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Suppressive effect of *Ginkgo biloba* extract (EGb 761) on topsin induced ovarian toxicity and oxidative stress in albino rats

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ABSTRACT

Topsin is a fungicide used against a wide range of pathogens in field crops, fruits, ornamentals and vegetables. The present work studied the effect of topsin on the ovary of Wistar rats and the possible ameliorative role of *Ginko biloba* extract (EGB). Many histopathological changes were seen in the ovary of rats after treatment with topsin. The number of ovarian follicles decreased and most of them degenerated which accompanied by increased of atretic follicles. Topsin significantly decreased the levels of both LH and FSH and increased estradiol. Histochemical results revealed an increase in carbohydrate content and decrease in total protein in the ovarian tissue. Moreover, topsin led to significant increase of LPO and CAT activity and non-significant increase of SOD. Treating animals with topsin and EGB effectively alleviated topsin-induced ovarian toxicity. In conclusion, this study provides evidence that topsin adversely damages ovarian tissue through increasing oxidative stress, while EGB treatment effectively attenuates the toxicity and oxidative effect of topsin in the ovary.

Key words: Topsin, Ovary, EGB, Histology, Oxidative stress.

INTRODUCTION

Fungicides are extensively used in industries, agricultures, home and gardens for many purposes including protection of seed grain during storage and germination. Although these fungicides have many benefit purposes, they cause adverse effects in both human and animals. Fungicide residues have been found on food for human consumption, mostly from post-harvest treatments (Brooks and Roberts, 1999). One of the most important fungicides groups is benzimidazole group which consists of thiophanate methyl (Topsin, benomyl and carbendazim). Dimethyl 4, 4,-(o-phenylene) bis (3-thioallophanate) (Topsin) is broad-spectrum fungicide controlling a wide range of pathogens. It is also used as a preservative in paint, papermaking, leather industry and as a preservative of fruits. It is effectively used against a wide variety of fungal diseases in vegetable and crops. Topsin is metabolized into benzimidazole compounds including the well known toxicant carbendazim (Traina *et al.*, 1998). Singh *et al*. (1987) evaluated the toxicity of topsin in rat at different doses. On postmortem examination, the capsular tissue of the liver showed excessive proliferation of fibers and mononuclear cell infiltration.Lungs showed pulmonary oedema, emphysema, congestion, hemorrhage and haemosidrosis. In addition, there was subcapsular oedema and reticular cell hyperplasia in the spleen. Fimognari *et al*. (1999) reported that topsin delayed cell proliferation and increased micronucleus induction and apoptosis in cultured human peripheral blood lymphocytes. Saquib *et al*. (2009) reported that topsin significantly increased the number of binucleated micronucleated cells with a dose dependent reduction in the nuclear division index in human lymphocyte. Maranghi *et al*. (2003) studied the histological and histomorphometric alterations in thyroid and adrenals of rat pups exposed in utero

to Topsin on pregnant rats. Thyroid histology showed necrotic cells with increased irregular nuclei and/or mitosis. Thyroid histomorphometry showed reduced follicular density, moderately increased follicular cell height and number of nuclei/follicle. The adrenal cortex showed increased karyomegaly and hydropic degeneration. The authors added that topsin may act as endocrine disrupter, suggesting that attention should be paid to delay endocrine alterations elicited by agrochemicals. Traina *et al.* (1998) reported that topsin at dose level of 700 and 1000 mg/kg/body weight for 35 consecutive days caused histopathological alterations in testes of mouse.

Recently, there was a great interest upon using herbs as an alternative medicine. Leaves of the plant *Ginko biloba* have been used for thousands of years as a traditional Chinese herbal medicine (Logani et al.2000). Recently, a standardized chemical product from these leaves was pharmacologically prepared containing two major functional constituents (24-25% flavonoid glycosides and 6% terpenoids) (Shen et al.1998). This extract or 761(EGB) was found to have many pharmacological proposes. It has neuroprotective, anticancer, cardioprotective, stress alleviating, memory enhancing effects and possible effects on tinnitus and psychiatric disorders (Chao and Chu, 2004, Yang *et al.,* 2005, Masteikova *et al.* 2007). The therapeutic mechanisms of action of Ginkgo leaf extract are suggested to be through its antioxidant, antiplatelet, antihypoxic, antiedemic, hemorrheologic and microcirculatory actions, where the flavenoid and terpenoid constituents may act in a complementary manner (Mahadevan and Park, 2008, Qi et al.2010, Shen et al.2011). Harputluoglu *et al. (*2006) reported that EGB ameliorated thioacetamide induced hepatic failure through its free radical scavenging effect. In another study, Sener *et al. (*2006) studied the possible protective effects of EGB extract against oxidative damage induced by irradiation in lung, liver, kidney and ileum of rats. The authors concluded that pretreatment of EGB attenuated irradiation-induced oxidative organ damage injury through its free radical scavenging and antioxidative properties. They suggested that Gb extract may have a potential benefit in enhancing success of radiotherapy. Tamborini and Taurelle (1993) reported that administration of EGB improved the premenstrual syndrome. In this respect, Oh and Chung (2006) stated that EGB exhibited estrogenic and antiestrogenic activity and had a biphasic effect on estrogen. Little informations seem to be available about the effect of topsin on ovary of mammals. This stimulated us to study the effect of topsin on ovary of albino rats and the possible ameliorative role of *Ginko biloba* extract in topsin-induced ovarian toxicity.

