ABSTRACT

**Background:** Erythrosine B (ErB), known as Red No. 3, is a synthetic cherry-pink food colorant widely used in foods, drugs, and cosmetics. It is considered potentially dangerous; and its use is restricted in most countries although its negative impact on the human body has not yet been proven.

**Aim:** This study aimed to assess the chronic toxic effects of ErB on reproductive organs (ovaries) of adult albino rats using body and reproductive relative organ weight parameters; combined with biochemical, histopathological, and immunohistochemical methods.

**Materials and methods:** Thirty-two adult female albino rats were divided as follows: female control groups (16 rats= “8 negative control & 8 positive control group”); female ErB-treated group (8 rats), administrated ErB dissolved in distilled water at a dose of 136 mg/kg once daily for 6 months, and ErB recovery group (8 female rats) that maintained life for 1 month after stopping of ErB.

**Results:** The present study revealed that ErB has chronic toxic effects on female reproductive organs (ovaries), illustrating the physical reduction in body weight and relative ovarian weight. Disturbed hormonal levels (a significant increase in follicular stimulating hormone and luteinizing hormone, while a significant reduction in progesterone and estradiol) and oxidative stress markers (highly significant reduction of glutathione and increase of malondialdehyde) in ovarian tissue homogenate were confirmed histopathologically by alternating the normal structure of ovarian tissue and immunohistochemically (positive reaction of caspase 3) in ovaries of the ErB-treated female group. These changes were improved in the recovery group.

**Conclusion:** Oral exposure of adult albino rats to ErB at a dose of 136 mg/kg daily for the period of the study (6 months) resulted in toxic hazards to the female reproductive organs (ovaries). These changes were partially improved after stopping ErB for 1 month.

**Keywords:** Food colorant, Erythrosine B, Ovary, Caspase 3, Oxidative stress, Rats.

1. **INTRODUCTION**

   Customer preferences may be significantly influenced by food coloring chemicals. However, food coloring may be compromised during processing. To make processed foods more visually appealing and to increase consumer demand, food colors are often added (**Ramesh & Muthuraman, 2018**).
Erythrosine B, sometimes known as Red 3, is a coal-based, cherry-pink food coloring ingredient. This is a xanthene dye with halogen added. Beverages, candies, and cake-decorating supplies often include them (Epelde-Elezcano et al., 2016; 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 (Covarrubias et al., 2008; Halliwell, 2011; Zhang et al., 2016).

Reproductive illnesses such as 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 coloring 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-Materials:

A. Chemicals:

- Erythrosine B powder is a biological stain (CAS Number:16423-68-0) (purity not less than 87 percent) and was acquired from the Thermo Fisher Scientific firm in India.

- Misr Chemical Industries Company supplied the distilled water (Cairo, Egypt).

- The experimental design study was approved by the Research Ethics Committee at the Faculty of Medicine, Benha University (REC-FOMBU), Egypt with approval number: MS 43-2-2022.

B. Animals:

This research involved 32 adults 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 experiment. 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 synchronization 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.

- Study design:

Eight rats were randomly assigned to each of the 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 distilled water orally once daily via a gavage tube for the duration of the trial.

Abdel Aziz et al. (1997) 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 a gavage tube for 6 months. The dose is 1/50 of LD50 which is 1840-7100 mg/kg bw (JECFA, 2011).

Female recovery Group (Group IV):

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 without ErB administration (Abdel Aziz et al., 1997).

II- Methodology:

A. Body weight was 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 end of the sixth month of therapy, scarified rats had their ovaries removed along with any remaining fatty tissue and blood arteries ovarian weight was assessed at the end of the experiment to measure the relative organ weight according to the equation “Relative organ weight = [organ weight/body weight] ×100” (Mossa et al., 2015).

B. Hormone study:

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 analyzed 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).

C. 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 homogenized 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 (50 mM potassium phosphate, pH 7.5) was added per gram of tissue and homogenized using a glass homogenizer. The homogenates were centrifuged at 4000 RPM for 15 min at 4 °C, using a high-speed centrifuge (Type 3-30K, Sigma, Osterode-am-Harz, Germany), and the resulting supernatant was removed and stored at -80 °C 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) reported 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.

III- Statistical analysis:

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. Multiple sets of numerical (parametric) data were compared using ANOVA (analysis of variance). The Kruskal-Walli’s test was employed to analyze 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".

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 figure (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).

