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Ameliorative Potential of Aqueous Extract of Broccoli Sprouts Against Triazophos Induced Ovarian Toxicity in Wistar Rats

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Traditional therapeutic procedures using antioxidant-rich fruits and vegetables have been in vogue for the development of evidence-based biomarkers for assessing reproductive health. Present investigation was designed to study the antioxidative potential of broccoli sprouts aqueous extract (BE), against ovarian toxicity in female rats induced by triazophos (TZ). In the experimental setup, six groups of rats were formed; Control (group 1), BE (group 2), TZ (group 3), and also BE+TZ groups such as BE1 (group 4), BE2 (group 5) and BE3 (group 6) groups. Body weight was weekly recorded of all the rats, while vaginal smear was observed daily during 30 days experiment. After sacrifice, oxidative stress (OS) biomarkers levels viz; catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GR), glutathione peroxidase (GPx), glutathione-S-transferase (GST) and lipid peroxidation (LPO) were determined along with histopathological and apoptotic observation. Results revealed differentially modified changes in OS biomarkers as CAT, SOD, GR, GPx, and GST, while LPO levels were significantly improved with broccoli supplementation compared to TZ group rats. Plasma progesterone and estradiol levels were also restored along with improved ovarian histoarchitecture among all BE+TZ treated rats. Reduced apoptotic granulosa cells with reduced atresia and normal ovarian surface epithelium height were also observed with BE treatment. BE exerts multi-mechanistic protective effects against TZ induced ovarian toxicity which is attributable to its antioxidant and protective actions.

Keywords: Apoptosis; Broccoli extract; Endogenous antioxidant; LPO; Ovary; Triazophos.

Organophosphorus (OP) pesticides are the most widely used insecticides globally for pest control and their exposure is a major threat to health care and general safety from toxicity[1,2]. OPs induces neurological disorders, and also act as endocrine disrupters (EDC), which have the potential to alter the normal functioning and development of the female reproductive system[3,4,5]. Organophosphorus pesticides exposure cause elevated generation of reactive oxygen species (ROS) and ultimately induces toxicity conditions in terms of oxidative stress (OS) in animals[6,7]. Endogenous antioxidants act as stress biomarkers and play an important role in female reproductive activities, as there abnormal activity levels have been found to influence female subfertility or infertility[1,2]. Further, supplementation of dietary antioxidants has been indicated as a possible

therapeutic strategy for treating reproductive disfuntions and infertility related disorders by controlling ROS production and oxidative stress[8].

Triazophos (TZ), is a broad spectrum OP insecticide and humans are exposed to TZ by number of food products including vegetables, drinking water, etc.[9,10] and recent literature states that due to its high stability, TZ accumulates in aquatic and other soil ecosystems, as a potential hazard [11,12]. TZ has been reported to induce the ovarian and reproductive toxicity[2], and also its *in utero* and lactational exposure has been evidenced to induce the reproductive system abnormalities in offsprings of *Rattus norvegicus*[13].

Natural plant products are rich in antioxidants and are traditionally in practice since long to strengthen the natural immunity. Further ameliorative studies with antioxidant-rich cruciferous vegetables, which contains pharmacologically active substances such as polyphenols, glucosinolates, vitamin C and flavonoids, has protective potential and stimulates defense mechanisms[14,15,16,17]. There are number of reports suggesting protective effects of natural antioxidants against different classes of pesticides and other environmental contaminants on reproductive stress biomarkers and strengthening potential of fertility parameters[14,18,19,20,21], but toxicokinetic assessment of oxidative stress induction in ovaries by OPs with laboratory animals, and reversal by aqueous broccoli extract are absent or limited. Therefore, the aim of this study was to assess the changes in ovarian stress biomarkers level, and to elucidate the ameliorative potential of broccoli sprouts extract against the TZ induced toxicity

MATERIALS AND METHODS

All the chemicals were purchased from SRL Pvt. Ltd, Sigma-Aldrich, SD Fine-Chem Ltd, or were either of high analytical grades. ELISA Kits for estimation of Estradiol and Progesterone hormones were purchased from Labor Diagnostika Nord, GmbH & Co. KG. Quantification Kit for apoptosis and necrosis was purchased from Biotium. Ashirwad Industries, Mohali, provided standard rat feed, while Triazophos as Truzo 40 EC was purchased from Meghmani Organics Limited, Chharodi, India.

Broccoli sample

Seeds of *Brassica oleracea var. italica*, were procured, grown and sprouts were harvested gently on 5 days and were further processed to a dry powdered form of broccoli extract (BE) as per Sharma and Sangha,[22] protocol. Quantification of glucosinolates (GSLs), from the powder, was done by Moller *et al*.[23], vitamin C by Adom *et al*.[24], total polyphenols by Ainsworth and Gillespie[25], and total flavonoids by Chang *et al*.[26] with slight modifications. Total glucosinolates (GSLs) content was estimated for 200 mg of dried sample and GSLs were considered as a base concentration alongwith other antioxidants in the extract [Table 1]. For experimentation, three different doses of 10, 20 and 30 µmol w.r.t. GSLs were made from dry BE powder in distilled water for subsequent use in present investigation.