Materials and Methods

Chemicals

Thiophanate methyl (Topsin)

Thiophanate methyl (Topsin) [70% WP Dimethyl 4,4(o-phenylene) bis (3-thioallophanate)] produced by Pilar Quim Co., (Shanghai), China was used.. It was dissolved in water and orally given at dose level $^1/_{10}$ LD_{50} (0.664 mg/kg/bw) (Hashimoto *et al*. 1972).

Ginkgo biloba extract

Ginkgo biloba extract used as Tanakan 761 (EGB) produced by Amriya for Pharmaceutical Industries, Egypt under license of Beaufour-ipsen International, Paris-France. EGB was orally administered at a dose level of 40 mg/kg/b.w (Zhou *et al*., 2006).

Animals and treatments

Adult female albino Wistar rats approximately three months old and weighting 140±5 g were used. The animals were kept under constant condition for two weeks before and through the experimental work. They were fed standard diet composed of 55% corn starch 20% casein, 15 % corn oil, 5% salt mixture and 5 % vitaminized starch and water was available *ad libitum.* All the experiments were done in compliance with the Guide for the Care and Use of Laboratory animals (National Research Council, 1985).The animals were divided into four groups of 20 rats each.

Group1: Animals of this group had been kept as normal without any treatment and considered as controls.

Group2: Animals of this group were orally administered EGB 40 mg/kg/bw daily for 4 weeks and animals were then sacrificed.

Group3: Animals of this group were orally given $^1/_{10}$ LD_{50} of topsin daily for 8 weeks.

Group4: Animals of this group were orally given $^1/_{10}$ LD_{50} of topsin for 8 weeks followed by EGB for 4 weeks and then animals were sacrificed.

Histological preparation

Immediately after decapitation, ovaries were quickly removed and fixed in alcoholic Bouin's fluid then dehydrated in an ascending series of alcohol, cleared in two changes of xylene and embedded in paraffin wax. Sections of 5 micrometers thickness were cut using rotary microtome and mounted on clean slides. For histological examination sections were stained with Ehrlich's haematoxylin and eosin. For histochemical study specimens were fixed in Carnoy's fluid. Periodic acid Schiff's reaction (Kiernan ,1981) was used for demonstration of polysaccharides. Total proteins were detected using the mercury bromophenol blue method (Pearse, 1972).

Morphometric analysis

Haematoxylin and eosin stained sections of ovaries of control and experimental animals were examined histologically and used for morphometric analysis. Differential count of types of follicles and corpora lutea was recorded according to Peters and Mc Natty (1980).

Biochemical analyses

For enzymes determination, blood samples was obtained from the inferior vena cava and then centrifuged. Sera were stored at -20 ºC until assayed for the biochemical parameters. FSH, LH and estradiol were quantitatively determined in sera by enzyme immunoassay kit (Medix Biotech Inc., USA). The extent of lipid peroxidation was estimated as the concentration of thiobarbituric acid reactive products (malondialedhyde) according to (Ohkawa et

al., 1979). Superoxide dismutase activity was measured using the methods of Rest and Spitznagel (1977). The principal of this method depends on the ability of SOD to inhibit the power of phenazine methosulphate-mediated to reduce the nitroblue tetrazolium. Catalase activity was determined from the rate of decomposition of H_2O_2 (Aebi et al., 1974).

Statistical analysis

The results were expressed as mean ± SD of different groups. The differences between the mean values were evaluated by ANOVA followed by Student's "t" test using Minitab 12 computer program (Minitab Inc., State Collage, P.A).

