RESEARCH ARTICLE

Ginkgo biloba extract protects against tartrazine-induced testicular toxicity in rats: involvement of antioxidant, anti-inflammatory, and anti-apoptotic mechanisms

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Abstract

The use of additives, especially colorants, in food and pharmaceutical industry is increasing dramatically. Currently, additives are classified as contaminants of emerging concern (CECs). Concerns have been raised about the potential hazards of food additives to reproductive organs and fertility. The present study investigates the reproductive toxicity of tartrazine (TRZ), a synthetic colorant, in male rats and aims to explore the curative effect of *Ginkgo biloba* extract (EGb) against TRZ-induced testicular toxicity. Twenty-four rats were divided into four groups: the control (0.5 ml distilled water), the EGb group (100 mg/kg EGb alone), the TRZ group (7.5 mg/kg TRZ alone), and the TRZ-EGb group (7.5 mg/kg TRZ plus 100 mg/kg EGb). The doses were administered orally in distilled water once daily for 28 days. Toxicity studies of TRZ investigated testicular redox state, serum gonadotropins, and testosterone levels, testicular 17 ß-hydroxysteroid dehydrogenase activity, sperm count and quality, levels of inflammatory cytokines, and caspase-3 expression as an apoptotic marker. Also, histopathological alterations of the testes were examined. TRZ significantly affected the testicular redox status as indicated by the increase in malondialdehyde and the decrease in reduced glutathione, superoxide dismutase, and catalase. It also disrupted serum gonadotropins (follicle stimulating hormone and luteinizing hormone) and testosterone levels and the activity of testicular 17ß-hydroxysteroid dehydrogenase. Additionally, TRZ adversely affected sperm count, motility, viability, and abnormality. Levels of tumor necrosis factor-α, interleukin-1β, interleukin-6, and expression of caspase-3 were increased in the testes. Histopathological examination of the testes supported the alterations mentioned above. Administration of EGb significantly ameliorated TRZ-induced testicular toxicity in rats. In conclusion, EGb protected against TRZ-induced testicular toxicity through antioxidant, anti-inflammatory, and anti-apoptotic mechanisms.

Keywords Tartrazine · Testicular toxicity · *Ginkgo biloba* extract · Oxidative stress · Inflammation · Apoptosis

Introduction

Infertility and subfertility are serious health problems which affect millions of people worldwide, of which 40–50% of cases are due to male factors (Krausz and Riera-Escamilla 2018). Concerns have been raised about the potential hazards of food additives to reproductive organs and fertility. More than 2500 items of food additives are used to improve the flavor and the artistic attributes of the food making it more attractive to the consumers. The worldwide increase in food consumption intensifies the occurrence of food additives and their metabolic products in the environment (Lempart-Rapacewicz et al. 2023). Recent literatures classified food additives as contaminants of emerging concern (CECs) (Salimi et al. 2017; Han et al. 2022). Colorants are a major class of food additives used widely in food industry and may also be used in non-food consumables such as cosmetics and pharmaceuticals (Delwiche 2004). There is no nutritional value for food colorants; however, interest in their adverse effects is growing (Tanaka 2006). A major group of food colorants is the synthetic azo dyes, including tartrazine (TRZ). FAO/WHO established an allowed daily intake of TRZ of 0–7.5 mg/kg (EFSA panel on food additives and nutrient sources added to food, 2009). Currently, data on TRZ toxicity are contradictory. Some studies reported no

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adverse effects for TRZ in both human and experimental models (Mpountoukas et al. 2010; Amin et al. 2010). However, several reports documented TRZ toxicity, including nephrotoxicity (Erdemli et al. 2017), neurotoxicity (Essawy et al. 2023), immunotoxicity (Abd-Elhakim et al. 2018), and mutagenic effects (Khayyat et al. 2017) when consumed moderately or in excess, or even at the allowed dose. Furthermore, adverse effects of TRZ on the male reproductive system have been reported. Wopara et al. (2021) mentioned that TRZ can alter testicular function and negatively affect sperm quantity and quality. It also disrupted the pituitary gonadotropins and decreased testosterone level (Mehedi et al. 2009). The toxicity of TRZ may be due to its direct covalent binding to proteins, causing denaturation of the active protein sites and misconfiguration (Saeed et al. 2011). Metabolic biotransformation of the azo bond can generate reactive amines that further generate free radicals and exaggerate oxidative damage, a driving force in TRZ toxicity (Demirkol et al. 2012; Albasher et al. 2020). Inflammation and apoptosis are also prime mechanisms in the pathophysiology of TRZ-induced testicular toxicity (Wopara et al. 2021; Abd-Elhakim et al. 2018).

Owing to their diverse beneficial effects, plant-derived phytochemicals have been used as key sources for the discovery of new drugs. *Ginkgo biloba* is an ancient tree species that originated from China and is now widespread all over the world. It has traditionally been used as a medicinal plant due to its wide spectrum of health benefits (Noor-E-Tabassum et al. 2022). Various bioactive ingredients with several therapeutic actions have been isolated from *Ginkgo biloba,* including flavonoids, terpenoids, alkylphenols, carboxylic acids, lignans, and polyprenols (Shu et al. 2020; Liu et al. 2021)*.* The therapeutic efficacy of *Ginkgo biloba* extract (EGb) in the treatment of health disorders such as bronchitis, stomach discomfort, nervousness, ischemic heart disease, and diabetes mellitus has been reported (Chan et al. 2007). Furthermore, studies reported the beneficial effects of EGb against toxicities in different animal models (Verma et al. 2019; Mohammed et al. 2020; Sherif et al. 2020; Essawy et al. 2022).

It becomes crucial to evaluate the safety limits and the potential toxicity of substances used as food additives. In line with this context, this study further investigates the reproductive toxicity of TRZ in male rats. To the best of our knowledge, the effect of EGb on testicular toxicity of TRZ in rats was not studied up to now. Therefore, the main objective here is to evaluate the potential ameliorative effect of EGb on TRZ-induced toxicity in the testes of rats referring to the mechanisms involved. To achieve our objective, testicular redox status, gonadotropin and testosterone levels, 17 ß-hydroxysteroid dehydrogenase activity, sperm count and quality, inflammatory cytokine levels, and caspase-3 expression were evaluated. As well, histopathological examinations of the testicular tissue were performed.