Animals and Experimental design

Female albino rats aging 9-12 weeks were obtained from the Department of Livestock Production and Management, GADVASU, Ludhiana. Two rats were housed in each polypropylene cages using bedding of paddy husk in the laboratory, where the optimized humidity of 55±5%, temperature around 25±2°C and a 12 to 12 hours light-dark cycle of photoperiod were maintained. Feed and water were provided to housed animals during experimentation and guidelines of CPCSEA, India were used for animal handling, while experiments were duly approved by the "Institutional Animal Ethics Committee (IAEC)", GADVASU, Ludhiana (date: 06.08.2012 and letter no. 3901-35).

After ten days acclimatization, female rats were segregated into six groups with eight rats in each group as Group I Control rats; Group II as BE rats received 10 µmol of BE; Group III as TZ rats; Group IV as BE1 rats received TZ alongwith 10 µmol of BE; Group V as BE2 rats received TZ and 20 µmol of BE and Group VI as BE3 rats received TZ and 30 µmol of BE. TZ was given as 1/10th of LD_{50} i.e. 8.2 mg/ kg b.w. in olive oil, while control and BE rats were provided with equal volume of olive oil. Rats with BE supplementation and TZ treatment were collectively referred as Br+TZ rats. During 30 days oral intubation experiment, body weights were recorded weekly, while vaginal smear was observed daily to check the cyclicity of all the rats.

Organs weight and Body weight

After experimentation, female rats were mildly anesthetized using chloroform and their blood sample was collected from heart directly in heparinized vials for its further processing. After that blood was centrifuged for 15 minutes at 2300 r.p.m. and the supernatant as plasma was used for hormonal analysis. Subsequently, reproductive organs were excised, and weighed after clearing off the adhering tissue.

Biochemical and Histological studies

After dissection, whole ovary samples from each rat were homogenized in 0.1 M PBS (pH 7.4), centrifuged and the supernatant was used for the biochemical parameters which were assayed by standard methods. Total proteins was estimated by Lowry *et all*[27]; CAT (catalase) by Aebi[28]; SOD (Superoxide Dismutase) by Marklund and Marklund[29]; GST (glutathione-S-transferase) by Habig *et al.*[30]; GR (glutathione reductase) by Carlberg and Mannervik[31]; GPx (glutathione peroxidase) by Hafeman *et al.*[32]; LPO (Lipid peroxidation) by Stocks and Dormandy[33]. Progesterone and Estradiol levels of all rats were assessed by ELISA Kits.

Ovary samples from four rats were used to get granulosa cells smears for analysis of the apoptosis and necrosis study as per the standardized protocol by Sharma *et al*.[2] with the help of fluorescent dyes reagents Annexin V (AV) and Ethidium Homodimer (EH) from Quantification Kit. Similarly, ethidium bromide and acridine orange were used in 0.1 M PBS and cells after one minute incubation, were washed with PBS and then smeared on a slide for observation under microscope. Photography was done by fluorescence Nikon ECLIPSE 80i microscope. Ovary tissues from four rats of each group were processed for 24 hours by placing in alcoholic Bouin's fixative. Using graded series of alcohols ovarian tissues were dehydrated and then cleared using benzene. Subsequently embedded in the paraffin wax having melting point around 58-60°C. Routine laboratory microtome was used to get the 5µm thick sections and further tissue sections were stained by routine procedures with hematoxylin and eosin, and slides

Table 1. Broccoli sprouts extract with glucosinolates and other components for dose formation

Components	Units/ g of dry BE powder	µ mol	30 µ mol of GSLs	20 µ mol of GSLs	10 µ mol of GSLs
Vitamin C	16.57 ± 1.45 µ mol	16.57	4.569	3.046	1.523
Total polyphenols	23.82 ± 2.33 mg gallic acid equivalents	0.14	0.039	0.026	0.013
Total flavonoids	0.045 ± 0.003 mg quercetin equivalents	$1.51*10^{-4}$	$4.16*10^{-5}$	$2.78*10^{-5}$	$1.39*10^{-5}$
Total glucosinolates	108.80 ± 3.13 µ mol	108.8	30	20	10

Values expressed as Mean ± SE. (GSLs: glucosinolates)