RESULTS

Histological Observations

Sections of ovary of control rat revealed that it consists of spindle shaped cells, fine collagen fibres and ground substance which together constitute the ovarian stroma. The peripheral zone of the stroma, the cortex, contains numerous follicles in various stages of development (primary, secondary and Graafian follicle). In addition, corpora lutea degenerate and forms corpus ablicantes and degenerated atretic follicles. The central zone of the ovarian stroma, the medulla, is highly vascular and contains hilus cells. The ovarian artery (a branch of aorta) and the ovarian branches of uterine artery form anastomoses in the mesovarium and the broad ligament. On the surface of the ovary, germinal epithelium is present (Fig.1A).

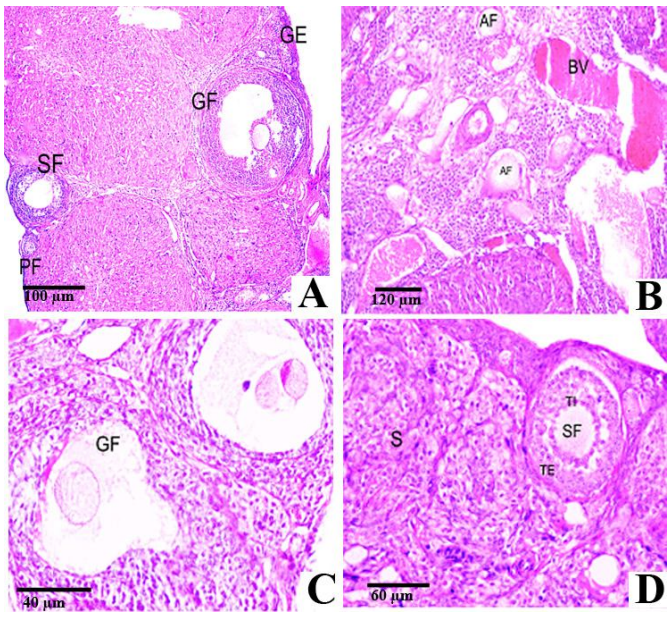

Fig.1. Sections in ovary of (A) control rat showing graaffian follicle(GF),germinal epithelium (GE),primary follicle (PF)and secondary follicle (SF). (B)&(C) after treatment with topsin showing atretic follicle (AF) and congested blood vessel (BV). (D) treatment with topsin + EGB showing secondary follicle (SF) with normal theca interna (TI) and well defined theca extrena (TE) merged in healthy stroma (S).

Ovarian sections of rats treated daily with EGB for 8 weeks showed normal structure of germinal epithelium as well as healthy follicles and stromal cells. Sections in ovaries of rats daily treated with topsin for 8 weeks revealed many deleterious histological changes. The germinal epithelium showed abnormal structure including the appearance of many degrees of invaginations along its surface. The cuboidal cells of the germinal epithelium became flattened with deeply stained nuclei and lost their arrangement. The ovarian stroma contained large number of vacuoles, atretic follicles of different sizes and congested blood vessels (Fig.1B). Some abnormal Graafian follicles appeared with enlarged antrum and degenerated zona pellucida and cumulus oophorus. Other Graafian follicles appeared with projections and contained two nuclei or faintly stained nuclei (Fig.1C). The corpora lutea occupied a large area in the section. Ovaries of rats treated daily with topsin for 8 weeks followed by EGB for 4 weeks exhibited marked improvement in the histological state compared with those of animals treated with topsin. Normal like stages of oogenesis, primary, secondary and Graafian follicles were observed. Secondary follicles appeared with almost normal theca interna and theca externa while Graafian follicles appeared with even thickness zona granulosa (Fig.1D).

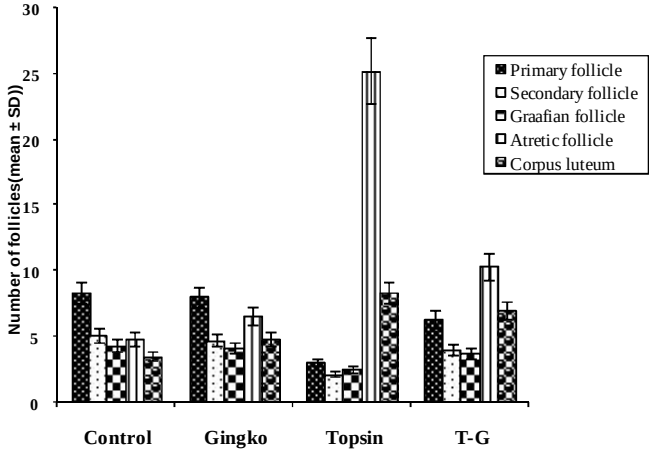

Fig.2. Effect of different treatments on the number of ovarian follicles and corpora lutea.

