Triazophos induced neuro-splenic toxicity and evaluation of antioxidative potential of aqueous Broccoli extract in Wistar albino rats

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Abstract
The present investigation was carried out to assess the antioxidative potential of Broccoli sprouts aqueous extract (BE) against triazophos (TZ) induced oxidative stress (OS) in brain and spleen. 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. Rats were orally intubated for 30 days as per experimental design. After sacrifice, OS biomarkers viz; catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GR), glutathione peroxidase (GPx), glutathione-S-transferase (GST), and lipid peroxidation (LPO) levels were determined in brain and spleen. Acetylcholinesterase (AChE) activity was observed in plasma and brain samples. Histological study of the spleen in TZ rats showed increased thickness of capsule, congestion and hypocellularity in follicles of spleen’s white pulp and the histoarchitecture was restored in TZ+BE group rats. TZ caused degenerative changes in brain histology and rats showed mild congestion along with haemorrhage in the cerebral cortex. Results suggest that TZ exposure is associated with neural toxicity along with altered spleen stress biomarkers, which further corroborates with histopathological findings. It is inferred that BE exerts multi-mechanistic protective effects against TZ induced neuro-splenic toxicity which is attributable to its protective antioxidant actions.

Keywords: Antioxidant enzymes, Brain, Broccoli Extract, Spleen, Triazophos

INTRODUCTION

Environmental contamination associated with irrational use of pesticides is a serious issue for global public health, as these are used for managed agricultural and other domestic practices worldwide. Excessive pesticides use not only affects target species but also has potential to affect the health of non-target species, through the generation of reactive oxygen species (ROS) which ultimately cause a condition of oxidative stress (OS) (Agrawal and Sharma, 2010; Ghosh and Philip, 2006). Organophosphorus (OP) pesticides account for more than half of the total world pesticides consumptions as they are cost-effective and used against a wide range of pests, thus their exposure is a major public health issue (Li et al., 2008). Triazophos, an OP, is being extensively used in agricultural and aquaculture activities throughout the globe, but it has a potential risk to human health as well as to ecological system due to its chemical stability and low degradation rate (Sharma et al., 2015; Wu et al., 2017). OPs are primarily recognized for their neurotoxic effects in mammals and higher vertebrates through the inhibition of acetylcholinesterase (AChE), which leads to the accumulation of neurotransmitter acetylcholine and have been found toxic to axonal transport of neurons along with neuroinflammation and cognitive impairment (Chen et al., 2017; Gao et al., 2017). OPs induced OS, is also assessed by number of stress biomarkers such as differential levels of malondialdehyde (MDA), i.e. end product of lipid peroxidation (LPO) and by modified endogenous antioxidants activity levels like catalase (CAT), superoxide dismutase (SOD), glutathione-S-transferase (GST) and glutathione peroxidase (GPx). Altered stress biomarkers can lead to the development of moderate to severe pathophysiological changes (Sharma and Sangha, 2014; Sharma et al., 2014). Fruits, vegetables, and herbal extracts rich in antioxid-
Antioxidants have been used traditionally to strengthen the natural immune system without fear of its negative effects on the body and slow down the oxidative damage caused by ROS (Cekic et al., 2011; Katiyar et al., 2012). These days much attention has been paid to the protective effects of natural antioxidants that fight chemical toxins and associated pathophysiological conditions (Gao et al., 2003; Vouldoukis et al., 2004). Studies with cruciferous vegetables rich in antioxidants such as broccoli, contain a large number of active antioxidants such as polyphenols, glucosinolates, vitamin C and flavonoids, suggest a protective potential that stimulates defense mechanisms through the elimination of ROS by inducing endogenous antioxidants (Jeffery and Araya, 2009; Koh et al., 2009).