Table 2. Effect of BE and TZ treatment on estrous cycle

Groups	No. of Cycles	Duration in days				Diestrus index
		Estrus	Metestrus	Diestrus	Proestrus	
Control	6.01 ± 0.23	7.09 ± 0.21	5.77 ± 0.13	11.04 ± 0.67	5.67 ± 0.31	37.89 ± 1.21
BE	6.41 ± 0.35	7.23 ± 0.17	5.78 ± 0.11	10.87 ± 0.77	5.73 ± 0.23	36.67 ± 1.43
TZ	4.17 ± 0.11*	5.21 ± 0.21*	4.72 ± 0.31	16.13 ± 0.77*	16.89 ± 0.67*	54.67 ± 1.63*
BE1	5.67 ± 0.21^	6.15 ± 0.23	5.47 ± 0.11	13.67 ± 0.67	13.67 ± 0.47*	46.78 ± 1.89*^
BE2	5.89 ± 0.24^	6.67 ± 0.19^	5.56 ± 0.23	13.23 ± 0.21^	13.33 ± 0.23*^	44.97 ± 1.67*^
BE3	6.21 ± 0.11^	6.89 ± 0.35^	5.67 ± 0.43	12.50 ± 0.69^	12.50 ± 0.69*^	41.78 ± 1.53^

Values expressed as Mean ± SE (n=8) (*Significant difference as compared to control; ^Significant difference as compared to TZ at $P \leq 0.05$)

were studied and analyzed under OLYMPUS CH20i microscope for photographs.

Statistical Analysis

All values are represented as mean ± standard error of the mean (SEM). Statistical evaluation of collected information was done by one-way ANOVA on a computer by using CPCS1 to check statistical significance at $P < 0.05$.

RESULTS

Non-significant changes in the final body weight and net body weight gain was observed in all the experimental group rats, but significant change in estrous cycle was observed along with decreased growth rate in TZ treated rats as compared to control rats at $P<0.05$ [Table 2]; [Table 3]. The number of estrous cycles was reduced significantly in TZ treated animals, and shorter estrus phase with prolonged diestrus was observed compared to control rats at $P < 0.05$. Estrus, diestrus, and proestrus were significantly improved in BE2 and BE3 rats, while diestrus index was decreased significantly in Br+TZ group rats at a dose-dependent manner of broccoli extract to TZ treated rats at $P < 0.05$ [Table 2]. There was no significant change observed in weight of ovary, oviduct, and vagina, while weight of uterus was slightly reduced in all Br+TZ group rats [Table 3].

Altered activity levels of various antioxidative parameters were observed in all TZ and Br+TZ treated group rats [Table 4]. Proteins levels in the ovary were non-significant in all group rats. CAT activity was significantly increased with TZ treatment and was restored slightly in all Br+TZ group rats at $P<0.05$ [Table 4]. SOD activity levels were significantly decreased in TZ group rats and were restored significantly in all Br+TZ treated rats. Significantly increased GST activity levels were reported in TZ group rats, which

Table 3. Effect of BE and TZ treatment on body weight (g) and reproductive organs weight (g/100g b.w.)

Parameters	Control	BE	TZ	BE1	BE2	BE3
Initial b.w. (g)	161.75 ± 4.19	162.50 ± 5.76	163.52 ± 4.57	160.75 ± 4.84	164.50 ± 3.87	164.75 ± 5.03
Final b.w. (g)	195.50 ± 4.15	196.75 ± 5.31	190.45 ± 3.43	187.72 ± 3.67	195.75 ± 3.21	193.50 ± 4.11
Growth rate (g/week/100g b.w./rat)	3.01 ± 0.15	3.03 ± 0.27	2.38 ± 0.35	2.43 ± 0.30	2.63 ± 0.14	2.47 ± 0.29
Ovary	0.017±0.001	0.016±0.001	0.018±0.001	0.016±0.001	0.017±0.000	0.016±0.001
Oviduct	0.007±0.000	0.007±0.001	0.005±0.000	0.006±0.000	0.006±0.000	0.006±0.000
Uterus	0.129±0.021	0.127±0.017	0.128±0.014	0.115±0.003	0.104±0.002	0.109±0.014
Vagina	0.064±0.005	0.071±0.003	0.068±0.005	0.065±0.005	0.065±0.004	0.066±0.005

Values expressed as Mean ± SE (n=8) (*Significant difference as compared to control; ^Significant difference as compared to TZ at $P \leq 0.05$)

Table 4. Effect of TZ and BE treatment on ovarian enzyme activity

Parameter	Control	BE	TZ	BE1	BE2	BE3
Protein	4.33±0.41	4.47±0.32	4.86±0.56	4.51±0.23	4.57±0.31	4.71±0.29
CAT	6.71±1.03	5.63±0.65	8.92±0.31*	6.83±0.72	7.64±0.54	8.06±0.45
SOD	2.53±0.15	2.61±0.15	1.53±0.51*	3.88±0.42*^	4.73±0.62*^	4.75±0.42 *^
GST	0.035±0.002	0.036±0.002	0.061±0.005*	0.075±0.003*	0.044±0.011^	0.039±0.006^
GPx	0.43±0.11	0.43±0.08	0.44±0.01	0.52±0.08	0.54±0.03	0.78±0.07 *^
GR	0.005±0.001	0.006±0.001	0.007±0.001	0.006±0.001	0.007±0.001	0.008±0.001

