Chronic Reproductive Toxic Effects of Erythrosine B on Adult Female Albino Rats: Biochemical, Histopathological and Immunohistochemical Study

Yasmin F. olama[a], Sheren S. El-kholy[a], Abdelmonem G. Madboly[a], Rasha M. El-sawi[b], and Rabab Shaban El-Shafey[a]
[a]Forensic medicine and Clinical Toxicology Department, Faculty of Medicine, Benha University, Egypt. [b]Pathology Department, Faculty of Medicine, Benha University, Egypt

***Corresponding Author:** Yasmin F. Olama. **Email:** yasoolama010@gmail.com

ABSTRACT

The synthetic cherry-pink food colourant Erythrosine B (ErB), often known as Red No. 3, is extensively used in the food, medicine, and cosmetic industries. However, the evidence of its harmful effects on humans is still lacking, thus its usage is limited in most nations. The purpose of this research was to evaluate the long-term harmful effects of ErB on the ovaries of adult albino rats by weighing their bodies and reproductive organs compared to one another, as well as by employing biochemical, histological, and immunohistochemical techniques. Methods and materials: Thirty-two adult female albino rats were split into four groups: a control group (16 rats = "8 negative control & 8 positive control group"); a group that received ErB dissolved in distilled water at a dose of ErB 136 mg/kg once daily for 6 months; a group that recovered from ErB treatment (8 female rats) and continued to live for 1 month after ErB was discontinued; and a group that served as a comparison. The current investigation demonstrated that ErB has persistent toxic effects on female reproductive organs (ovaries), as shown by a decrease in both total body mass and ovarian weight. Alternating the normal structure of ovarian tissue with immunohistochemistry (positive reaction of caspase 3) confirmed the alterations in hormone levels (significant increase in follicular stimulating hormone and luteinizing hormone, while significant reduction in progesterone and estradiol) and oxidative stress markers (highly significant reduction of glutathione and increase of malondialdehyde) in ovarian tissue homogenate. The alterations were enhanced in the recuperation group. Ovary toxicity was seen when adult albino rats were given a daily oral dosage of 136 mg/kg ErB for the duration of the trial (7 months). After discontinuing ErB for a month, several of these alterations returned to normal.

Keywords: Oxidative stress, Caspase 3, Casomorphin, ovary, erythrosine B, and food colouring.

INTRODUCTION

Customer preferences may be significantly influenced by food colouring chemicals. However, food colouring may be compromised during processing. To make processed foods more visually appealing and to increase consumer demand, food colours are often added (Ramesh & Muthuraman, 2018).

Due to their resistance to pH, oxygen, and light, synthetic food colours are widely used in the food business rather than their natural counterparts. There is a reduction in both production costs and the potential for microbial contamination as a result of these processes (Downham & Collins, 2000; Lok et al., 2011; Gomes et al., 2013).

Erythrosine (E127) (ErB), sometimes known as Red 3, is a coal-based, cherry-pink food colouring ingredient. This is a xanthene dye with halogen added. Beverages, candies, and cake decorating supplies often include it (Epelde-Elezcano et al., 2016; Neeta, 2018).

Hyperthyroidism, higher levels of thyroid hormone, changes in how sensitive one is to light, changes in how one learns, and antipathetic reactions are all possible side effects of erythrosine(E127) (Neeta, 2018).

Inhibition of some metabolic enzymes in human microsomes and oxidative stress effect by the excess production of free radicals have been linked to protein, nucleic acid, lipid, membrane, and apoptotic cell death (Mizutani, 2009; Covarrubias et al., 2008; Halliwell, 2011; Zhang et al., 2016) as shown by Mizutani (2009).

Reproductive illnesses such endometriosis, polycystic ovary syndrome, and infertility have been linked to an imbalance of prooxidants and antioxidants (Maryanti et al., 2014).

Few studies have been done in Egypt on the toxic effects of ErB on the reproductive organs of male and female albino rats, so the purpose of this study was to use biochemical, histopathological, and immunohistochemical techniques to examine the effects of ErB as a food colouring agent on adult female albino rats and to see if any changes had occurred after the rats stopped receiving the substance for a month.

MATERIAL & METHODS

I-material:

A. -Chemicals:

- Both the biological stain (CAS Number:16423-68-0) and the erythrosine B powder (purity not less than 87 percent) were acquired from the Thermo Fisher Scientific firm in India.
- Misr Chemical Industries Company supplied the distilled water (Cairo, Egypt).

B. Animals:

This research involved 32 adult female Wistar albino rats from the Animal Department, Faculty of Veterinary Medicine, ranging in weight from 120 g to 150 g at the start of the trial. To ensure the health of the rats used in the research and to weed out any sick ones, they were given a week to acclimate at the Animal Department of Benha University's School of Veterinary Medicine. Wheat, bread, and milk were given to all the animals for 12 hours during the day and 12 hours at night. All animals were given ErB at the same time in the afternoon.

Estrus synchronisation was carried out throughout the first four days. Animals were grouped according to their estrous status, which was assessed by microscopy of vaginal swabs (weihe, 1987). Day 0 marks the beginning of the trial. Animals were considered to be in estrus on the day they were observed, with the next predicted estrus occurring on Day 5 of the research.

Methodology development and animal classification:

Eight rats were randomly assigned to each of four groups.

There was no intervention with the rats in Group I (the negative control group), and they had unrestricted access to food and distilled water throughout the research.