Materials and methods

Chemical and reagents

Tartrazine (purity ≥ 95%) was procured from Sigma-Aldrich (USA). *Ginkgo biloba* extract was procured from Puritan Pride (USA). Kits and reagents used in the analyses were purchased from Bio-diagnostic Co. (Egypt). Enzyme-linked immunosorbent assay (ELISA) kits for measuring inflammatory cytokines were procured from Sigma-Aldrich Chemie (Germany). All reagents and chemicals used were of analytical grade.

Animals and experimental design

Twenty-four adult male Wistar albino rats (3 months, 180–200 g) were obtained from the facility house of the Faculty of Drug and Pharmacy, Pharos University, Alexandria, Egypt. The animals were housed in stainless steel cages and kept at 25 ± 1 °C and a 12-h dark/light cycle with access to the standard diet and tap water. The handling and experimental procedures of the study comply with and were approved by the Animal Ethical Committee of Alexandria University (approval number AU04190226101). After 2 weeks of acclimatization, the rats were assigned to four groups. The control group was given 0.5 ml of distilled water for 28 consecutive days; the EGb group received 100 mg/kg EGb alone; the TRZ group administered 7.5 mg/kg TRZ alone; and the TRZ-EGb group received 7.5 mg/kg TRZ; and 100 mg/kg EGb separately. The dose of TRZ was selected as the upper limit of the allowed daily intake (0–7.5 mg/kg/day), while the dose of EGb was selected according to Abd El-Maksoud et al. (2019). TRZ and EGb doses were administered orally in 0.5 ml of distilled water once daily for 28 consecutive days. The dose the, route, and the duration of TRZ administration were selected according to Essawy et al. (2023).

Blood collection and preparation of testicular homogenate

After completion of the treatment protocol, the rats were fasted overnight prior to anesthesia (light diethyl ether) for the euthanization. Blood was collected by cardiac puncture in non-heparinized tubes. The blood was centrifuged (3000 rpm for 10 min.) for isolation of the sera which were stored at − 20 °C for measurement of follicle stimulating hormone (FSH), luteinizing hormone (LH),

and testosterone levels. For the preparation of testicular tissue lysate, the testes were immediately isolated from the rats and washed with cold saline. A 0.5 g from each testis was homogenized in 5-ml-cold phosphate buffer using a polytron. The homogenates were then centrifuged at 12,000 rpm for 15 min using a cooling centrifuge (Hettich model EBA12R, Germany). The supernatants were stored at − 20 °C for further analysis. A portion of the rat's testes was immediately fixed in 10% formalin for histological and immunohistochemical examinations.

Determination of the testicular redox state

For evaluation of the testicular redox state, the level of malondialdehyde (MDA), the reduced glutathione (GSH) content, and the activities of superoxide dismutase (SOD) and catalase (CAT) were determined in testicular tissue. MDA was determined spectrophotometrically according to Ohkawa et al. (1979) and is expressed as nmol/g tissue. The GSH content, expressed as μmol/g tissue, was measured as described by Beutler et al. (1963). The activities of SOD and CAT, expressed as U/mg protein, were measured as described by Nishikimi et al. (1972) and Aebi (1984), respectively.

Measurement of reproductive hormones and 17ß-hydroxysteroid dehydrogenase

To evaluate the effect of TRZ with and without EGb on reproductive hormones, serum levels of FSH and LH were determined as described by Simoni et al. (1997) and Kosasa (1981), respectively. They are expressed as mIU/ml. Furthermore, the serum testosterone level (ng/ml) was quantitatively measured using ELISA technique based on competitive binding testosterone in the sample and a defined amount of horseradish peroxidase–conjugated testosterone. The specific activity of 17ß-hydroxysteroid dehydrogenase (17 β-HSD) was estimated in the testicular tissue as described by Penning et al. (1984). Enzyme activity is expressed as U/mg protein.

Determination of testicular inflammatory cytokines

Tumor necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6) levels were quantitatively determined in testicular tissue using ELISA kits, as described in the manufacturer's instructions. They are expressed as pg/g tissue.

Sperm collection and evaluation of sperm quality

For sperm collection, the left testes and the cauda epididymis were immediately isolated from the rats. The epididymis was chopped in 5 ml Ham's F12 medium, and the sperm suspension was incubated for 5 min at 35 °C. After repeated washings in Ham's F12 medium, the collected sperm were evaluated for count, motility, viability, and abnormalities. For sperm count (millions/ml), one drop of sperm suspension was transferred to hemocytometer and allowed for 10 min to settle the sperm. The sperm mobility (%) was evaluated in at least 10 hemocytometer slide fields (Slott et al. 1991). Sperm viability (ratio of live and dead sperm) expressed as percentage was assessed according to the method of Talbot and Chacon (1981). Briefly, 0.2 ml of the sperm suspension was added to 1% trypan blue stain and incubated for 15 min at 37 °C, and then 0.1 ml of the mixture was loaded in hemocytometer chamber, allowed to settle for 60 s, and then the viable sperms were counted. Abnormal sperm shapes were observed in eosin/nigrosin-stained smears. One hundred sperms were randomly examined in different fields to measure the percentage of abnormal sperm (Filler 1993).

Histological examination

One testis from each rat was immediately fixed in 10% formalin, dehydrated in ascending grades of ethyl alcohol, fixed, embedded in paraffin, and sectioned at 5-μm thickness. Sections were stained with routine hematoxylin and eosin (H&E) and examined to record the histological changes (Sakr et al. 2011).

Immunohistochemical investigation

Successive 5-μm thick testis paraffin sections were mounted on positively charged slides and incubated at 37 °C to assess the immunoreactivity of caspase-3. Anticaspase-3 antibody (ab4051, Abcam, Inc.) was used, as described in the avidin–biotin complex (ABC) technique (Hsu et al. 1981).

Statistical analysis

All the results are presented as *mean ± standard error of the mean (SEM)*. Statistical Package for the Social Sciences (SPSS, version 28) software was used for the analysis. One-way analysis of variance (ANOVA) followed by the least significant difference (LSD) test was applied to compare between the experimental groups. Differences are significant when $p < 0.05$.