Units: Proteins (mg/100 mg tissue), CAT (µmole of H_2O_2 decomposed/min/mg protein), SOD (U/mg protein), GST (µmoles of GSH-CDNB conjugate formed/ min/mg protein), GR (µmoles of NADPH oxidized/ min/mg protein), GPx (U/mg protein).
Values expressed as Mean ± SE (n=8) (*Significant difference as compared to control; ^Significant difference as compared to TZ at $P \leq 0.05$)

were also improved significantly with broccoli extract supplementation in BE2 and BE3 group rats at P<0.05. Glutathione reductase activity was comparable, while GPx activity was significantly high and improved in BE3 group rats, as compared to control and TZ group rats at P<0.05 [Table 4]. TZ caused significant increase in the ovarian MDA levels as a result of lipid peroxidation and was restored and improved significantly in all Br+TZ treated rats at P<0.05 [Figure 1]. Plasma estradiol levels were significantly increased and progesterone levels were significantly reduced in TZ group rats compared to control group rats at P<0.05. Further estradiol and progesterone levels were restored significantly in BE2 and BE3 experimental group rats as compared to TZ treated rats (P<0.05) [Figure 2]; [Figure 3].

All phases of the follicular development were observed in the ovarian tissue sections [Figure 4]; [Table 5]. Increased follicular atresia was observed in TZ and BE1 group rats as compared to control rats. The follicular diameter of secondary and tertiary follicle was abnormal and reduced in TZ treated rats and all Br+TZ treated group rats [Table 5]. Ovarian interstitial glands were also observed in TZ, BE1, and BE2 group rats, while degenerating oocytes were observed in BE1 group rats and degenerating oocytes were numerous in TZ group rats [Figure 4]; [Table 5]. Control rats ovarian surface epithelium (OSE) was well organized with cuboidal cells single layer, while OSE height was increased TZ treated rats, and was also restored significantly in BE3 group rats compared to TZ group rats at P<0.05 [Figure 5]; [Figure 6]. Granulosa cells smear of ovarian tissues showed only a few apoptotic cells in control rats [Table 6] and gave green fluorescence with annexin V and also fewer necrotic cells, which gave red fluorescence with ethidium homodimer [Figure 6] were observed. Significant increase in the number of apoptotic and necrotic cells was observed in TZ group rats ovarian granulosa cells, while reduced apoptotic and necrotic cells were noticed in all Br+TZ experimental group rats [Table 6]. Also, more live cells in all Br+TZ group rats were observed, compared to TZ treated rats at P<0.05 [Table 6]; [Figure 7].

DISCUSSION

OPs are the most widely used pesticide of choice in agricultural practices due to their effectiveness and relatively low persistence[34], and many of these tested pesticides have been

Table 5. Histopathological changes in ovary of TZ and BE treated rats.

S. No.	Feature	Control	BE	TZ	BE1	BE2	BE3
1	Follicular atresia	+	+	+++	+++	++	++
2	Degenerating oocyte	-	-	+++	++	++	+
3	Apoptotic and necrotic granulosa cells	+	+	+++	+++	++	++
4	Increased height of ovarian surface epithelium	-	-	+++	++	+	+
5	Ovarian interstitial glands development	-	-	++	+	+	-
6	Follicular diameter of Secondary and Tertiary follicle	N	N	↓, +++	↓,++	↓, ++	↓, +

- nil; + minimal (<10%); ++ mild (<25%); +++ moderate (<40%); N: normal; ↓: Reduced.

Table 6. Apoptosis and Necrosis observation in ovarian granulosa cells of TZ and BE treated rats

Treatment	Control	BE	TZ	BE1	BE2	BE3
Apoptotic Cells	12.50 ± 1.14	12.37 ± 1.47	28.43 ± 3.23*	27.71 ± 2.37*	24.66 ± 2.95*	23.13 ± 2.48*
Necrotic Cells	25.50 ± 3.04	24.67 ± 3.54	67.50 ± 8.50*	54.22 ± 5.47*	50.57 ± 4.41*^	46.75 ± 4.71*^
Apoptotic and Necrotic Cells	6.50 ± 2.29	7.27 ± 1.27	20.70 ± 3.41*	18.07 ± 1.54*	16.67 ± 1.57*	16.13 ± 1.05*

All values are expressed as Mean ± SE from 4 rats in each group from smeared granulosa cells (200µl sample). (*Significant difference as compared to control; ^Significant difference as compared to TZ at P ≤ 0.05)

proved more toxic to females of a species[35]. Apart from allergenic sensitization, pesticides toxicity can cause cancer, number of genetic disorders alongwith number of birth defects, sterility and miscarriage related issues can also develop in response to OS[1,6,36,37]. Broccoli and other cruciferous vegetables, contains pharmacologically active dietary antioxidants such as vitamin C, polyphenol-rich compounds, glucosinolates as the precursor of sulforaphane (SFN) and flavonoids, has protective potential and stimulates defence mechanisms[16,38,39,40]. The glucosinolates have been shown to provide protection both in *in vitro* and *in vivo* system from oxidative stress through scavenging ROS and have capability to minimize the risk of stress and diseased conditions by inducing endogenous antioxidant defenses[41,42,43,44]. Non-significant changes in body and organs weight are in agreement with literature, where pesticides toxicity related outcomes were not been found as potential threat to pose significant effects on body and organs weight of exposed animals[45,46]. The altered estrous cyclicity with prolonged diestrus phase and reduced estrus are correlated with secretion of estrogen and reduced secretion of gonadotropins, thus causing hormonal imbalance[2]. Similar observations for the number of reduced estrous cycles, and significant increase in the duration of diestrus and diestrus index were also reported in rats treated with