The effects of toxins, associated with organs toxicity and stress, are known to cause major physiological or anatomical changes (Mansour and Mossa, 2010). The high lipid content in the brain makes it more vulnerable for free radicals mediated insult (Pajovic et al., 2003), while spleen plays an important role in establishing immunity (Wang et al., 2017). Number of studies have been made to evaluate the neuroprotective and immunity strengthening potential of natural antioxidants against different classes of pesticides and other environmental contaminants (Khan et al., 2017; Samida et al., 2017; Saoudi et al., 2017), but studies with laboratory animals regarding toxicokinetics and oxidative stress induction by TZ in brain and spleen are lacking. Therefore present investigation was carried out to study the ameliorative potential of aqueous extract of broccoli sprouts against the TZ induced toxicity in the brain and spleen of female Wistar rats.

MATERIALS AND METHODS

Chemicals
All chemicals were purchased from SRL Pvt. Ltd, Sigma-Aldrich, SD Fine-Chem Ltd, or were among the highest grades of analysis. Ashirwad Industries in Mohali provided standard rat feeds, while Triazophos as a Truzo 40 EC was purchased from Meghmani Organics Limited, Charodi, India.

Plant sample preparation
Seeds of Brassica oleracea var. italica, were procured from Department of Vegetable Sciences, Punjab Agricultural University, Ludhiana, 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, (2018) protocol. Quantification of glucosinolates (GSLs), from the powder, was done by Moller et al. (1985), vitamin C by Adom et al. (2002), total polyphenols by Ainsworth and Gillespie (2007), and total flavonoids by Chang et al. (2007) with slight modifications. Total glucosinolates (GSLs) content was estimated for 200 mg of dried sample and GSLs were considered as a base concentration along with other antioxidants in the extract (Table 1). For experimentation, three different doses of 10, 20 and 30 µmols with respect to GSLs were made from 200 mg dried BE powder in distilled water for subsequent use in the present investigation.

Experimental design
Female albino rats ageing 9-12 weeks and weighing 140-170 grams were obtained from the Department of Livestock Production and Management, Guru Angad Dev Veterinary and Animal Sciences University, 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 LD₅₀, i.e. 8.2 mg/ kg b.w. in olive oil, while control and BE rats were provided with an equal volume of olive oil. Rats with BE supplementation and TZ treatment were collectively referred as BE+TZ rats. During 30 days oral intubation experiment, body weights were also recorded weekly.

Organs weight, body weight and blood plasma preparation
After experimentation, female rats were mildly anesthetized using chloroform and their blood sample was collected from the heart directly in heparinized vials for further processing. After that, blood was centrifuged for 15 minutes at 2300 r.p.m. and the supernatant as plasma was used for biochemical analysis. After dissection, brain and spleen were excised immediately, cleared off the adhering tissue and weighed.

Biochemical and histological studies
Eight rats from each group were analyzed for biochemical studies of the spleen, while five rats from each group were evaluated for the brain. For organs homogenate, 0.3 gm of spleen and 0.5 gm of the brain was homogenized in 2 ml and 3 ml of 0.1 M PBS (pH 7.4) were used respectively, centrifuged and the superna-
RESULTS

Body weight changes were comparable in all the experimental group rats, but a reduced growth rate was observed in TZ and all BE+TZ rats as compared to control at P<0.05 (Table 2). Brain weight was comparable while spleen weight was slightly reduced in TZ group rats and restored in group V and VI rats compared to TZ rats (Table 2). Investigation of spleen revealed that total protein content was comparable, while significantly reduced levels of CAT, GST, GPx and GR were observed in TZ treated rats compared to control rats at P<0.05 (Table 3). CAT and GR levels were restored significantly in BE3 group rats. GPx activity levels were also significantly improved in all BE+TZ group rats compared to TZ treated rats. SOD activity levels were comparable in all experimental group rats. The activity levels of GST were significantly (at P<0.05 ) restored in BE2 and BE3 group rats. LPO levels were high in TZ treated rats and were reversed significantly in all rats of BE+TZ groups as compared to TZ treated rats at P<0.05 (Table 3). Significant inhibition of plasma and brain AChE activity in TZ treated rats was noticed, and supplementation of broccoli extract significantly (at P<0.05) restored AChE levels in brain of BE3 group rats and was also improved in plasma of BE+TZ group rats compared to TZ group rats (Table 4). Total protein content of brain was comparable in all the experimental group rats (Table 4). Significantly elevated levels of CAT were observed in all TZ and BE+TZ treated rats, while SOD activity levels were reduced significantly in TZ group rats, and were significantly restored in BE3 group rats. GPx activity levels were significantly reduced with TZ treatment and were improved statistically in all BE+TZ group rats. GR and GST activity levels were comparable in all experimental group rats (P<0.05) (Table 4). Significantly high LPO levels were observed in the brain of TZ treated rats and were significantly restored in all BE+TZ group rats at P<0.05 (Table 4).

