعالجة بالتارترازين ظهر المبيض في فترة ما بعد الولادة أصغر في الحجم مع ظهور اثار لتدمير في طبقة القشرة، و ظهرت عدة اثار تدمير في الحويصلات الأولية والثانوية للخلايا الحبيبية وأيضا أظهرت الخصية والأنابيب المنوية انخفاضًا ملحوظًا في العدد، واضطرابًا في معظم الخلايا التناسلية. اما المجموعة المعالجة بإضافة السمسم أظهرت دور السمسم الضعيف في الحماية ضد التارترازين في ذرية الإناث حيث ظهرت بعض تدميرات في الحويصلات الأولية والثانوية و الخلايا الحبيبية للمبيض بينما في الشهر الأول كانت درجة التدمير أقل مقارنة بالمجموعة المعاملة بالتارترازين ولكن في ذكور الجرذان ظهرت الخلايا التناسليه في الأنابيب المنوية افضل من المعالجه بمجموعه التارترازين ولوحظ وجود خلايا متغلظه ومتدهوره. مادة caspase كان هناك رد فعل إيجابي معتدل في كل من المجموعة المعالجة مع في أنسجة المبيض بالتارترازين والمجموعة المعالجة بالسمسم والتارترازين (في أنسجة الخصية كان هناك انخفاض معنوي للغاية في المنطقة المعروضة PCNA) مع ماده مقارنة بالمجموعة الضابطه وانخفاض معنوي معتدل مقارنة بالمجموعة المعالجة بالتارترازين والسمسم.

خاتمة: أظهرت النتائج التي تم الحصول عليها في هذه الدراسة أن التارترازين يسبب اضطرابات وتشوهات في خلايا المبيض والخصية، كما أن إعطاء زيت السمسم كان له تأثير ضعيف في حماية هذه الانسجه.