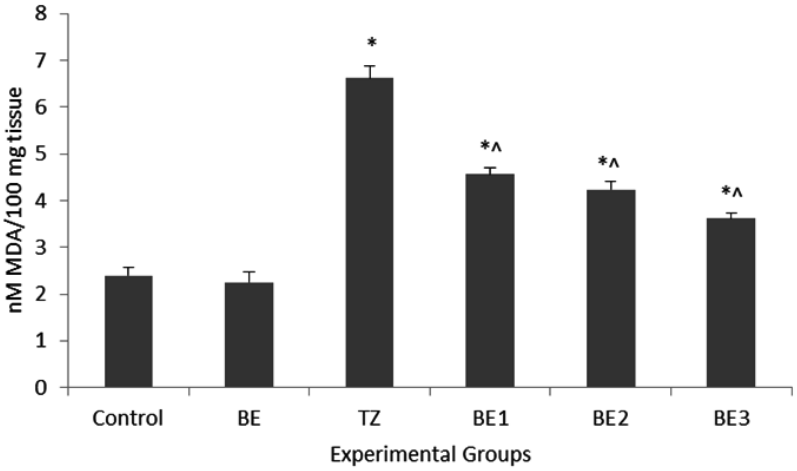

Fig. 1. Effect of BE and TZ oral intubation on ovarian LPO levels in rats.

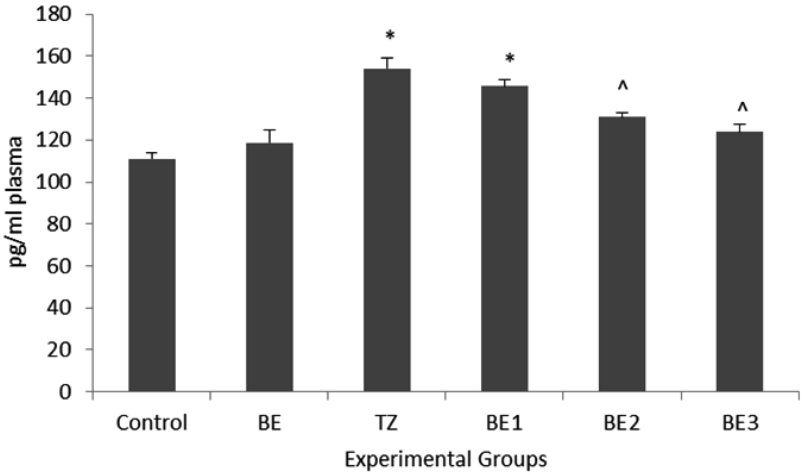

Fig. 2. Effect of BE and TZ treatment on plasma estradiol levels in rats.

organophosphate pesticides[47,48] further consolidate our findings and their reversal in all Br+TZ rats can be corroborated to the antioxidative potential of broccoli sprouts. Similarly, daily intake of antioxidant-rich vegetables, fruits, legumes, and other plant products having high levels of antioxidants, has been confirmed to have positive effects on improving fertility potential by reducing the adverse effects of OS[49,50] also consolidate our present findings.

Broccoli extract rich in glucosinolates such as sulforaphane and other antioxidants as vitamin C has an extraordinary ability to induce expression of several indirect enzymes via the

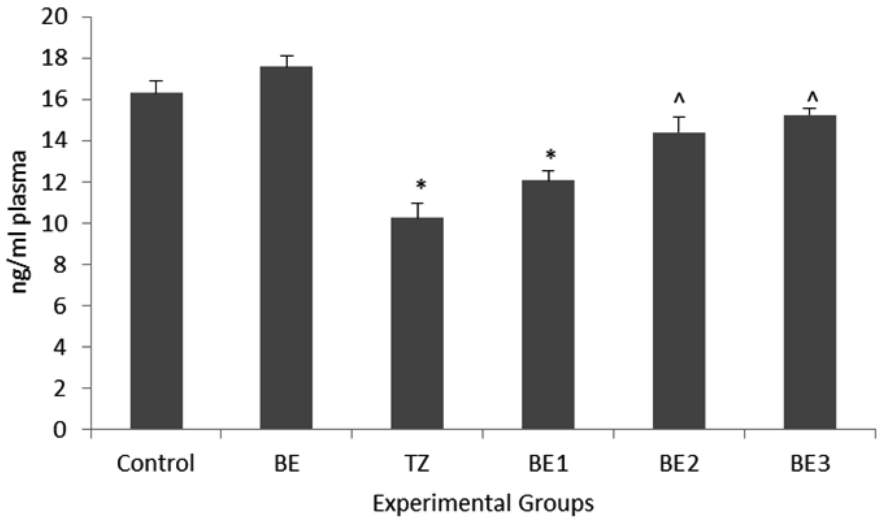

Fig. 3. Effect of BE and TZ oral intubation on plasma progesterone levels in rats.