Results

EGb ameliorated TRZ-induced changes in the testicular redox status

To assess testicular redox state under the influence of TRZ and EGb, level of lipid peroxidation (LPO), as MDA, in addition to the antioxidants GSH, SOD, and CAT were measured (Fig. 1). Oral administration of 7.5 mg/kg TRZ daily for 28 triggered oxidative stress indicated by a significant increase of 301.6% in MDA level compared to the control group. Furthermore, TRZ-treated rats had significantly lower levels of antioxidants. They decreased by 66.9%, 83.9%, and 52.8% for GSH, SOD, and CAT, respectively relative to the control group. Supplementation with EGb plus TRZ significantly improved the redox state of the testes of rats. It decreased the level of MDA by 58.4% while GSH, SOD, and CAT increased by 101.8%, 342.6%, and 31.6%, respectively compared to the TRZ alone group. Notably, no significant changes in testicular redox indices were found in rats treated with EGb alone relative to the control group.

EGb improved TRZ-induced alteration in serum reproductive hormones and testicular 17 β-HSD activity

According to Fig. 2, TRZ administration significantly decreased serum LH and testosterone levels by 63.1 and 52.8%, while serum FSH level increased significantly by 70.9% compared to the control group. Furthermore, testicular 17 β-HSD activity decreased significantly by 37.6% compared to the control group. Interestingly, combined administration of EGb plus TRZ significantly improved these indices. Compared to the TRZ group, LH and testosterone levels and 17 β-HSD activity increased by 137.4%, 25.9%, and 23.1%, respectively, while FSH level decreased by 28.9%. No significant changes were observed in these parameters after EGb administration alone.

Effect of TRZ with and without EGb on the quantity and quality of sperm

Indices to diagnose the quantity and quality of the sperm were determined to assess the testicular function (Table 1). The results showed significant deterioration in sperm quality in rats administered TRZ. The sperm count, motility, and viability significantly decreased by 54.8%, 49.0%, and 54.9%, respectively while sperm abnormality increased significantly by 140.5% compared to the control group. Concurrent administration of TRZ plus EGb significantly increased the count, motility, viability of sperm and decreased the percentage of sperm abnormalities compared to the TRZ group. It is notable that administration of EGb alone significantly increased the count and decreased the abnormalities of sperm, while the motility and viability of sperms were insignificantly increased compared to normal control.

Fig. 1 Effect of TRZ with and without EGb treatment on testicular redox state. The experimental groups were tested for levels of MDA (**a**), GSH (**b**), SOD (**c**), and CAT (**d**). The results are presented as *mean ± SEM* (*n* = 5). [a] and [b] represent statistical significance versus the control and TRZ groups, respectively using one-way ANOVA followed by the least significant difference (LSD) test. Abbreviations: CAT, catalase; EGb, *Ginkgo biloba* extract; GSH, reduced glutathione; SOD, superoxide dismutase; and TRZ, tartrazine
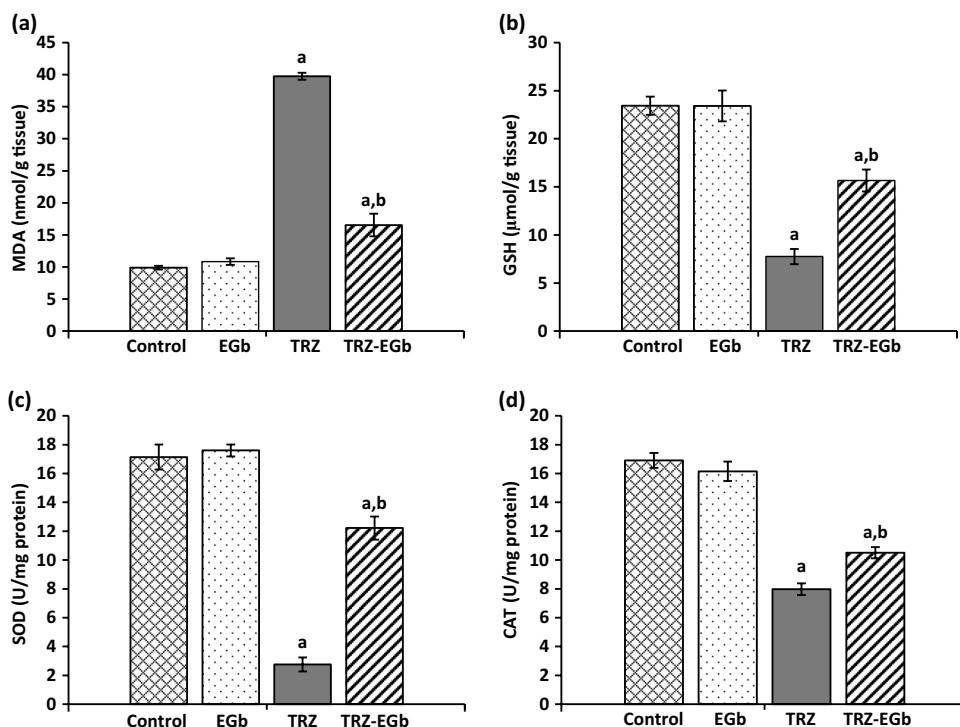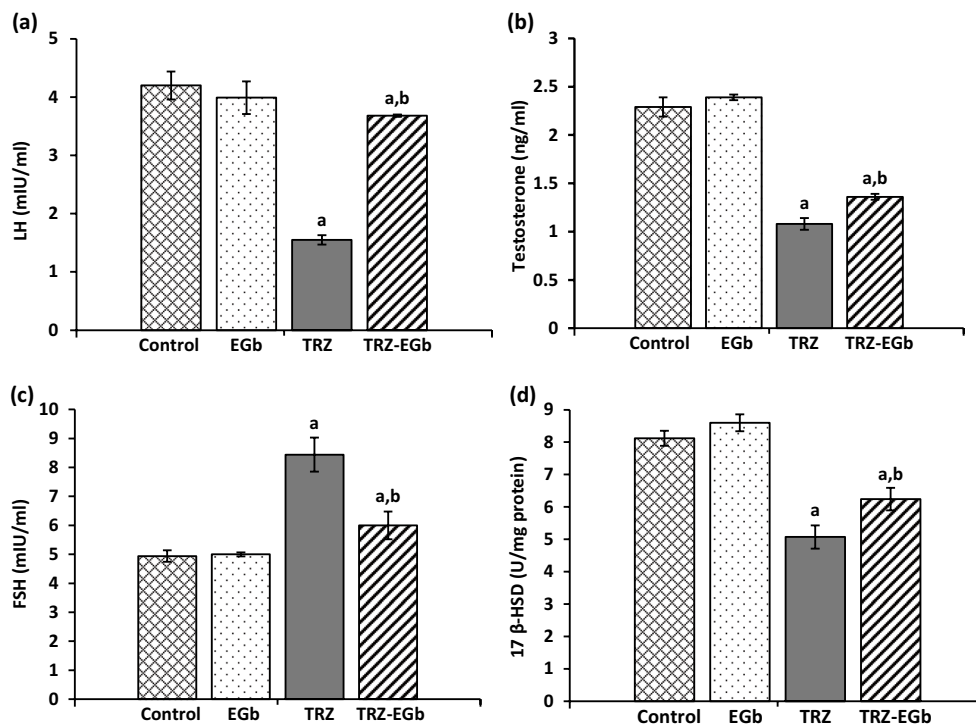
Fig. 2 Effect of TRZ with and without EGb on serum levels of LH (**a**), testosterone (**b**), and FSH (**c**), and testicular 17 β-HSD activity (**d**). Values are presented as the *mean ± SEM* ($n=4$). [a] and [b] represent the statistical significance versus the control and TRZ groups, respectively, using one-way ANOVA followed by the least significant difference (LSD) test. Abbreviations: EGb, *Ginkgo biloba* extract; FSH, follicle stimulating hormone; LH, luteinizing hormone; and 17 β-HSD, 17-beta-hydroxysteroid dehydrogenase