Morphometric results

Data in figure (2) showed that treatment of animals with EGB induced insignificant difference in numbers of the ovarian follicles compared with those of control animals. On the other hand, ovaries of animals treated with topsin revealed a significant decrease in the numbers of primary, secondary and Graafian follicles while significant increase in the number of both atretic follicles and corpora lutea was recorded. Animals treated with EGB after topsin, exhibited a great degree of amelioration in the numbers of follicles when compared with topsin -treated rats.

Histochemical Observations

Total carbohydrates

Examination of ovary of control rats revealed that the germinal epithelial cells and the stromal cells showed a slight PAS-positive reaction. The ovum of primary, secondary and Graafian follicles showed a moderate reactivity while the cytoplasm of their

granulosa was slightly stained. The zona pellucida encircling the oocyte in the different types of the follicles had a marked reaction. The corona radiata and the theca folliculi showed slightly positive PAS-reaction whereas the antrum of the follicles was negatively stained. The luteal cells of the corpora lutea appeared slightly reactive with PAS-reaction. The core of the atretic follicles showed a moderate PAS-positive reaction (Fig.3A). Sections in ovaries of animals treated with topsin showed marked increase of PAS-positive materials in the different parts of the ovary as compared with ovary of both control and Ginkgo treated rats. A marked increase in PAS material was observed in stromal cells, degenerated Graafian follicles, cores of atretic follicles as well as the luteal cells (Fig.3B). Examination of ovary of rats treated with topsin and EGB showed reduction in the total carbohydrate contents of the stromal cells, the ovum of primary, secondary and Graafian follicles compared with those of topsin treated animals (Fig.3C).

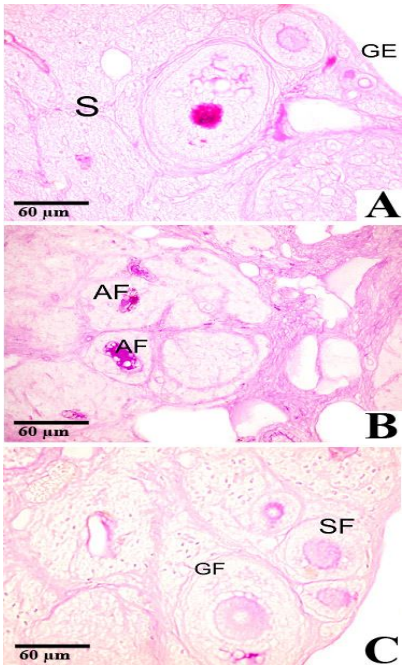

Fig.3. Sections in ovary stained with PAS showing (A) slight total carbohydrate contents in germinal epithelium (GE) and stroma (S) of a control rat, (B) an increase of total carbohydrates after treatment with topsin, (C) treatment with topsin+ EGB

Total proteins

Examination of ovaries of control rats stained with mercury bromophenol blue showed a positive affinity in most of the ovarian components. The cytoplasm of germinal epithelial cells and the stromal area were strongly stained. The primary, secondary and Graafian follicles and zona pellucida exhibited a strong stain. The corona radiata and theca folliculi exhibited a moderate reaction (Fig.4A). Ovaries of EGB treated rats showed, to great extent, normal total protein contents in most of the ovarian components. Examination of ovaries of topsin treated rats exhibited marked reduction in total protein contents in most of the ovarian components. The germinal epithelium, stroma, degenerated Graafian follicles and Corpus luteum were moderately stained (Figs.4B). Examination of ovaries of rats treated with topsin for followed by EGB showed marked improvement compared with animals treated with topsin. The protein contents were increased in all parts of ovarian tissue; germinal epithelium, stromal cells, primary, secondary and Graafian follicles as well as corpus luteum (Fig.4C).