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

<table>
<thead>
<tr>
<th></th>
<th>group 1 F control (n=8)</th>
<th>Treated group (n=8)</th>
<th>Recovery group (n=8)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Organ weight</td>
<td>0.32</td>
<td>0.07</td>
<td>0.14</td>
<td>0.04</td>
</tr>
<tr>
<td>Post-hoc</td>
<td></td>
<td></td>
<td>P1&lt;0.001*</td>
<td></td>
</tr>
<tr>
<td>Relative Organ weight</td>
<td>0.0013</td>
<td>0.0003</td>
<td>0.007</td>
<td>0.0002</td>
</tr>
<tr>
<td>Post-hoc</td>
<td></td>
<td></td>
<td>P1=0.003*</td>
<td></td>
</tr>
</tbody>
</table>

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.

Figure (1): Comparison between the mean values of body weights among different groups (F= female).
According to 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 study showed 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 not significantly decreased in comparison to the control group table (2).

### 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):

<table>
<thead>
<tr>
<th></th>
<th>Group 1 F Control (n=8)</th>
<th>Treated group (n=8)</th>
<th>Recovery group (n=8)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT (ng·ml)</td>
<td>0.65       0.13</td>
<td>0.62       0.12</td>
<td>0.63       0.26</td>
<td>0.9</td>
</tr>
<tr>
<td>Post-hoc</td>
<td></td>
<td>P1=0.8</td>
<td>P1=0.9</td>
<td>P2=0.9</td>
</tr>
<tr>
<td>LH (IU·ml)</td>
<td>2.31       0.24</td>
<td>3.59       1.26</td>
<td>2.73       0.60</td>
<td>0.02</td>
</tr>
<tr>
<td>Post-hoc</td>
<td></td>
<td>P1=0.005*</td>
<td>P1=0.3</td>
<td>P2=0.04*</td>
</tr>
<tr>
<td>FSH (IU·ml)</td>
<td>0.48       0.16</td>
<td>1.28       0.30</td>
<td>0.65       0.22</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Post-hoc</td>
<td></td>
<td>P1=&lt;0.001**</td>
<td>P1=0.2</td>
<td>P2=&lt;0.001*</td>
</tr>
<tr>
<td>PG (ng·ml)</td>
<td>0.34       0.11</td>
<td>0.20       0.07</td>
<td>0.29       0.10</td>
<td>0.02</td>
</tr>
<tr>
<td>Post-hoc</td>
<td></td>
<td>P1=0.005*</td>
<td>P1=0.4</td>
<td>P2=0.04*</td>
</tr>
<tr>
<td>E2 (Pg·ml)</td>
<td>111.65    9.11</td>
<td>97.98     7.42</td>
<td>108.64     11.85</td>
<td>0.03*</td>
</tr>
<tr>
<td>Post-hoc</td>
<td></td>
<td>P1=0.01*</td>
<td>P1=0.6</td>
<td>P2=0.04*</td>
</tr>
</tbody>
</table>

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.

### Histopathology

Ovarian tissue from the control group showed normal ovarian histology, including the presence of the ovarian cortex, interstitial tissue, blood vessels, and many primary follicles, as determined by histopathological research. ErB-treated female rats showed vascular congestion and perivascular edema, as well as atretic follicles surrounded by cellular vacuolation, bleeding, and vacuolation of parenchymal cells. As can be seen in figures (2, 3, and 4), while the recovery group showed only mild improvement with diffuse congestion of ovarian blood vessels with vacuolation of some germinal epithelium cells; in addition to, degenerated 2ry follicles.

### Immunohistochemical results

Immunohistochemical examination of peroxidase-stained ovarian tissue of rats of the control group showed a negative reaction for caspase-3. While ovarian sections of the ErB-treated female group 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 figures (5, 6 and 7).
Fig. (2): A Photomicrograph of a section of ovary of female rat of control group showing normal ovarian cortex, interstitial tissue and blood vessels with multiple primary follicle (arrows head) (H&E x 200).

Fig. (3): A Photomicrograph of a section of ovary of erythrosine-β treated female rat showing degenerated follicle with deep basophilic follicular core (arrow) and surrounding cellular vacuolation (arrows head) (H&E x 200).

Fig. (4): A Photomicrograph of a section of female rat ovary of erythrosine-B recovery group showing diffuse mild congestion of ovarian blood vessels (arrows) (H&E x 400).
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 colors 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 colorant, erythrosine (E127) is a synthetic xanthene dye. Diabetes, increased tumor 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 colors in high serum or tissue concentrations (Dixit & Goyal, 2013; Dafallah et al., 2015; Merinas-Amo et al., 2019).