Rats in Group II (the solvent control group) were given one millilitre of distilled water orally once daily via gavage tube for the duration of the trial.

Abdel Aziz et al. report that in group III (the erythrosine B-treated females), rats were given a single dosage of ErB

(136 mg/kg) diluted in distilled water orally through gavage tube for 6 months (1997).

Female Recuperation Group (Group IV):

According to Abdel Aziz et al. (1997), rats in this group were given a single daily dosage of ErB (136 mg/kg) dissolved in distilled water orally by gavage tube for 6 months, after which they were given full access to food and water for another month before being left without ErB administration.

Methodology (research variables) Part II:

Body weight and ovarian weight were assessed in rats before and after treatment, and then every two weeks during the research period, using a delicate balance to ensure accuracy. At the conclusion of the sixth month of therapy, scarified rats had their ovaries removed along with any remaining fatty tissue and blood arteries.

B. Hormone test based on biochemical research:

After administering ether to put the animals to sleep, we positioned them on the proper apparatus and drew blood from their hearts using 5-milliliter syringes. Blood samples were stored in anticoagulant-free containers at 37 degrees Celsius for 15 minutes. Serum samples were taken from centrifuged blood, wrapped in parafilm, labelled, and refrigerated for three days before being analysed for various hormones (Picard et al., 2008).

Enzyme-Linked fluorescence assay (Anckaert et al., 2002) was used to measure testosterone, follicle-stimulating hormone (FSH), luteinizing hormone (LH), oestrogen (estradiol), and progesterone levels in serum using VIDAS commercial kits (BIOMERIEUX Company, France) (BIOMERIEUX Company, France).

Evaluation of Oxidative Stress Markers in Tissue Homogenate:

Animals were killed by scarification, and their ovaries were removed, weighed, and diced into minute pieces before being homogenised in a phosphate-buffered saline (PBS) solution at a pH of 7.4 containing 0.16 mg/ml heparin. Next, 5-10 ml of cold buffer (i.e., 50 mM potassium phosphate, pH 7.5) were added per gramme of tissue and homogenised using a glass homogenizer. The homogenates were centrifuged at 4000 RPM for 15 min at 4 oC, using a high-speed centrifuge (Type 3-30K, Sigma, Osterode-am-Harz, Germany), and the resulting supernatant was removed and stored at -80 oC for later use in determining the oxidative stress parameters (GSH and MDA) levels in the organ tissue using commercially available colorimetric methods (diagnostic kits supplied by Bio Diagnostic Company, Egypt (Hussein et al., 2018).

Bancroft and Gamble (2008) report that ovaries were fixed in Bouin's solution, a morphological analysis fixative, for their C-level histopathological research. The Pathology Department of the Animal Health Research Institute in Zagazig, Egypt, preserved the tissue samples for 6-8 hours in 70% alcohol before processing them via automated dehydration, paraffin embedding, sectioning, and staining in histology.

D- Immunohistochemical analysis: proliferating cell nuclear antigen (PCNA) was used in immunohistochemical (IHC) experiments to detect DNA replication (Happerfield et al., 1993). Positive cellular nuclei responses include brown and stringent ones.

Analyzing the Data

SPSS version 16 was used to tabulate and analyse the gathered data (SPSS Inc., Chicago, ILL, Company). Mean SD and range were used to represent quantitative data. Parametric and non-parametric variables were assessed using the student t-test and Mann-Whitney U (ZMWU) test, respectively (respectively). Multiple sets of numerical (parametric) data were compared using ANOVA (analysis of variance). The Kruskal Wallis test was

employed to analyse continuous, non-parametric data, and post hoc analysis was done to identify significant differences between groups. According to Greenberg and colleagues, a P value below 0.05 was deemed to indicate statistical significance in this study (1996).

RESULTS

The results of both negative and positive control female groups did not show any statistically significant differences, so, the data obtained for both groups were expressed in the figures and tables as one group "control".

I. **According to the body weight and relative organ weight,** The present work showed a significant ($p < 0.05$) decrease in mean values of body weight of the female ERB-treated group in comparison to the female negative control group after treatment for 6 months. However, there was a significant ($p<0.05$) increase in mean values of body weight of the female ErB-recovery group in comparison to the female ErB-treated group and a non-significant ($p > 0.05$) change in mean values of body weight of the female ErB-recovery group in comparison to the negative control group, as illustrated in **fig. 1.** The current study showed a significant ($p <0.05$) decrease in mean value of relative organ weight of female rats of the ErB-treated group in comparison to the negative control group at the end of 6th month. While, there was a significant increase in mean value of relative organ weight of female rats of the ErB-recovery group in comparison to the ErB-treated group and a non-significant decrease in mean values of ovary weight of the ErB-recovery group in comparison to the negative control group, as shown in **table 1.**