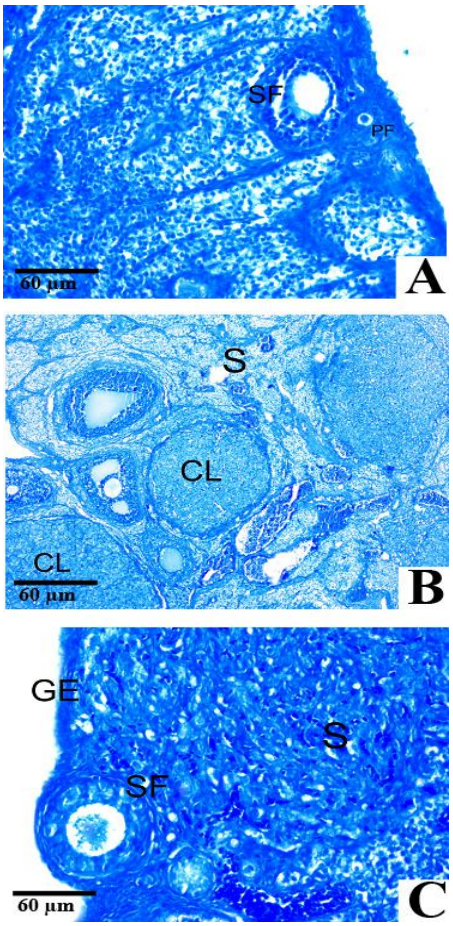

Fig.4. Sections in ovary stained with bromophenol blue showing (A) total protein contents in primary follicle (PF) and secondary follicle (SF) of control rat, (B) a reduction of total proteins after treatment with topsin, (C) treatment with topsin+ EGB.

Biochemical Results

Changes in FSH, LH and estradiol

Treating animals with topsin revealed significant decrease in FSH level compared with control group. Animals treated with topsin and EGB showed insignificant change in FSH level when compared with topsin treated rats (Fig. 5A). Concerning the change in LH level, figure (5B) showed significant decrease in sera of animals treated with topsin while animals treated with EGB showed significant increase when compared with topsin treated rats. Figure (5C) showed highly significant increase in estradiol level in sera of animals treated with topsin compared with control and significant decrease in sera of animals treated with topsin ans GBE. Treating animals with EGB showed non-significant difference in levels of FSH, LH and estradiol when compared with control group.

Changes in serum MDA, CAT and SOD

Treating animals with tospsin caused significant ($P<0.05$) increase in the level of MDA compared with control group, the

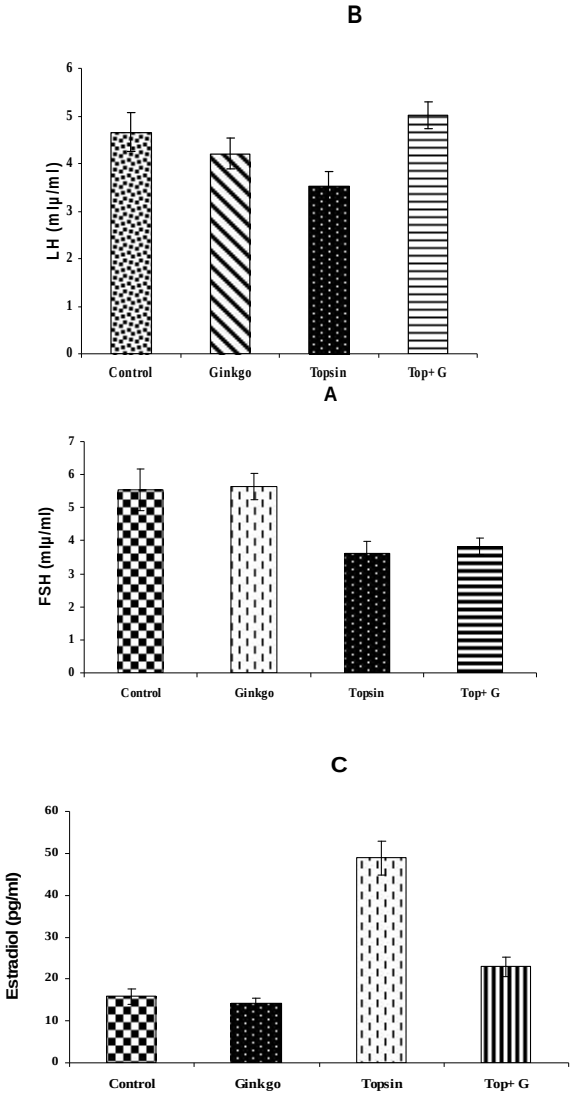

Fig.5. Change in (A) FSH, (B) LH and (C) Estradiol in different animal groups (mean ±SD).

mean value was 9.15 ± 0.27 nmol/ml. Animals treated with topsin and EGB showed significant decrease in MDA level in sera compared with topsin group; the mean value was 5.92 ± 0.2 (Fig.6A). No significant change was recorded between control and EGB treated group. Similarly, a significant increase was recorded in the activity of CAT in sera of topsin-traeted animals compared with control group. The mean value of CAT was 33.33 ± 2.3 in topsin group after 8 weeks. Animals given topsin and EGB showed significant decrease in the activity of CAT (11.33 ± 0.43) compared with topsin group (Fig.6B). Insignificant increase was recorded in SOD activity after treatment with topsin and insignificant decrease was observed in rats given topsin and EGB (Fig.6C). No significant change was obtained in SOD and CAT activity in sera of rats treated with EGB for 8 weeks.