Table 1 Effect of TRZ with and without EGb on the sperm count and quality in male rats

	Control	EGb	TRZ	TRZ + EGb
Sperm count (million/ml)	83.60 ± 1.81	90.60[a] ± 2.16	37.80[a] ± 1.20	70.60[ab] ± 3.23
Sperm motility (%)	80.80 ± 2.29	85.70 ± 2.11	41.20[a] ± 1.16	70.80[ab] ± 1.20
Sperm viability (%)	77.60 ± 2.50	81.60 ± 2.44	35.00[a] ± 2.05	60.40[ab] ± 2.79
Sperm abnormality (%)	22.20 ± 1.74	13.40[a] ± 1.83	53.40[a] ± 2.98	30.20[ab] ± 2.27

Values are presented as the *mean ± SEM* ($n=5$ for the control group, $n=6$ for the other groups). [a] and [b] are the statistical significance versus the control and TRZ groups, respectively using one-way ANOVA followed by least significant differences (LSD) test. Abbreviations: EGb, *Ginkgo biloba* extract; TRZ, tartrazine

EGb ameliorated TRZ-induced inflammation in the testes of rats

TRZ was evaluated for its inflammatory effect in the testes of rats by monitoring cytokine levels. According to Fig. 3, TRZ administration significantly increased TNF-α, IL-1β, and IL-6 levels by 81.7%, 110.6%, and 134.1%, respectively compared to the control group. Treatment with EGb plus TRZ significantly decreased the levels of TNF-α, IL-1β, and IL-6 levels by 21.6%, 26.0%, and 26.2% respectively relative to TRZ alone group. Administration of EGb alone does not have an effect on cytokine levels in the testes of rats.

Effect of EGb on TRZ-induced histopathological alterations

Histological alterations of the testes were observed in H&E stained sections. Sections from the control and EGb-treated groups (Fig. 4) showed the normal structure of the seminiferous tubules. The tubules have regular outlines and are surrounded by a thin regular basal lamina. The interstitial tissue showed blood vessels and clusters of normal Leydig cells. Within the seminiferous tubules, the spermatogenic cells are found in an organized manner with spermatogonia, primary spermatocytes, spermatids, and tufts of spermatozoa appeared in the lumen. Sertoli cells appeared elongated
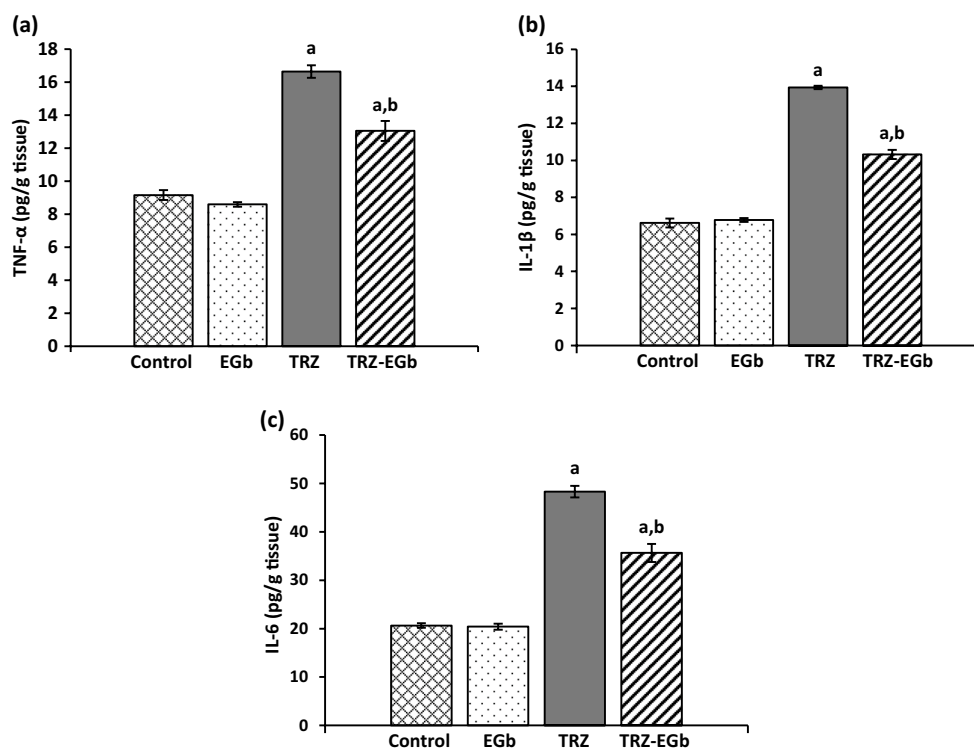
Fig. 3 Effect of EGb on TRZ-induced inflammatory mediators in the testes of rats. The experimental groups were assessed for levels of TNF-α (**a**), IL-1β (**b**), and IL-6 (**c**). Values are presented as $mean \pm SEM$ ($n=4$). [a] and [b] represent the statistical significance versus the control and TRZ groups, respectively, using one-way ANOVA followed by the least significant difference (LSD) test. Abbreviations: EGb, *Ginkgo biloba* extract; IL, interleukin; TNF-α, tumor necrosis factor-alpha; and TRZ, tartrazine