Abdel-samie et al. (2015) and Khiralla et al. (2015), both reported that treatment with ErB resulted in a statistically significant decrease in body weight at all time intervals, which agreed with the current study. Albino rats, on the other hand, gained weight once therapy was discontinued for two weeks. These findings are consistent with those of EFSA, (2009), and Amin et al. (2010), who found that exposure to any synthetic food colorant led to a statistically significant (P<0.05) drop in body weight compared to the control group that this was due to the colorants' ability to disrupt multiple metabolic pathways.

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

Other food colorants that don't influence the thyroid have been linked to increased food intake and weight gain, according to research by Meheldi 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 in 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. 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 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).

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 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, estrogen, 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 PG 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) catalyzes the biosynthesis of this hormone from androgens (Tomic et al., 2007). Because ErB reduced aromatase activity, estradiol production was hampered
In response to elevated estradiol, gonadotropin-releasing hormone (GnRH) neurons increase luteinizing hormone (LH) production (Sulistina et al., 2014).

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

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.

Reduced glutathione 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 colored with artificial colors 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 defense 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).

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 coloring.

Modest histopathologic abnormalities, including mild vacuolation of ovarian cells, were also seen in female rats treated with tartrazine, as reported by Elekima & Nwachuku (2017). This suggests that long-term, everyday exposure to even accepted daily intake 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 colors was also seen in other investigations, which was consistent with our findings (Sherif and Al-Gayyar, 2013; Raposa et al., 2016).

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

Oral administration of ErB in a dose of 136 mg/kg produced a toxic effect on the reproductive function which was proved by the affection of the body weight and relative organ weights (testis & ovary) of both male and female albino rats, and imbalance in reproductive hormones (testosterone, FSH, LH, estrogen and progesterone) in both sexes and confirmed by the histopathological and immunohistochemical changes in both testis and ovary. These changes improved in the recovery groups of both male and female adult albino rats.

RECOMMENDATIONS

- Creating a national strategy to limit the use of ErB while encouraging environmentally and socially responsible alternatives.
- International and national authorities should reevaluate the accepted daily intake 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 labeled by government rules and consumer protection authorities.
- The usage of food colors is an issue that must be brought to the attention of both marketers and consumers and find alternatives as natural food colors.
- Raise people's awareness of 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.

ACKNOWLEDGMENT

We would like to express our sincere appreciation to everyone who worked with us at Benha University's Department of Forensic Medicine and Clinical Toxicology at the School of Medicine.

http://www.fmed.bu.edu.eg

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التأثيرات السمية الإنجابية المزمنة للإريثروسين B على إناث الجرذان البيضاء البالغة: دراسة بيوكيميائية ولهستوباثولوجية وكميائية مناعية

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الإريثروسين B أو الأحمر 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 السريري على جسم الإنسان لم يتم تقديره. هذه الدراسة تهدف إلى تقييم التأثيرات السامة المزمنة للإريثروسين B على الأعضاء التناسلية (المبيض) لإناث جرذان التجارب البيضاء البالغة باستخدام الطرق الكيميائية والنسيجية والمناعية. تتم الدراسة على عدد اثنان وثلاثين من إناث الجرذان البالغة تم تقسيمهم بشكل عشوائي على أربع مجموعات على النحو التالي: مجموعة ضابطة سلبية، مجموعة ضابطة إيجابية، مجموعة معالجة تم علاجها بالإريثروسين 136 ملجم / كجم بالفم مرة واحدة يوميا لمدة شهرين، ومجموعة تعاونية تم علاجها بالإريثروسين 136 ملجم / كجم بالفم مرة واحدة يوميا لمدة شهرين ثم تركها لمدة شهر بدون الإريثروسين.

النتائج: أظهرت الدراسة أن الإريثروسين B قد أثر سامًا على الأعضاء التناسلية الأنثوية (المبيض) من خلال انخفاض ملحوظ في وزن الجسم والوزن النسبي للمبيض وارتفاع مستوي الهرمونات (زيادة كبيرة في هرمون البروجسترون والإستراديول) وعلاقات الإجهاد التأكسدي (انخفاض كبير للغاية في الغلوتاثيون وزيادة مالون داي الدهيدو). تأكدت هذه التغيرات من خلال فحص الأنسجة المبيض والأدلة الكيميائية المناعية (إيجابية لتفاعل الكابس-3) في إناث جرذان التجارب البيضاء البالغة المجموعة المزمنة للإريثروسين B. هذه التغيرات في مجموعة التعاونية: أدى التعرض الفموي للإناث إلى حدوث التغيرات المذكورة لـ الإريثروسين بجرعة 136 مجم / كجم يوميًا أثناء مدة الدراسة (7 أشهر) إلى أخطار سامة على الأعضاء التناسلية للإناث (المبيض). هذه التغيرات قد تحسن زعماً بعد إيقاف الإريثروسين لمدة شهر.