DISCUSSION

The increasing use of pesticides for agricultural use and public health purposes is among the major factors of pollution in different countries, including Egypt. Fungicides are now produced and enter the environment in greater quantities to control fungi. Topsin is a fungicide used against a wide range of pathogens in field crops, fruits, ornamentals and vegetables (Rockett *et al.* 2006).In the present study, many histopathological changes were seen in the ovary of albino rats after treatment with topsin. The number of ovarian follicles decreased and most of them degenerated which accompanied by increased of atretic follicles. These results are similar to that obtained by some investigators using different fungicides and other pesticides. Armenti *et al.* (2008) concluded that exposure to organochlorine pesticide, methoxychlor, resulted in reduced ovulation and fertility and premature aging, possibly by altering ovarian gene expression and folliculogenesis. The authors added that methoxychlor reduced serum progesterone, increased luteinizing hormone and down-regulated Cytochrome P_{450} side-chain cleavage. Juliani et al. (2008) indicated that the herbicide, atrazine induced impaired folliculogenesis and increased follicular atresia in rats. The authors added that it act as endocrine disruptor. Moreover, Shibayama et al. (2009) reported that atrazine (in a two-week repeated dose) led to histopathological findings such as decrease in the numbers of corpora lutea, increase in large-sized atretic follicles and swelling of the luteal cells. The authors suggested that atrazine had an ovulatory effect through suppression of the luteinizing hormone surge. Harazono and Ema (2000) reported that tributyltin chloride administration significantly decreased serum progesterone levels and suppressed the uterine decidual cell. Güney *et al*. (2007) found that subchronic administration of methidathion pesticide to female rats caused ovarian damage. They added that elevation of lipid peroxidase enzyme may be one of the molecular mechanisms involved this toxicity.

In the present study, topsin significantly decreased the levels of both LH and FSH. Sakr *et al.* (2009) reported that mancozeb fungicide decreased LH in sera of rats. On the contrary, Goldman *et al*. (1989) recorded an elevation of FSH level and Rajeswary *et al*. (2007) reported non significant difference in the level of serum LH after carbendazim treatment in male rats. Topsin treatment significantly increased serum estradiol level compared with control group. This increase may be due to enhanced synthesis or impaired metabolism. This result come in accordance with Morinaga *et al. (*2004) who reported that carbendazim induced aromatase activity in human ovarian granulosa-like tumor cell line which responsible for maintaining the homeostatic balance between androgens and estrogens.. On the other hand, Spencer *et al.* (1996) reported unchanged estradiol after carbendazim administration. Oduma *et al*. (2006) mentioned that heptachlor, a chlorinated hydrocarbon pesticide, suppressed the production of progesterone and estradiol in female rat or in isolated ovaries *in vitro*.

Histochemical results showed that when animals treated with Topsin for 8 weeks, an increase in carbohydrate content and

decrease in total protein were observed. The increased carbohydrate contents may be due to disturbance in carbohydrate metabolism. In this respect, Singh *et al. (*1987) recorded increase in both blood glucose and liver glycogen in Topsin treated rats. In addition, Rajeswary *et al*. (2007) recorded decrease in glucose-6-