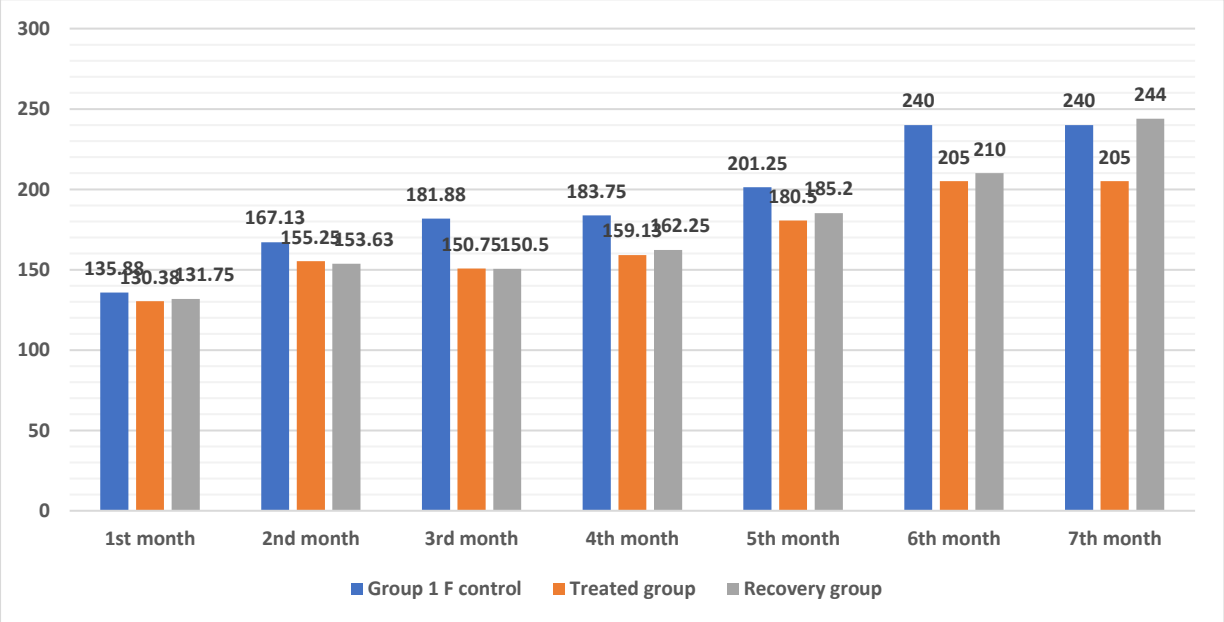

Figure (1): Comparison between the mean values of body weights among different groups (F= female).

Table (1): Comparison between the mean values of relative ovarian weight among different groups (n = 8, for each):

	group 1 F control (n=8)		Treated group (n=8)		Recovery group (n=8)		P value
	Mean	SD	Mean	SD	Mean	SD	
Organ weight 1	0.32	0.07	0.14	0.04	0.36	0.09	**<0.001***
Post-hoc			P1<0.001*		P1=0.32 P2<0.001*		
Relative Organ weight	0.0013	0.0003	0.007	0.0002	0.0014	0.0004	**<0.001***
Post-hoc			**P1=0.003***		**P1=0.79 P2<0.001****		

Data expressed as mean ± SD F:female
P:Probability *:significance <0.05 **:highly significance <0.001
P1: Significance vs Group 1 M control, P2: Significance vs Treated group

According to this study's hormonal assays, the ErB-treated group had lower mean values of testosterone than the control group, although the difference was not statistically significant ($p > 0.05$). Additionally, when comparing the treated and recovery groups, there was no statistically significant difference in mean testosterone level. Female rats given ErB had significantly lower mean levels of E2 and PG compared to controls (p 0.05). The female rats in the recovery group had significantly higher mean values of PG and E2 than those in the treatment group.

The present investigation found that the mean values of LH and FSH in ErB-treated female rats were significantly higher than those in control rats (p 0.05). Mean values of LH and FSH in female rats in the ErB recovery group were lower than those in the treatment group and the control group, respectively (table 2), with the difference between the two groups not being statistically significant ($p > 0.05$).

Table (2): Comparison between the mean values of hormonal levels (testosterone (TT), luteinizing hormone (LH), follicle-stimulating hormone (FSH), progesterone (PG) and estradiol (E2)) among different studied groups (n=8; for each):

	Group 1 F Control (n=8)		Treated group (n=8)		Recovery group (n=8)		P value
TT (ng-ml)	0.65	0.13	0.62	0.12	0.63	0.26	0.9
Post-hoc			P1=0.8		P1=0.9 P2=0.9		
LH (U IU-ml)	2.31	0.24	3.59	1.26	2.73	0.60	0.02
Post-hoc			P1=0.005*		P1=0.3		

					P2=0.04*		
FSH (U IU-ml)	0.48	0.16	1.28	0.30	0.65	0.22	<0.001**
Post-hoc			P1=<0.001**		P1=0.2 P2=<0.001*		
PG (ng-ml)	0.34	0.11	0.20	0.07	0.29	0.10	0.02
Post-hoc			P1=0.005*		P1=0.4 P2=0.04*		
E2 (Pg-ml)	111.65	9.11	97.98	7.42	108.64	11.85	0.03*
Post-hoc			P1=0.01*		P1=0.6 P2=0.04*		

Data expressed as mean ± SD F:female
P:Probability *:significance <0.05 **:highly significance <0.001
Test used: One way ANOVA followed by post-hoc LSD
P1: Significance vs Group 1 M control, P2: Significance vs Treated group

When comparing the female rats exposed to ErB to the female rats exposed to the negative control, there was a statistically significant (P0.001) rise in the mean values of malondialdehyde (MDA) and a decrease in the mean values of reduced glutathione (GSH) level. In the meanwhile, a females in the recovery group showed a statistically significant (P0.001) drop in MDA and an increase in GSH relative to females in the treatment group.