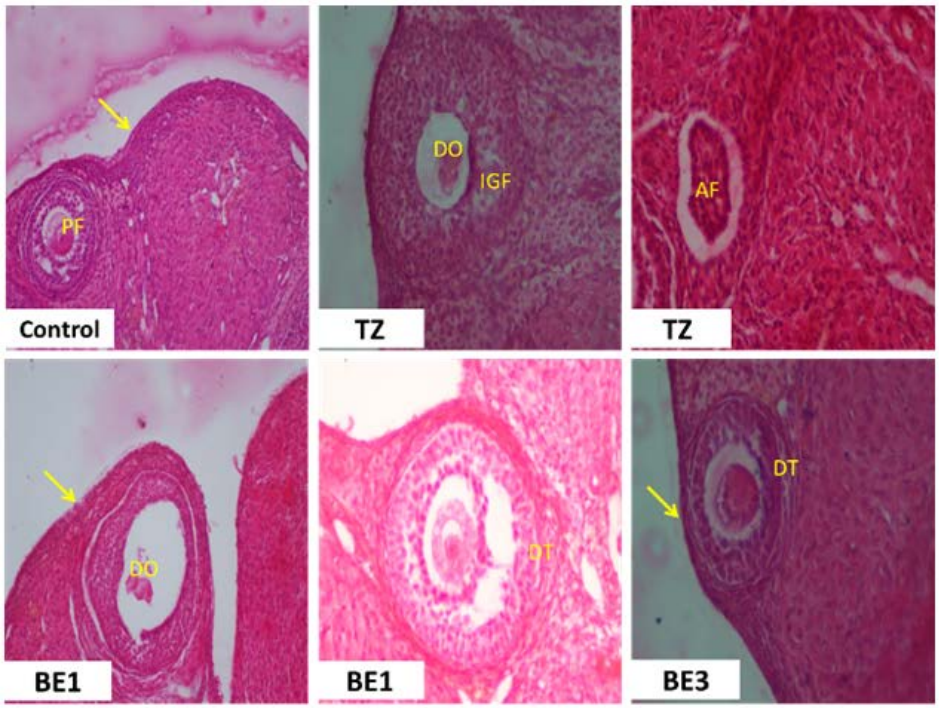

Fig. 4. Effect of BE and TZ treatment on ovarian histoarchitecture

KEAP1, Nrf2, ARE pathway, which play important role in toxicokinetics of xenobiotic substances[51,52]. Further, broccoli extract upregulates various Nrf2/ ARE genes and forms phase II detoxification enzyme systems by causing their expression, and have protective affinity against the OS by maintaining redox homeostasis and activity of free radical scavengers[53] and thus, can be exploited for strengthening immune defenses against pesticide induced toxicity. The activities of superoxide dismutase, catalase, and glutathione peroxidase constitute first line antioxidant defense system,