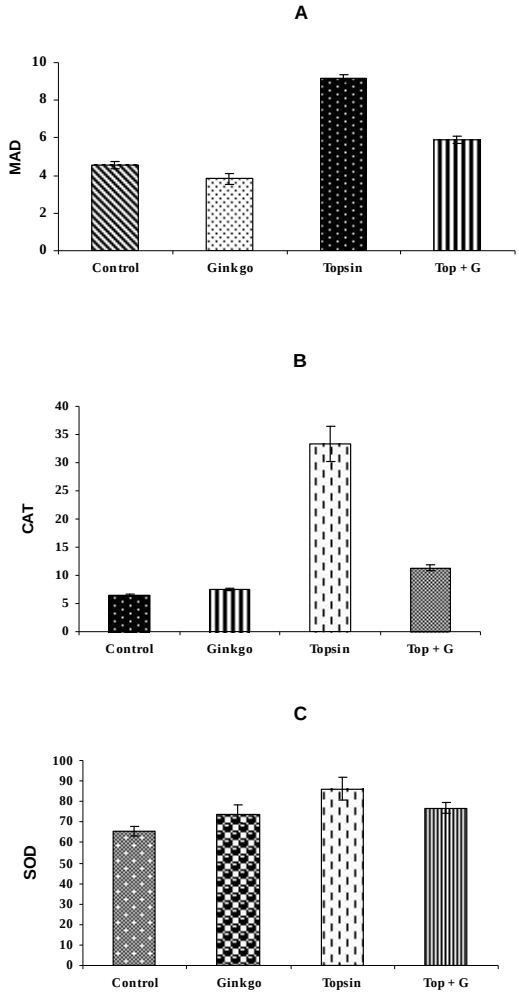

Fig.6. Effect of different treatments on (A). MAD, (B). CAT and (C). SOD (nmol/ml).

phosphate dehydrogenase in testes of rats treated with carbendazim (metabolite of Topsin). The reduction of total proteins observed in the present study, in ovary of Topsin treated rats may be due to change in enzymes related to protein degradation. In this concern, Igbedioh and Akinyele (1996) recorded significant increase in alanine and aspartate transaminases activities (concerned with protein degradation) in rats treated with benomyl. Sakr *et al.* (2004) also observed reduction of total proteins in liver of benomyl-treated rats. The authors suggested that the reduction may be due to either arrested metabolism or to use it to build up new cells or enzymes to reduce the stress. Similar results were obtained after benomyl treatment; by Igbedioh and Akinyele, (1992) and Spencer *et al*. (1996) in liver and uterus of rats and by Marinovich *et al*. (1994) in human leukemic cell line (HL-60 cell). Mahadevaswami *et al*. (2000) reported that mancozeb fungicide caused a significant decrease in the levels of protein, glycogen, total lipid, phospholipids, and neutral lipid in the liver, uterus, and ovary. In addition to the decrease in the compensatory ovarian hypertrophy, mancozeb treatment reduced the number of healthy follicles with a concomitant increase in the number of atretic follicles.

Topsin led to significant increase of LPO and CAT activity and non-significant increase of SOD when compared with control group. Similarly, Rajeswary *et al*. (2007) recorded elevation in lipid peroxidase and reactive oxygen after carbendazim administration. On the contrary, Muthuviveganadavel *et al*. (2008) reported that carbendazim administration caused significant decrease in lipid peroxidation in liver tissue of male rats. The increased activities of CAT and SOD after topsin treatment may be a defense mechanism against oxygen free radical damage. In this concern, Jin *et al. (*2001) reported that both of SOD and CAT have a beneficial effect against various diseases mediated by reactive oxygen species. Liochev and Fridovich (2007) explained the effects of overproduction of SOD on the basis of increased H_2O_2 production by the catalyzed dismutation of O_2^-. In addition, Halliwell and Gutteridge (1990) decided that catalase, a cellular defender finishes the work started by SOD, serves to remove hydrogen peroxide by breaking it to water and oxygen to ovoid generation of hydroxyl radicals. Tate *et al*. (1995) also suggested that the generation H_2O_2 may act as intracellular signal which leads to increase levels of key antioxidant enzymes and other proteins important for protecting the cells from oxidative enzymes. Moreover, Rohrdanz *et al*. (2001) reported that the impairment of mitochondrial functions become obvious only for higher concentrations of H_2O_2 and this led to increase in catalase, manganese superoxide dismutase and glutathione peroxidase expression levels. The ovarian toxicity recorded in the present study may be due to the oxidative stress resulted from the toxicity of topsin or its metabolite, carbendazim. In this respect, Hanukoglu *et al*. (1993) reported that lipid peroxidation and reactive oxygen species are produced by electron leakage outside the electron transfer chains and these oxygen radicals can initiate lipid peroxidation, to inactivate P_{450} enzymes. Mathews *et al.* (2000) reported that the damage occurred in the cell membrane by hydroxyl radicals induced oxidation of polyunsaturated fatty acids in membrane lipid in a process called lipid peroxidation. Moreover, Banks and Soliman (1997) recorded increase in serum hydroperoxides and decrease in reduced glutathione after benomyl toxicity in rats. The authors added that the *in vivo* toxicity of benomyl may be associated with oxidative stress.