Ovarian tissue from the control group showed normal ovarian anatomy, including the presence of the ovarian cortex, interstitial tissue, blood vessels, and many primary follicles, as determined by the histopathological research. ErB-treated female rats showed vascular congestion and perivascular edoema, as well as atretic follicles surrounded by cellular vacuolation, bleeding, and vacuolation of parenchymal cells. As can be seen in figs. 2, 3, and 4, the moderate congestion of ovarian blood vessels also improved in the recovery group.

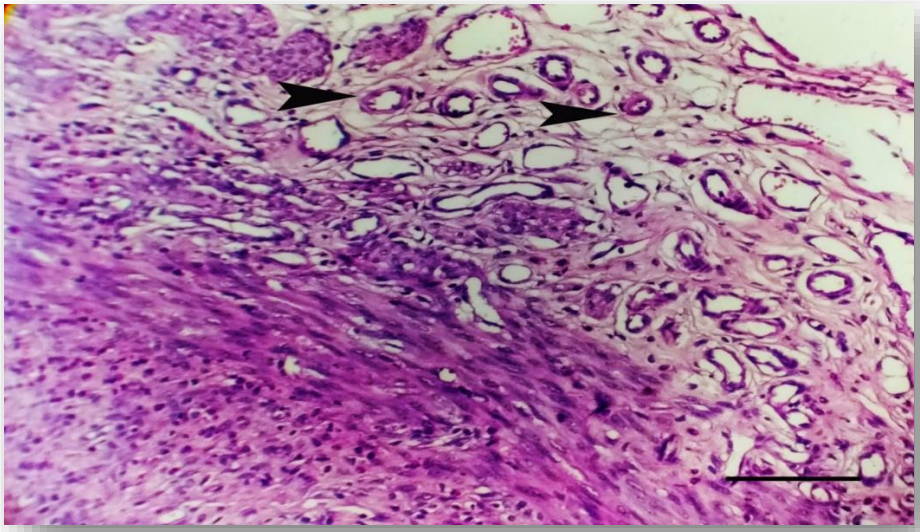

Fig. (2): Several initial follicles (shown by the arrows' tips) can be seen in the normal ovarian cortex, interstitial tissue, and blood vessels in this photomicrograph from a female rat in the control group (H&E x 200).

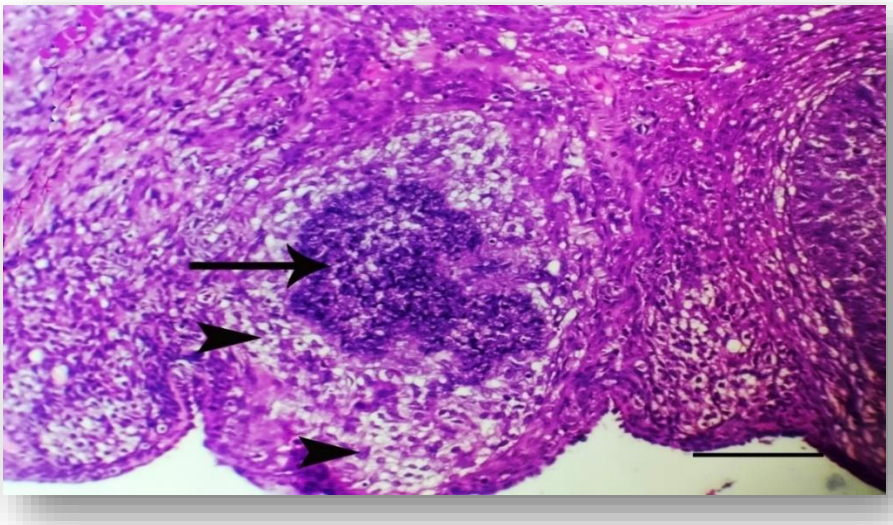

Fig. (3): Degenerated follicle with deep basophilic follicular core (arrow) and surrounding cellular vacuolation (arrows head) are shown in this photomicrograph from the ovary of an erythrosine- treated female rat (H&E x 200).

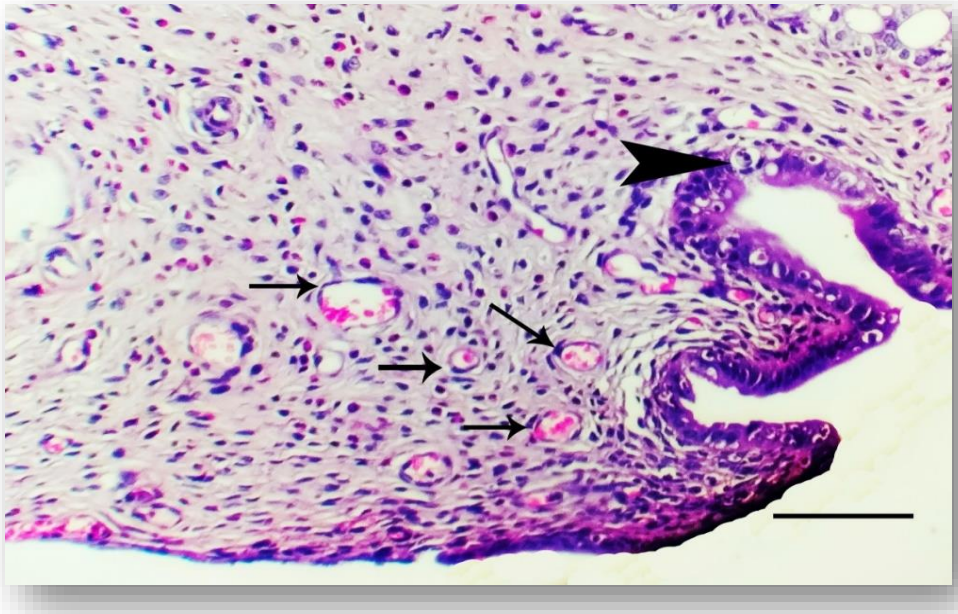

Fig. (4): Diffuse, slight congestion of ovarian blood vessels (arrows) is shown in this photomicrograph of a slice of the ovary from a female rat in the erythrosine-B recovery group (H&E x 400).