with oval basal nuclei. On the other hand, testicular sections from TRZ-treated rats (Fig. 5) revealed severe histopathological alterations, as reflected by deformed and atrophied seminiferous tubules with irregular outline and wide spaces between the tubules. Deformed germ cells with pyknotic nuclei, irregularly arranged and degenerated spermatogenic cells with vacuolations among them, wide separation between germ cells, many Sertoli cells with densely stained nuclei, portions of clustered and abnormal scanty elongated spermatids, and absence of sperm were observed within the seminiferous tubules. In addition, degeneration of Leydig cells and congested blood vessels were observed. Testicular sections of rats treated with TRZ and EGb (Fig. 6) revealed preservation of the normal architecture of many seminiferous tubules with well-organized germ cells, normal appearance of spermatogenic cells and Leydig cells, and improved interstitial tissue.

Effect of TRZ with and without EGb on caspase-3 reactivity in rat's testis

To examine apoptotic activity in the testes, immunoreactivity of caspase-3 was observed. As shown in Fig. 7, sections in testes from the control (a) and the EGb alone (b) groups

revealed absence or weak caspase-3 immunoreactivity. On the contrary, the TRZ-treated group (c) shows strong immunoreactivity for caspase-3. Combined administration of TRZ and EGb (d) significantly decreased caspase-3 expression in the testes compared to the TRZ-only group.

Discussion

Food additives are classified as CECs because they are only partially metabolized inside the human body and excreted into the sewage (Birch et al. 2015). Adverse effects associated with the use of food additives on the reproductive system and fertility have been reported (Mehedi et al. 2009; Wopara et al. 2021). Therefore, it is recommended to precisely assess the toxicity of food additives in an attempt to avoid their possible health hazards. Currently, we investigate the toxic effect of TRZ, a widely used synthetic colorant, on the rat testes. Additionally, the study examined the protective effect of EGb, a natural phenolic compound, against TRZ-induced testicular injury. The results confirmed the toxic effect of TRZ, as shown by deterioration of testicular redox status, disruption of reproductive hormones, decline in sperm count and quality, increased production
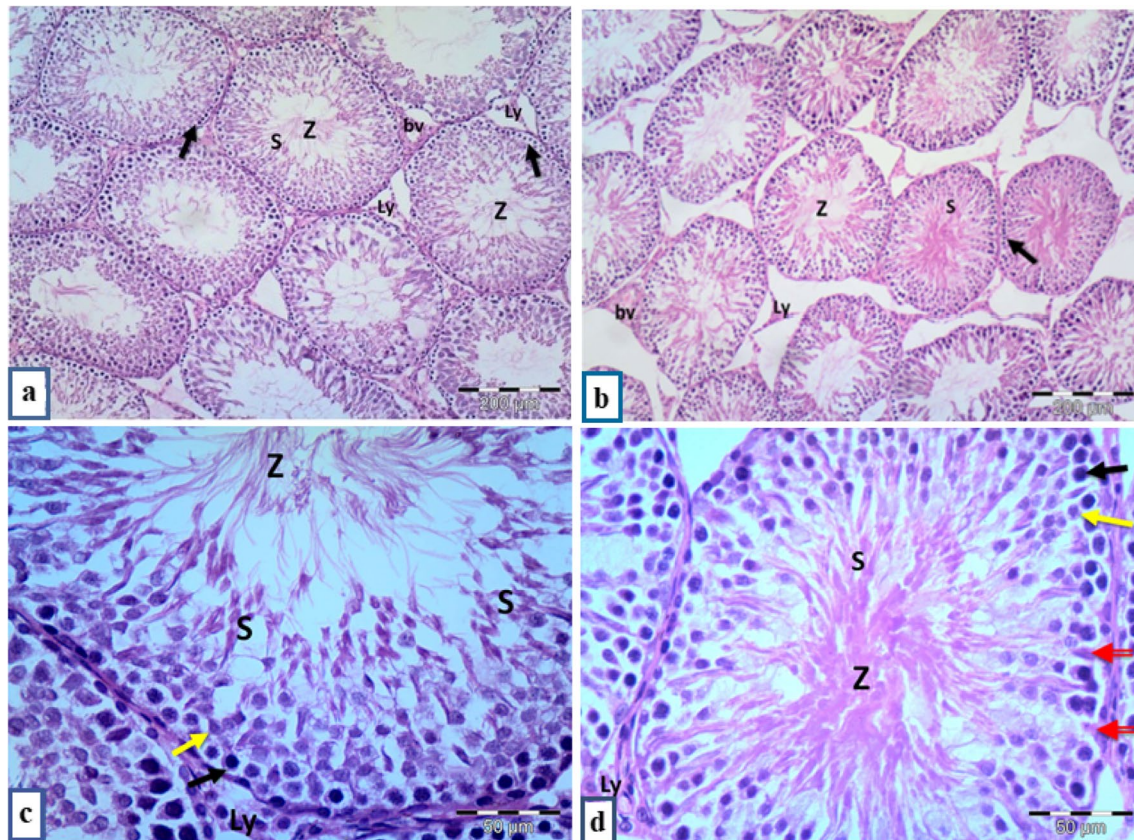
Fig. 4 Photomicrographs of testicular tissue from the control group (**a** and **c**) and EGb-treated rats (**b** and **d**). Figs. **a** and **b** show the normal architecture of seminiferous tubules, interstitial tissue containing normal Leydig cells (Ly), normal blood vessels (bv), normal spermatogonia (black arrow), and spermatids (S), and lumen filled with spermatozoa (Z). Figs. **c** and **d** show higher magnification of the seminiferous tubules with normal and complete spermatogenic cells arranged in a regular manner with spermatogonia and primary spermatocytes (yellow arrow), spermatids (S), spermatozoa (Z), and normal Sertoli cells (double red arrow). H&E, scale bar: 200 μm (**a** and **b**) and 50 μm (**c** and **d**)

of pro-inflammatory mediators, and increased expression of caspase-3 in addition to histopathological alteration in testicular tissue. Concurrent EGb administration significantly improved the testicular structure and function and protected against TRZ toxic effects.