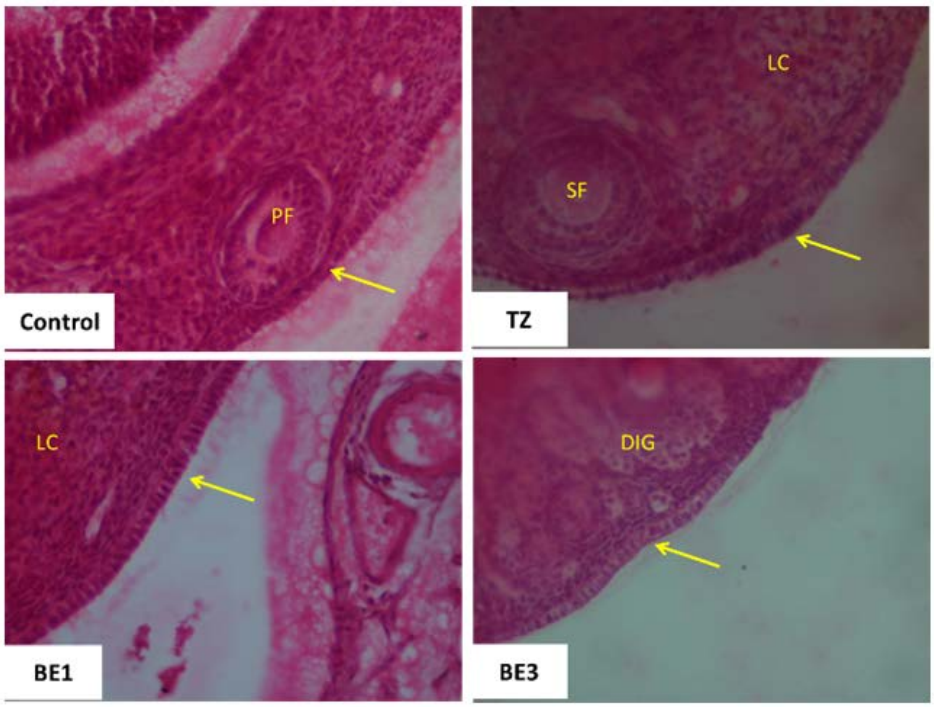

Fig. 5. Effect of BE and TZ oral intubation on ovarian surface epithelium

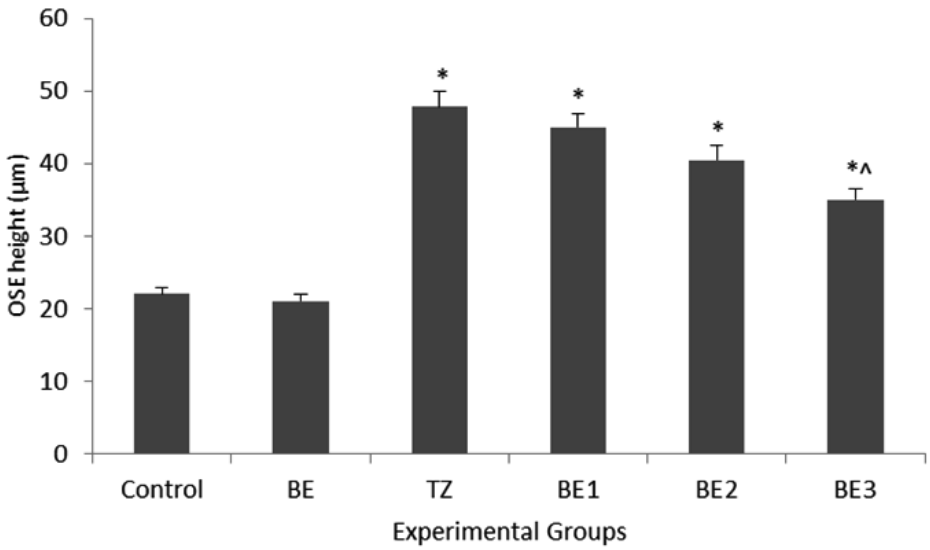

Fig. 6. Effect of BE and TZ treatment on ovarian surface epithelium (OSE)

which plays a major role in the total defense mechanisms in biological systems[54]. CAT activity was increased with TZ treatment and was restored slightly with BE supplementation may be assumed to block the free radical load[55], while SOD activity increased significantly in all Br+TZ group rats, which may be due to SOD gene upregulation by antioxidants of broccoli sprouts, and supposed to counter the toxic effects of TZ. Similarly, phoxim and methomyl mixture has also been evidenced to induce reproductive toxicity through reduced SOD activity[56] and intake of antioxidants rich white grape juice has been confirmed to improve metabolic status in women by increasing SOD levels, and has been attributed to reducing the risk of number of diseases[57] also consolidate present findings. Further, the GST activity levels were altered, along with GPx levels and these were restored in all Br+TZ treated group rats. Studies have demonstrated that oleic acid as natural antioxidant contributes to the cellular antioxidant defenses and was observed beneficial against mitochondrial OS through cellular glutathione peroxidase activation and enhances clearance of ROS,[58] which further strengthens our findings. Significant increase in ovarian MDA levels by TZ was also reversed significantly in all Br+TZ treated rats. Increased MDA levels following OPs exposure, has been linked to the increased production of ROS in ovaries and can induce OS,[59,60] and TZ induced OS can be reversed with the application of glucosinolates rich broccoli extract. Further, the protective role of natural antioxidants have been evidenced in ovarian toxicity[61] also supports our findings.

Most of the OPs act as a potential endocrine disrupter (EDC) and in severity can induce the developmental and other defects of female reproductive system[2,3,62]. Restored estradiol and progesterone levels in BE2 and BE3 group rats were reported compared to TZ rats. Similarly, the protective antioxidant properties of *Pistacia lentiscus* oil (PLO), on chlorpyrifos induced changes in the reproductive hormone levels of female rats, are in agreement with the present investigation, where progesterone levels were restored significantly[20]. TZ induced toxicity decreases progesterone levels in the plasma, probably by direct damage to the granulosa cells structure and function, while the formation of interstitial glands has induced the over-expression of genes responsible of elevated levels of estrogens. It has been observed that broccoli supplementation has the potential in ameliorating the TZ induced oxidative damage and thus consolidates its antioxidative properties against pesticides toxicity.