Immunohistochemical study:

Immunohistochemical examination of peroxidase-stained ovarian tissue of rats of control group showed negative reaction for caspase-3. While, ovarian sections of the ErB-treated female grous showed moderate to severe positive reaction for caspase-3 in ovarian follicles and negative reaction in other areas. The recovery rats revealed a negative reaction for caspase-3, as illustrated in **fig. 5-6-7.**

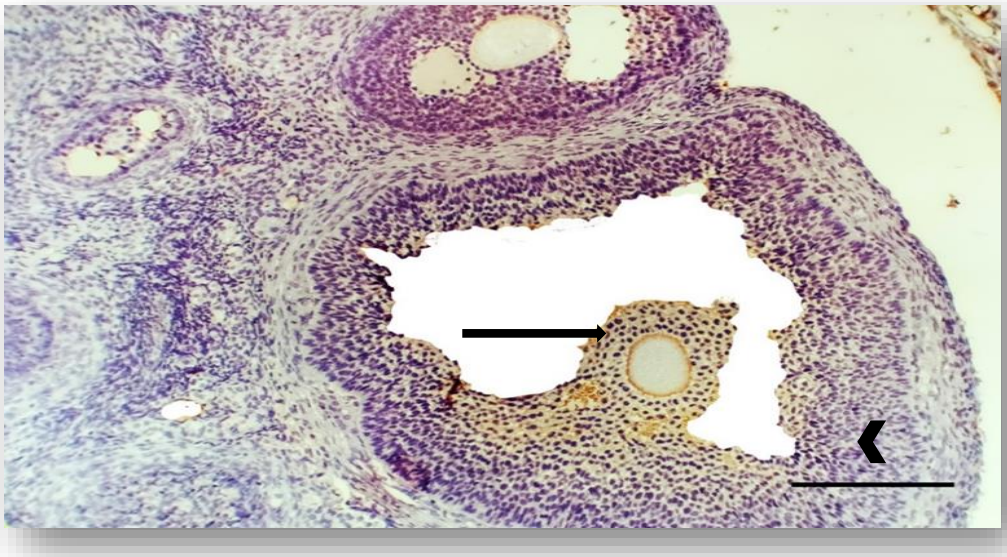

Fig. (5):Photomicrograph of peroxidase stained section of female rat ovary of control group showing negative reaction (-) for caspase-3 (arrow head) , mild positive reaction around oocyst (arrow) could be detected **(IHC x 400).**

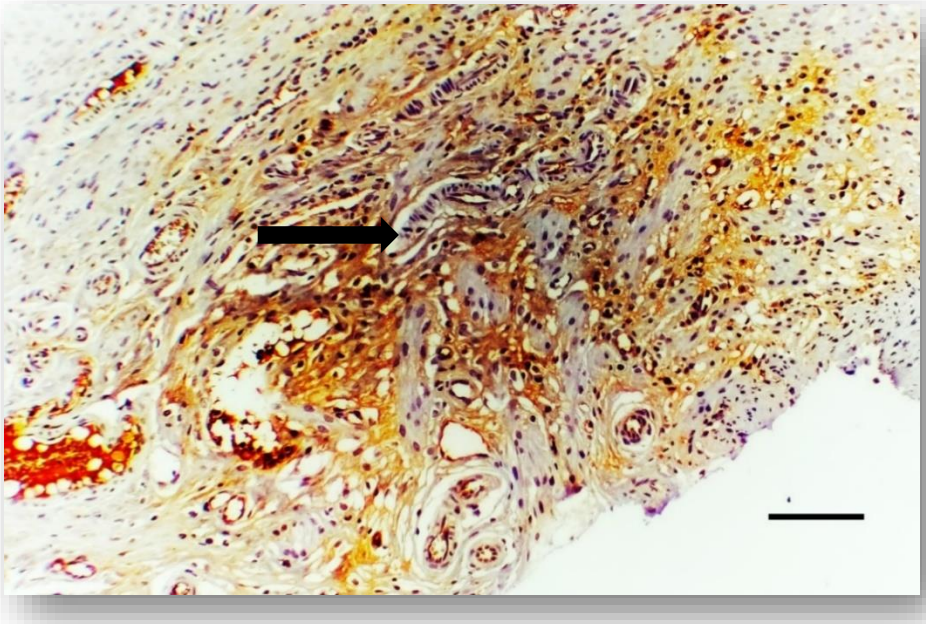

Fig. (6) : Photomicrograph of peroxidase stained section of female rat ovary of erythrosine-B treated group showing severe positive reaction (+++) for caspase-3 in focal areas (arrow)of interstitial tissue and some follicles while it declared negative reaction (-) in other areas **(IHC x 200).**