About 30–80% of men infertility cases have been linked to increased ROS and the oxidative damage they induce (Tremellen 2008). The results of the current study points to oxidative stress as a prime mechanism in mediating the testicular toxicity of TRZ. Oral administration of TRZ triggered oxidative stress in the testes, as shown by the higher level of MDA, a product of LPO that indicates oxidative stress. Our results support previous studies reporting TRZ-induced oxidative stress (Amin et al. 2010; Albasher et al. 2020; Mansour et al. 2021). The testicular tissue is eminently liable to oxidative injury due to its high content of polyunsaturated fatty acids (Sikka 1996). The metabolism of azo dyes catalyzed by reductases and peroxidases, resulting in the formation of semiquinone radicals and aromatic amines, may be the reason behind TRZ-induced oxidative stress (Demirkol

et al. 2012). Furthermore, semiquinone radicals promote the formation of other radicals such as superoxide and hydroxyl radicals, and hydrogen peroxide which exaggerate oxidative stress.. Our results illustrated a marked improvement in MDA level in testes of rats co-administered TRZ and EGb. Similarly, previous studies reported that EGb can confer protection against methotrexate-induced testicular damage (Sherif et al. 2020; Mansour et al. 2021). The observed antioxidant effect of EGb is closely related to its constituents of terpenoids and flavonoids, which are highly efficient ingredients in the scavenging of free radicals, including superoxide, hydroxyl, and peroxyl radicals (Yeh et al. 2009).

Depletion of cellular antioxidants is strongly associated with oxidative stress and progression of several disorders. The results reported here revealed decrease in GSH content and activities of SOD and CAT. Overconsumption of antioxidants to remove increased levels of oxidants may explain the decline in the cellular antioxidants. Supplementation of EGb significantly enhanced the measured antioxidants in TRZ-treated rats. EGb possibly ameliorated TRZ-induced

Fig. 5 Photomicrographs of testicular tissue from rats treated with TRZ alone (**a**, **b**, **c**, and **d**). Figs. **a** and **b** show deformed and atrophied seminiferous tubules with irregular outline, wide spaces between the tubules (filled star), markedly disarranged spermatogenic cells (double black arrow), vacuoles between spermatogenic cells (V), lack of spermatozoa (Z), congested blood vessels (bv), and degenerated Leydig cells (red arrow). Figs. **c** and **d** illustrate higher magnification of the seminiferous tubules showing spermatogonia with pyknotic nuclei (green arrow), separation between germ cells (double headed arrow), clustered spermatids (S), and elongated scanty spermatids (curved orange arrow). H&E, scale bar: 200 μm (**a** and **b**) and 50 μm (**c** and **d**)
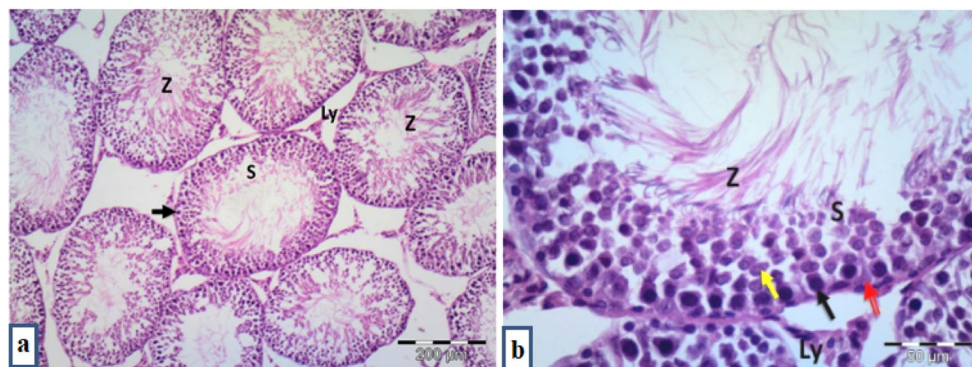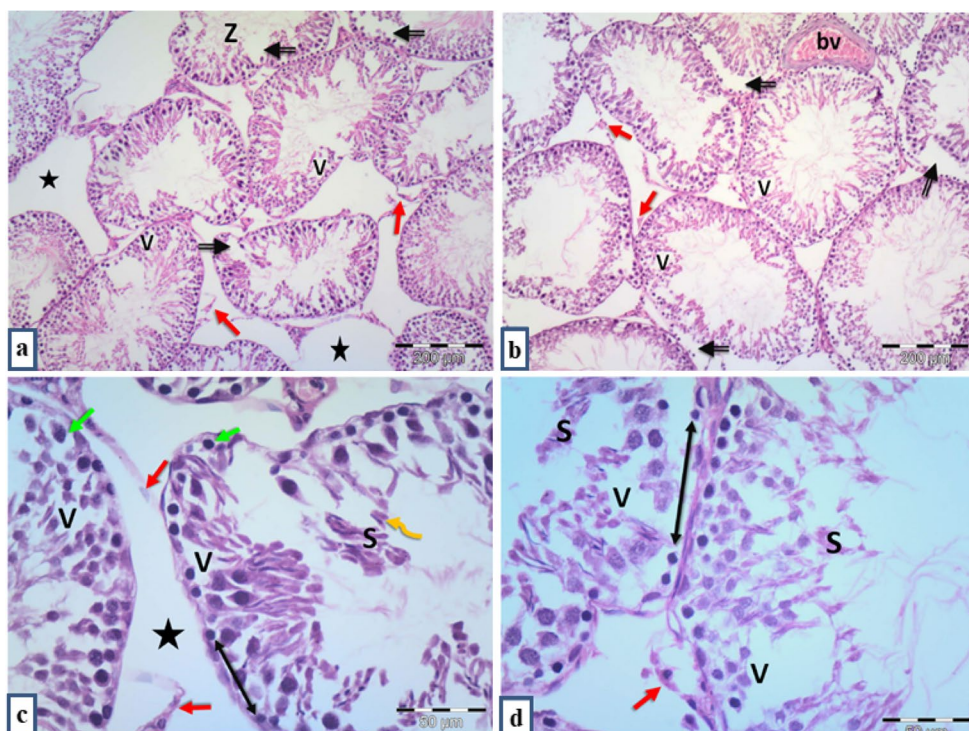

Fig. 6 Photomicrographs of sections from testes of rats treated with TRZ and EGb. Fig. **a** shows improved architecture of seminiferous tubules with normal arrangement of spermatogenic cells, spermatogonia (black arrow), normal Leydig cells (Ly), spermatids (S), and sperm (Z). Fig. **b** shows higher magnification of seminiferous tubules with normal primary spermatocyte (yellow arrow) and Sertoli cells (double red arrow). H&E, scale bar: 200 μm (**a**) and 50 μm (**b**)

oxidative stress through recovery of the testicular antioxidants. In line with our results, EGb was reported to attenuate the testicular toxicity associated with chemotherapies by restoring normal testicular antioxidants along with decreasing the level of MDA (Sherif et al. 2020). EGb has been reported to upregulate testicular antioxidant enzyme by increasing the gene expression of nuclear factor erythroid 2-related factor 2 (Nrf2), a transcription factor that regulates cell oxidative stress and inflammation through the expression of genes encoding detoxifying and antioxidant enzymes (Mansour et al. 2021).