Physiologically, ROS levels fluctuates in different phases of ovarian cycle such as after

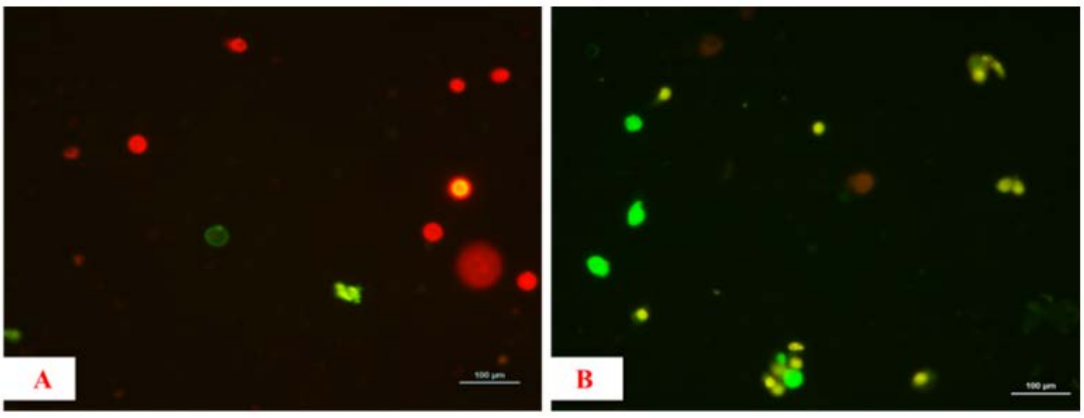

Fig. 7. Effect of BE and TZ oral intubation on ovarian granulosa cells apoptotic observations

preovulatory gonadotropin surge and during steroidogenesis, but are neutralized by regulatory endogenous or exogenous antioxidant molecules[63,64] to maintain homeostasis, as it is a pre-requirement for assuring the regulated reproductive functions in a healthy organism[36,65]. OS characterized by the changes in antioxidants status or ROS scavenging enzymes, is detrimental as it can damage macromolecules such as nucleic acids, lipids, and proteins, which can threaten the integrity of cell membranes and cytosolic organelles[1]. Ovarian histoarchitecture observation showed restored features of different stages of follicles including follicular diameter, reduced follicular atresia and degenerating oocytes in all Br+TZ treated group rats. Recent findings has shown that subchronic exposure of OPs has the potential to accumulate and damage ovarian integrity and histoarchitecture[2,66]. Similarly increased ROS levels can bind with DNA genetic material and increase preapoptotic signals which subsequently induces externalization of phosphatidylserine (PS) along with elevated LPO levels in live cells in response to toxicants exposure[63,67]. These act as death signals and activates caspase cascade, which leads to cell death or apoptosis in granulosa cells[68]. Fluorescent-labeled annexin V used to visualize the externalization of PS on the granulosa cells[2], and present investigation revealed reduced apoptotic and necrotic cells in all Br+TZ rats and further it has been attributed to the protective potential of BE. Similar observations with antioxidants Vitamins C and E supplementation has been reported, where decreased oxidative stress mediated granulosa cells apoptosis was observed due to its efficiency to diminish glyphosate induced oxidative stress and thereby, preventing associated fertility disorders[14]. These corroborate with our present findings and free radical-scavenging activity of BE protects the tissues from oxidative damage by restoring the levels of MDA, SOD, GPx, CAT, etc. which confirms its strong antioxidizing potential against TZ toxicity. TZ is thought to be estrogenic and is responsible for increased ovarian surface epithelium (OSE)[2]. OSE expresses estrogen receptors and also act as the site susceptible for ovarian cancer development[47]. Further, increased levels of estradiol and increased height of OSE in TZ were restored by the broccoli extract supplementation, which might have role in the down-regulation of bcl-2 gene expression and thus can play a protective role in ovarian cancer development in OSE cells, because OSE has been found associated with the development of number of ovarian cancers and its cystic derivatives[69] and thus confirms antioxidative properties of broccoli sprouts.

Overall health, in females, is determined by the proper physiological functioning of ovaries and reproductive system. Similarly, ROS generated during ovarian physiological metabolism, and endogenous antioxidants maintain the balance between ROS generation and their clearance from living system[8]. Present study demonstrate that TZ alters the levels of endogenous stress biomarkers including reproductive hormones and also influences estrous cyclicity by causing substantial oxidative damage in the ovarian tissues. BE can ameliorate the detrimental OS biomarkers changes with reduced MDA levels and apoptotic granulosa cells along with improved histoarchitecture. Protective effects of BE is probably due to the antioxidant properties of major compounds of BE, such as glucosinolates, vitamin C, polyphenols, etc., which scavenge and helps in clearance of excessively produced free radicals and prevent the oxidative damage by TZ induced toxicity.

CONCLUSION

Aqueous extract of broccoli sprouts has strong antioxidant affinity to reverse the TZ toxic effects as BE increases cell viability by reducing ROS generation and suppresses granulosa cells apoptosis, by inhibition of lipid peroxidation and restoring estrogen/progesterone balance. Endogenous antioxidants play an important role in follicular growth, oocyte maturation, and cyclicity, and moreover scientific attention is required to elucidate the underlying molecular mechanisms for better understanding of the possible protective roles of natural antioxidants, which can be used for treating pesticides induced female infertility and associated disorders with *in vivo* antioxidant supplementation.

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Conflict of interest

None.

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