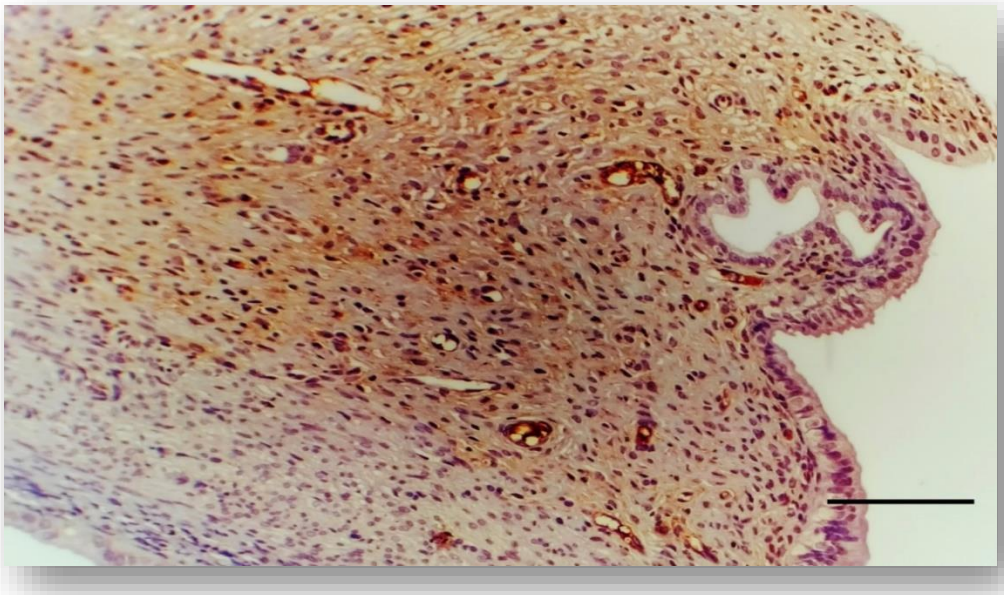

Fig. (7) : Photomicrograph of peroxidase stained section of female rat ovary of erythrosine-β recovery group showing negative reaction (-) in ovarian follicles **(IHC x 200).**

DISCUSSION

During production, several different synthetic food colours are often added to enhance the visual appeal of processed foods. Due to their great tinctorial strength, chemical stability, and cheap manufacturing cost, synthetic hues quickly gained popularity in the food industry. However, with continuous usage, many of them become poisonous

and may cause health issues (Ammar et al., 2021).

Commonly employed as a food colourant, erythrosine (E127) is a synthetic xanthene dye. Diabetes, increased tumour cell development, elevated blood glucose, decreased rates of high-density lipoprotein cholesterol (HDL-C), decreased plasma immune system operators, elevated oxidative stress, and reproductive toxicity have all been linked to the consumption of artificial food colours in high serum or tissue concentrations (Dixit & Goyal, 2013; Dafallah et al., 2015; Merinas-Amo et al., 2019).

The current study found that the mean values of body weight for female ErB-treated rats were significantly lower than those for female negative control groups (P0.05). While the mean values of body weight of the ErB-recovery group of female rats were significantly higher than those of the female treatment groups (P>0.05). In addition, the female ErB-recovery group's mean values of body weight differed from those of the female negative control group, although the difference was not statistically significant (P>0.05).

Abdel-samie et al. (2015) and Khiralla et al. (2015), both of whom reported that treatment with ErB resulted in a statistically significant decrease in body weight at all time intervals, found findings consistent with those presented here. Albino rats, on the other hand, gained weight once therapy was discontinued for two weeks.

These findings are consistent with those of EFSA, ANS, and Amin et al. (2010), who found that exposure to any synthetic food colourant led to a statistically significant (P0.05) drop in body weight compared to the control group and hypothesised that this was due to the colourants' ability to disrupt multiple metabolic pathways.

These results are consistent with those of MA (1995), who reported that mice exposed to artificial food colourants gained weight rapidly up until the fourth month, when they began to lose weight.

Other food colourants that don't influence the thyroid have been linked to increased food intake and weight gain, according to research by Mehedi et al. (2009) and El-Malky et al. (2014). Differences in the dietary additives studied, as well as dosage and length of administration, might account for variations with our results.

Loss of body mass is an established and sensitive sign of poisoning. Thus, the current study's weight loss may serve as an early indicator of dye's deleterious consequences. Possible bacterial cell surface binding of synthetic food colourants in the rat gut. This resulted in a reduction in the number of viable bacterial cells in the gut, which in turn reduced the intestinal surface's ability to absorb nutrients from meals (El-Wahab & Moram, 2013).

The present research found that the relative ovary weight of women exposed to ErB was significantly (P0.05) lower than that of those exposed to a negative control. While the female ErB-recovery group had significantly higher mean values of relative ovarian weight compared to the female ErB-treated group, the ErB-recovery group's mean values of relative ovary weight were not significantly different from the negative control group (P>0.05).

Consistent with previous research, our findings showed that ErB significantly reduced the relative weight of testes compared to the control group.

The ErB component of the food causing excessive consumption of a non-nutritive chemical may account for the much lower body mass and relative organ masses of the treated group of rats compared to the control. In addition, ErB may lead to free radical production, which in turn produces oxidative stress, which in turn causes metabolic abnormalities and overall losses in body mass (El-Desoky et al., 2017).

When comparing the female treated group to the female negative control group, the present findings showed a statistically significant (P0.05) rise in mean values of follicular stimulating hormone (FSH) and luteinizing hormone (LH) levels in the treated group. Mean levels of FSH and LH were lower in the ErB recovery group compared to the treatment group, although the difference was not statistically significant (P>0.05). Female rats treated with ErB had significantly lower mean levels of PG and E2 compared to female rats in the negative control group (P 0.05). In contrast, the female recovery group's mean value of PG and E2 levels was significantly higher than that of the female treatment group (P 0.05).