Concerning the effect of *Ginkgo biloba*, when animals treated with topsin followed by EGB marked improvement in the histological picture of ovary and the number of healthy follicles was seen as compared with ovary of animals treated with topsin. Moreover, an improvement of carbohydrates and proteins was recorded in ovarian tissues. This result come in accordance with Ji *et al*. (2009) who reported that EGB prevented glucose-induced accumulation of extracellular matrix by lowering the levels of transforming growth factor beta 1, insuline like growth factor 1 and

connective tissue growth factor of high glucose. Moreover, Tang *et al*. (2009) concluded that Gb extract has a protective effect against glomeruloscleroses in diabetic nephropathy of mesangial cells due to the ability of reduction of collagen IV, laminin and mRNA levels. On the contrary, Rudge *et al*.(2007) reported that Gb at 200 mg/kg /day failed to modify the diabetes-associated increase in maternal glycemia. Ahlemeyer and Krieglstein (2003) reported that this improvement in DNA and RNA is due to bilobalide which prevented DNA fragmentation due to hydroxyl radical, β-amyloid and hydrogen peroxide. Ginkgolides A and B decreased the legend binding capacity, protein, and mRNA expression of peripheral benzodiazepine receptor (PBR) which led to decreased corticosteroid synthesis and subsequently the circulating levels of glucocorticoids (Amri *et al*., 1996). Moreover, a time dependent induction of hepatic Cytochrome P450 (CYP) enzyme activity and protein expression was observed after a single dose of 30 mg/kg bilobalide in rats by Taki *et al*. (2009).

Ginkgo administration significantly improved estradiol levels compared with topsin treated rats. In this respect, Oh and Chung (2006) proved that EGB has potential estrogenic activities and exhibited estrogenic and antiestrogenic activity and had a biphasic effect on estrogen. The authors added that EGB contains 24% phytoestrogens (kaempferol, quercetin and isorhamnetin) which could be a part of selective estrogen receptors and modulators. Hodek *et al.* (2002) stated that phytoestrogens show estrogenic activity owing to the structural similarity with estrogen skeleton, mimicking natural estrogens which bind to estrogen receptors and modulate its activity.

EGB treatment ameliorated LPO, CAT and SOD activities. Many authors reported that EGB could be helpful in both therapy and prevention of diseases and other degenerative processes associated with oxidative stress (Onen *et al*., 1999; Schindowski *et al*., 2001 and Kusmic *et al*., 2004). In this respect, Maitra *et al*. (1995) and Haung *et al.* (2000) stated that EGB could counteract the function of ROS, directly scavenge superoxide anion, hydroxyl radicals, peroxyl radical species and nitric oxide. Moreover, EGB has an SOD like activity and a hydroxyl radical scavenging activity (Diamond *et al*., 2000 and Wu *et al*., 2002) and significantly decreased MDA levels and histopathologic scores of the pancreatitis in rats (Zeybek *et al*., 2003). Zhou *et al*.(2006) reported that a possible mechanism of EGB, in 2,4,6-trinitrobenzene sulfonic acid-induced colitis in rats, is that it could scavenge oxidative-free radicals, down-regulate some of the inflammatory mediators involved in the intestinal immune and inflammatory response, including TNF-α, NF-kBp65 and IL6 resulting in the improvement of ulcerative colitis. Moreover, Harputluoglu *et al*. (2006) reported that EGB improved tissue damage in acetic acid induced-colitis and also has antagonistic activity on platelet-activating factor receptors. In addition, Mustafa *et al*.(2006) added that treatment with EGB for five consecutive days attenuated the mucosal damage in rats and subsequently reduced myeloperoxidase activity in colonic damage due to reduce neutrophil infiltration in inflamed colonic tissue and increase GSH levels.

Li *et al*. (2005) concluded that EGB has protective effect against ethanol-induced oxidative injury in rat testes. Pener *et al*. (2005) concluded that EGB with its potent free radicals scavenging and antioxidant properties seems to be a highly promising agent in protecting hepatic tissue against oxidative damage and in preventing hepatic fibrosis and dysfunction due to obstructive jaundice. Iraz *et al*. (2006) stated that EGB has a potent antioxidant and antifibrotic activity in the model of bleomycin-induced lung fibrosis in rats. Moreover, Sun *et al*. (2006) proved that EGB extract could inhibit inducible nitric oxide synthase expression and have neuroprotective effect by preventing nerve cells from apoptosis. EGB extract also protected brain, lung, liver and kidney tissue against mercury (II)-induced oxidative damage in rats (Sener *et al*., 2007).

In conclusion, this study provides evidence that topsin adversely damages ovarian tissue through increasing oxidative stress, while EGB treatment effectively attenuates the toxicity and oxidative effect of topsin in the ovary.

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