On histological study of the spleen in control rats showed well marked vascular organization and cellular composition in the white and red pulp of the spleen. TZ toxicity induced many histopathological conditions such as the increased thickness of capsule, congestion and hypocellularity in follicles of spleen's white pulp (Fig. 1 and 2). TZ treatment induced mild to moderate distortions in germinal centers of the spleen and the histarchitecture was restored in all TZ+BE group rats (Fig. 1, Table 5). Peripheral regions of the spleen in TZ treated rats showed disturbed outer cortex with increased marginal sinus thickness/ vacuolation compared to control (Fig. 1), which was improved by BE treatment in a dose-dependent manner TZ+BE group rats (Table 5).

Brain histology showed TZ caused degenerative changes.
Table 2. Effect of Triazophos and broccoli extract treatment on body weight (b.w.) and organ weight (g/100 g of b.w.) of treated female Wistar rats.

| Parameter         | 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 ± 4.145 | 196.75 ± 5.31 | 190.45 ± 3.43 | 187.72 ± 3.67 | 195.75 ± 3.21 | 193.50 ± 4.11 |
| Growth rate (g/day/100g b.w./rat) | 0.43 ± 0.09 | 0.44 ± 0.02 | 0.34 ± 0.06 | 0.35 ± 0.04 | 0.37 ± 0.03 | 0.35 ± 0.06 |
| Spleen            | 0.239±0.011 | 0.246±0.005 | 0.224±0.011 | 0.233±0.013 | 0.241±0.002 | 0.243±0.009 |
| Brain             | 0.952±0.019 | 0.957±0.023 | 0.946±0.012 | 0.951±0.021 | 0.955±0.026 | 0.957±0.029 |

Values expressed as Mean ± SE (n=8); *Significant difference (P ≤ 0.05) as compared to control; ^Significant difference (P ≤ 0.05) as compared to TZ; BE : Broccoli extract; TZ: Triazophos; BE1: 10 µmol of broccoli extract and Triazophos; BE2: 20 µmol of broccoli extract and Triazophos; BE3: 30 µmol of broccoli extract and Triazophos.

Table 3. Effect of TZ and broccoli extract treatment on spleen biochemical stress markers.

| Parameter   | Control | BE    | TZ    | BE1 | BE2 | BE3 |
|-------------|---------|-------|-------|-----|-----|-----|
| Protein     | 6.35±0.45 | 6.67±0.43 | 5.87±0.56 | 7.02±0.67 | 6.85±0.41 | 6.70±0.47 |
| CAT         | 9.89±0.17 | 10.59±0.13 | 4.09±0.09* | 5.67±0.09* | 5.81±0.12* | 7.66±0.09** |
| SOD         | 18.55±0.54 | 19.32±0.52 | 20.79±0.63 | 19.78±0.57 | 19.05±0.69 | 19.28±0.46 |
| GST         | 0.076±0.004 | 0.081±0.005 | 0.030±0.003* | 0.035±0.003* | 0.053±0.005** | 0.068±0.008^ |
| GPx         | 0.52±0.01 | 0.45±0.05 | 0.23±0.04* | 0.35±0.02^ | 0.48±0.08^ | 0.57±0.08^ |
| GR          | 0.075±0.004 | 0.077±0.004 | 0.049±0.005* | 0.053±0.005* | 0.059±0.006* | 0.077±0.006^ |
| LPO         | 4.08±0.01 | 4.03±0.05 | 6.65±0.02* | 4.39±0.04^ | 4.32±0.03^ | 4.24±0.06^ |