Treatment with rhodamine B xanthene dye increased FSH and LH responses of estradiol levels in female reproductive function in the present research, correlating with previous findings by L Brevini et al. (2005) and Sulistina et al. (2014).

Maryanti et al. (2014) and Sharma (2015) found that xanthene dye reduced estradiol levels in adult female Wistar rats, which was consistent with the findings of the present investigation.

After 30 days of treatment with food dyes in female rats, both sets of researchers found a decrease in LH, oestrogen, and FSH levels, but a rise in progesterone.

In contrast to the current study's findings, Tanaka (2006) and Elekima & Nwachuku (2017) found no statistically significant differences in tartrazine-treated and control female rat PROG and E2 concentrations at 30, 60, and 90 days of chronic therapy.

Xanthene dye promotes apoptosis and follicular atresia by oxidative damage to cells, which in turn lowers estradiol levels (Kaipia and Hsueh, 1997).

Estradiol is a steroid hormone necessary for pregnancy and birth. Aromatase (an enzyme located in the endoplasmic reticulum of granulose cells) catalyses the biosynthesis of this hormone from androgens (Tomic et al., 2007). Because ErB reduced aromatase activity, estradiol production was hampered (Satoh et al., 2008). In response to elevated estradiol, gonadotropin-releasing hormone (GnRH) neurons increase luteinizing hormone (LH) production (Sulistina et al., 2014).

ErB destroys oocytes, which throws off the body's endocrine system, leading to lower levels of oestrogen and progesterone and higher levels of follicle-stimulating hormone and luteinizing hormone (L Brevini et al., 2005).

Compared to the female negative control group, female rats exposed to ErB showed statistically significant (P0.001) increases in mean values of malondialdehyde (MDA) and decreases in mean values of reduced glutathione (GSH) levels associated with oxidative stress. Meanwhile, in the female ErB-recovery group, there was a large drop in MDA level and a marked rise in GSH compared to the female ErB-treated group (P0.001).

Results were consistent with those of El-Wahab and Moram (2013) and Marwa et al. (2019), who found elevated MDA and reduced GSH in the serum of ErB-treated rats, respectively. Due to its usage in conjugation with foreign molecules entering the body as ErB dye, glutathione (GSH) levels are depleted and malondialdehyde (MDA) levels are elevated, indicating the presence of oxidative stress (Maryanti et al., 2014).

Abd-Elhakim et al. (2019) found that ErB treatment led to a reduction in antioxidants and an increase in lipid peroxidation products in rats. In addition, Demirkol et al. (2019) showed that cells treated with food dyes had elevated levels of MDA.

GSH levels fell and MDA levels rose after treatment with ErB, consistent with previous studies (Dafallah et al., 2015; Demirkol et al., 2019; Gupta et al., 2019).

These findings are consistent with those of (Selvakumar et al.,2006;

Okwudiri et al.,2012), who found that consuming meals coloured with artificial colours decreased their levels of reduced glutathione and increased their levels of lipid peroxidation.

The increased oxidative stress is thought to be due to ErB's ROS production. Tissue homogenate GSH levels altered because antioxidant defence systems, such as GSH, were depleted trying to protect cells from death at the hands of newly formed reactive oxygen species (ROS). On the other hand, ROS impact on membrane lipids led to elevated MDA levels due to lipid peroxidation (Wang et al., 2006; El Golli, 2016).

Ovarian tissue from females exposed to ErB revealed congestion of blood vessels with perivascular edoema, atretic follicles with surrounding cellular vacuolation, haemorrhage, and vacuolation of parenchymal cells, according to a histological analysis. Additionally, there is a little enhancement in ovarian histology in ErB recovery groups, as seen by the vacuolation of certain germinal epithelial cells and reduced congestion of ovarian blood vessels.

Ovarian tissue from the female ErB-treated group revealed a moderate to severe positive response for caspase-3 in ovarian follicles and a negative reaction in other regions, as determined by immunohistochemistry. In addition, a negative response for caspase-3 in ovarian follicles may be seen in an immunohistochemical analysis of peroxidase-stained ovaries from the ErB-recovered group of female rats.

Maryanti et al. (2014) found that giving female rats xanthene dye dramatically reduced the number of primary, secondary, and De Graaf follicles compared to the control, and our findings corroborate this.

These findings corroborated those of Rohmawati et al. (2021), who showed that xanthene dye reduced the number of primary follicles in the ovaries of white rats, indicating that it may have a role in the prevalence of infertility condition. As well as follicular atresia with modest vacuolations, Ara et al. (2022) found that ovarian structure was disrupted following treatment with food colouring.

Modest histopathologic abnormalities, including mild vacuolation of ovarian cells, were also seen in female rats treated with tartrazine, as reported by Elekima & Nwachuku (2019). This suggests that long-term, everyday exposure to even ADI levels of food dyes may cause hormone disruptions.

Caspase-3 activation, an apoptotic marker, appears to be an index of unwanted mesenchymal cell clearance via a number of pathways, which is consistent with the findings of Li and Yuan (2008) and Wopara et al. (2021), who showed that caspase-9 gene expression significantly increased in the rats treated with erythrosine (Araki et al., 2003; Qui et al., 2018).