Regulation of the male reproductive function necessitates a multiplex array of endocrine interactions. The most significant regulatory pathway is the hypothalamic-pituitary–gonadal axis, which regulates vital functions through the release of gonadotropins (FSH and LH), in addition to maintaining high levels of testosterone from Leydig cells to control normal spermatogenesis (Anderson and Baird 2002). The results reported here revealed that TRZ administration significantly decreased LH and testosterone levels. Low levels of LH can negatively affect the release of testosterone from Leydig cells. Disruption in reproductive hormones

Fig. 7 Photomicrographs of sections in rats' testes showing immunoreactivity to caspase 3. Sections of the control group (**a**) and EGb group (**b**) show a negative or faint positive reaction. Testicular sections from TRZ-treated group (**c**) show strong immunoreactivity for caspase 3 (red arrows) while TRZ-EGb treated group (**d**) demonstrated weak expression to caspase 3. Scale bar is 50 μm
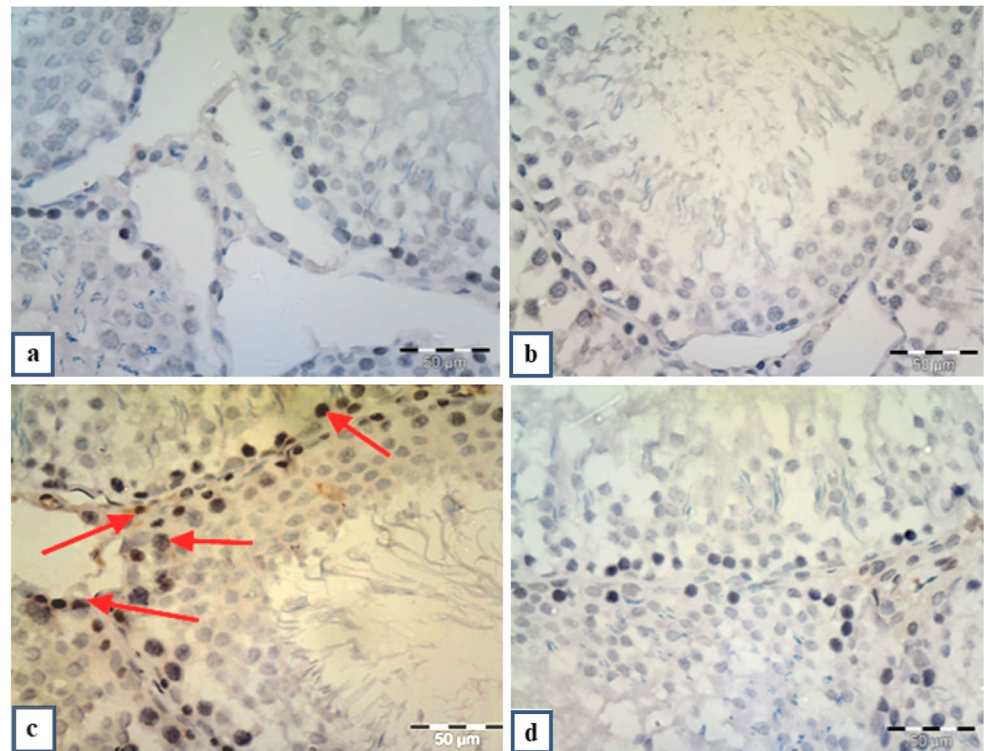
after TRZ administration can markedly affect steroidogenesis and spermatogenesis processes, which can be reflected in the significant decrease in sperm count and quality. Surprisingly, TRZ administration increased the level of FSH in our study. The results reporting the effect of TRZ on the levels of reproductive hormones are contradictory. Elekima and Nwachuku (2019) reported that they had no detrimental effect on reproductive hormones after TRZ administration. Wopara et al. (2021) reported increased levels of FSH, LH, and testosterone when TRZ was administered in combination with erythrosine, a synthetic food colorant. 17β-HSD is an enzyme that catalyzes the synthesis of testosterone in the Leydig cells (Hu et al. 2010). The present data showed a decline in testicular 17β-HSD activity in rats treated with TRZ which may explain the observed decrease in testosterone level in our study. Ayad et al. (2022) reported that the decrease in LH level can negatively affect 17β-HSD activity. Therefore, it can be suggested that the decrease in LH level and subsequent inhibition of 17β-HSD activity can result in a decreased level of testosterone. Administration of EGb plus TRZ for 28 days improved serum FSH, LH, and testosterone levels as well as testicular activity of 17β-HSD. These findings are consistent with those of Mansour et al. (2021), who reported that EGb ameliorated methotrexate-induced disruption of reproductive hormones. EGb conserves the volume and morphology of Leydig cells and increases the level of LH (de Souza Predes et al. 2011). Furthermore, all these parameters were significantly improved

by administering quercetin, a bioactive component of EGb, which has been reported to improve testicular steroidogenesis (Martin and Touaibia 2020).

High levels of inflammatory cytokines can aggravate the progression of testicular diseases (Loveland et al. 2017). The results reported in our study, which agree with Wopara et al. (2021), showed that TRZ-treated rats had higher levels of TNF-α, IL-1β, and IL-6 in the testes, indicating testicular inflammation. Inflammatory cytokines can contribute to testicular cell degeneration, which can adversely affect spermatogenesis and reproductive hormone production (Suescun et al. 2023). Wopara et al. (2021) reported significant upregulation in the expression of testicular TNF-α, IL-1α, and IL-1β genes in rats administered TRZ and erythrosine. TNF-α is an essential cytokine that regulates different processes in the mammalian testes, including spermatogenesis. Additionally, it can also promote the release of IL-1β and IL-6 which can induce oxidative stress and adversely affect spermatogenesis (Hong et al. 2004). Oxidative stress and inflammation are interrelated mechanisms. Excess ROS production has been documented to activate nuclear factor-kappa B (NF-κB), a transcription factor that can mediate upregulation of genes encoding the synthesis of pro-inflammatory mediators and is considered the primary regulator of inflammation (Singh et al. 2022). Therefore, TRZ-induced testicular inflammation can be mediated through ROS-induced upregulation of NF-κB and subsequent increased synthesis of gene products such as inflammatory cytokines.

Raposa et al. (2016) reported that TRZ could significantly increase the expression of NF-κB in the liver of male and female mice. Our finding reported the anti-inflammatory effect of EGb by decreasing testicular TNF-α, IL-1β, and IL-6 levels which can be mediated through inhibition of NF-κB expression as previously reported by Fu et al. (2019). The anti-inflammatory effect of EGb was previously reported against methotrexate-induced testicular damage (Mansour et al. 2021).

Infertility is a health problem that encounters about 15% of couples globally of which, the male factor is involved in almost half of the cases (Krausz and Riera-Escamilla 2018). Decreased sperm count, motility, viability, and abnormal morphology are frequent attributes that diagnose male infertility. Our results showed marked decreases in sperm count, motility, and viability, while the percentage of sperm abnormalities significantly increased in rats treated with TRZ. Oxidative damage may be one of the main contributors to decreased sperm count and quality. Sperms are vulnerable to oxidative damage due to their high content of unsaturated lipids in the plasma membrane (Aitken et al. 2012). Peroxidation of lipids and subsequent loss of membrane integrity can cause substantial damage to sperm (O'Flaherty 2020). Furthermore, ROS can adversely affect the blood-testis barrier which contributes to disruption of spermatogenesis and decrease sperm quality (Liu et al. 2022). In light of this evidence, TRZ-induced ROS and oxidative stress may explain the observed deterioration in sperm quality. Similarly, Mehedi et al. (2009) reported a significant decrease in sperm quality in the mice after ingestion of TRZ. The decline in the sperm antioxidant system may be another reason for the observed decrease in the count and quality of the sperm. High level of testosterone is critically essential for spermatogenesis and maintenance of normal sperm quality. Therefore, the decline in sperm quality and quantity may be associated with impaired spermatogenesis due to disruption in testosterone secretion, severe alterations in the seminiferous tubules, and/or excessive sperm apoptosis. Concomitant administration of TRZ and EGb significantly improved sperm quality parameters. In line with our results, Mansour et al. (2021) found that EGb significantly improved the deterioration in sperm quality induced by methotrexate administration. The ameliorative effect of EGb may be attributed to its antioxidant effect.

Testicular dysfunction caused by exposure to TRZ was supported by histopathological examination of H&E stained sections, which revealed severe changes and loss of regular architecture of the testes. Atrophied seminiferous tubules, deformed germ cells, degenerated spermatogenic cells, absence of sperm, and Leydig cell degeneration were observed, which confirm testicular injury. Mehedi et al. (2009) demonstrated similar histological changes in seminiferous tubules and Leydig cells from TRZ-treated mice. These histological alterations can be caused by the direct effect of TRZ and its metabolites on testicular tissue or indirectly by the disruption of pituitary gonadotropins. Furthermore, peroxidation of lipids can decrease membrane fluidity and affect membrane integrity and functionality, causing significant pathological changes (Sadžak et al. 2020). Abd-Elkareem et al. (2021) reported similar deterioration in the testicular tissue of rats administered the food additive monosodium glutamate. Supplementation with EGb significantly improved the histological architecture of the testes of TRZ-treated rats. Seminiferous tubules with well-organized germ cells, normal appearance of spermatogenic cells and Leydig cells, and improved interstitial tissue were observed. EGb has been reported to improve histopathological changes in the testes of rats induced by ischemia/reperfusion injury (Akgül et al. 2008), doxorubicin (Yeh et al. 2009), and methotrexate (Mansour et al. 2021). The radical scavenging, antioxidant, and anti-inflammatory properties of EGb may explain the improvement in testicular tissue.

Apoptosis is a process of precisely controlled cell death that is essential for preserving tissue homeostasis. It is triggered by sequential activation of caspases, a group of endoproteases that become activated when the cell decides to undergo apoptosis. Caspase-3 is a frequently activated protease that catalyzes DNA fragmentation and cleavage of many cellular proteins. To examine the ability of TRZ to induce apoptosis in our study, the immunoreactivity of caspase-3 was examined in the testes of rats. The results showed a strong positive immunoreactivity to caspase-3 which may be attributed to increased oxidative stress. Abd-Elhakim et al. (2018) reported that the increase in caspase-3 expression in rats exposed to TRZ may be due to increased ROS that can initiate the mitochondrial caspase-3 apoptosis cascade. Administration of EGb in the current study exhibited anti-apoptotic action as manifested by downregulation of caspase-3 expression in the testicular tissue. These findings confirm the anti-apoptotic effect of EGb and its potency to inhibit apoptosis by affecting the caspase-3 pathway. The anti-apoptotic effect of EGb was previously documented in the testis and other organs (Yeh et al. 2009; Wu et al. 2015; Gomaa et al. 2020).

Conclusion

In conclusion, this study indicated that exposure to tartrazine, even within the acceptable daily intake range, adversely affected testicular structure and function. It induced oxidative stress, testicular dysfunction, inflammation, and apoptosis, as well as histological deterioration in the testicular architecture. The study also provided evidence for the protective effect of *Ginkgo biloba* extract against tartrazine-induced testicular toxicity. The

mechanism(s) by which *Ginkgo biloba* extract protected involve antioxidant effect by modulation of the testicular redox sate, anti-inflammatory effect through inhibition of inflammatory mediators, and apoptotic action by down-regulation of caspase-3 expression. These data strongly recommend limiting the intake of tartrazine as a synthetic azo dye and highlight the beneficial effects for supple-mentation of the herbal *Ginkgo biloba* against tartrazine-induced testicular toxicity. A limitation of our study is that Nrf2, NF-κB, and the gene expression of antioxidant enzymes were not determined currently. A further study is needed including these molecular studies to have a deeper insight and confirms the exact mechanism(s) by which EGb affords its protective effect.

Declarations

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