Increased caspase-3 activity after exposure to artificial food colours was also seen in other investigations, which was consistent with our findings (Raposa et al., 2016; Sherif and Al-Gayyar, 2013).

Researchers Mottram et al. (2012) and Abd-Elhakim et al. (2018) found that oxidative stress (increased reactive oxygen species) promotes ROS-mitochondria-Casp3-apoptosis cascades in rats exposed to food dyes.

Caspase3 is the death-dealing caspase in apoptosis, activating downstream inducers of cell death such as cytoskeletal protein breakdown, nuclear membrane permeabilization, DNA destruction, and so on (Elmore, 2007).

CONCLUSION

Due to a decrease in GSH and an increase in MDA in ovarian tissue homogenate, as well as disruptions in hormonal activity (testosterone, FSH, LH, oestrogen, and progesterone), the present research indicated that ErB had a longterm deleterious impact on female

reproductive function in adult albino rats. In addition, the ovary had notable histological and immunohistochemical alterations. After discontinuing ErB for a month, these modifications showed promise.

RECOMMENDATIONS

- Creating a national strategy to limit the use of ErB while encouraging environmentally and socially responsible alternatives.
- It is crucial to provide input on the establishment of appropriate tolerable limits for the use of these food additives to the committees responsible for the accepted daily intake (ADI), including the Food and Agriculture Organization of the United Nations and the World Health Organization (FAO/WHO) and The Joint FAO/WHO Expert Committee on Food Additives (JECFA).
- International and national authorities should reevaluate the ADI dose of ErB in terms of the ADI dosage throughout a lifetime.
- Food dyes and food items with additives should be regulated and their concentrations and ADI should be clearly labelled by government rules and consumer protection authorities.
- The food sector, as a producer, has to be kept firmly within the rules by means of regular inspection by a reputable agency.
- The usage of food colours is an issue that has to be brought to the attention of both marketers and consumers.
- Raise people's sensitivity to the health risks associated with ErB.
- To determine the extent of ErB's toxicity to the reproductive system and other organs, further research is needed.

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الملخص العربي

التأثيرات السمية الإنجابية المزمنة للإريثروسين ب على الفئران الاناث البيضاء البالغة: دراسة بيوكيميائية وهستوباثولوجية وكيميائية مناعية

ط/ ياسمين فتحي علما[أ]، أ.د/شيرين صبحي الخولى[أ], أ.د/عبدالمنعم جودة مدبولي[أ], أ.م.د/ رشا محمد الصاوى[ب], أ.م.د/رباب شعبان الشافعى[أ]

قسم الطب الشرعي والسموم الاكلينيكية، كلية الطب البشري – جامعة بنها – مصر[أ]

قسم الباثولوجي، كلية الطب البشري – جامعة بنها – مصر[ب]

الاريثروسين ب او الاحمر رقم 3 هو ملون غذائي اصطناعي بلون الكرز الوردي يستخدم على نطاق واسع في الأطعمة والأدوية ومستحضرات التجميل. ويعتبر خطراً محتملاً، ولذلك تم تقييد استخدامه في معظم البلدان، على الرغم من أن تأثيره السلبي على جسم الإنسان لم يثبت بعد. هدفت هذه الدراسة إلى تقييم التأثيرات السامة المزمنة للإريثروسين

ب على الأعضاء التناسلية (المبيض) لإناث جرذان التجارب البيضاء البالغة باستخدام الطرق الكيميائية والنسيجية والمناعية. تمت الدراسة على عدد اثنان وثلاثين من إناث التجارب البيضاء البالغة تم تقسيمهم بشكل عشوائي على اربع مجموعات على النحو التالي: مجموعة ضابطة سلبية، مجموعة ضابطة ايجابية، مجموعة معالجة تم علاجها بالإريثروسين 136 ملجم / كجم بالفم مرة واحدة يوميا لمدة ستة شهور. ومجموعة التعافي تم علاجها بالإريثروسين 136 ملجم / كجم بالفم مرة واحدة يوميا لمدة ستة شهور ثم تركها لمدة شهر بدون الإريثروسين.

النتائج: أظهرت الدارسة الحالية ان الاريثروسين له تأثير سام مزمن على الأعضاء التناسلية الأنثوية (المبايض) من خلال انخفاض ملحوظ في وزن الجسم والوزن النسبي للمبيض و اضطراب مستوي الهرمونات (زيادة كبيرة في الهرمون المنبه للجريبات والهرمون اللوتيني مع انخفاض كبير في هرمون البروجسترون والإستراديول) وعلامات الإجهاد التأكسدي (انخفاض كبير للغاية في الغلوتاثيون وزيادة مالون داي الدهيدو) في أنسجة المبيض وتأكدت هذه التغيرات من خلال فحص لأنسجة المبيض والدراسة الكيميائية المناعية (موجبة لتفاعل الكاسباس-3) في مبايض إناث جرذان التجارب البيضاء البالغة المجموعة الأنثوية المعالجة ثم تحسنت هذه التغيرات في مجموعة التعافى. الخلاصة: أدى التعرض الفموي للفئران البيضاء البالغة لـ الاريثروسين بجرعة 136 مجم / كجم يوميًا أثناء مدة الدراسة (7 أشهر) إلى أخطار سامة على الأعضاء التناسلية للإناث (المبيض). هذه التغيرات قد تحسنت جزئيا بعد إيقاف الأريثروسين لمدة شهر.