Units: Proteins (mg/100 mg tissue), CAT (µmole of H2O2 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), LPO (nM MDA/100 mg tissue); Values expressed as Mean ± SE (n=8); *Significant difference (P ≤ 0.05) as compared to control; ^Significant difference (P ≤ 0.05) as compared to TZ; BE : Broccoli extract; TZ: Triazophos; BE1: 10 µmol of broccoli extract and Triazophos; BE2: 20 µmol of broccoli extract and Triazophos; BE3: 30 µmol of broccoli extract and Triazophos.

changes in the brain of rats (Fig. 3-6). TZ treated rats showed mild congestion and haemorrhage in internal regions of the cerebral cortex, and all TZ+BE treated group rats showed improvement with broccoli extract supplementation (Fig. 3,4). Congestion and haemorrhage were characterized by the number of microglial cells found in association with normal looking neurons in CNS due to TZ induced neural toxicity (Fig. 3, Table 6). The cerebral cortex of TZ treated rats showed a number of degenerating neurons, pyknotic neurons, shrunken neurons and neuron swelling along with vacuolation, which were reduced in all BE+TZ treated rats (Fig. 4, Table 6). In the hippocampus region of brain the unique monomorphic pattern of neurons within its pyramidal layer was slightly compressed in TZ treated rats as compared to control and TZ+BE group rats (Fig. 5), while subcommissural organ, which is helpful in clearance of certain compounds from the circulation of the cerebrospinal fluid, was observed with distorted ependymal cells in TZ treated rats (Fig. 6, Table 6).

**DISCUSSION**

Organophosphorus pesticides are most widely used insecticides and have been implicated in numerous toxicity based public health problems (Leibson and Lifshutz 2008; Sharma et al., 2015a). Further ameliorative studies have shown that increased consumption of antioxidant-rich vegetables as broccoli, can reduce the risk of stress and disease conditions (Chen et al., 2008; Sajeesh et al., 2011). Decreased growth rate with TZ treatment, which was further improved with BE supplementation, is in accordance with many studies (Uzun and Kalender 2011; Sharma and Sangha 2014), which suggest that OP intoxication can cause decreased body weight and growth rate in animals. Broccoli extract has the ameliorative potential to restore the normal growth rates and organs weight, which is supported by a number of studies where broccoli sprouts were found beneficial in rats (Sharma and Sangha 2018; Paško et al., 2018).

Spleen is a more sensitive organ of the body to pesticides exposures and exposure of OPs are more hazardous at the environmentally-relevant low dose, as OPs act as the inhibitor of the cellular immune response in the spleen and can lead to a number of unknown immune system associated disorders (Wang et al., 2017). Also, the spleen is the site of direct and indirect toxicity and a target for some carcinogens (Babaei et al., 2015). Endogenous antioxidants are involved in
Table 4. Effect of TZ and Broccoli extract treatment on brain biochemical stress.

| Parameter | Control | BE | TZ | BE1 | BE2 | BE3 |
|-----------|---------|----|----|-----|-----|-----|
| Protein   | 8.18±0.56 | 8.22±0.67 | 7.41±0.43 | 8.01±0.31 | 7.78±0.67 | 7.80±0.56 |
| CAT       | 15.15±0.36 | 15.87±0.34 | 19.68±0.86* | 21.87±1.04* | 21.74±1.87* | 21.66±1.17* |
| SOD       | 5.21±0.09 | 5.29±0.09 | 2.31±0.22* | 2.44±0.20* | 2.87±0.16* | 3.64±0.14** |
| GST       | 0.036±0.001 | 0.037±0.001 | 0.036±0.002 | 0.039±0.002 | 0.039±0.002 | 0.042±0.002 |
| GPx       | 0.52±0.01 | 0.50±0.03 | 0.23±0.03* | 0.37±0.03** | 0.48±0.03** | 0.67±0.04** |
| GR        | 0.006±0.001 | 0.006±0.000 | 0.007±0.001 | 0.008±0.001 | 0.008±0.001 | 0.008±0.001 |
| LPO       | 3.02±0.06 | 2.98±0.04 | 4.84±0.04* | 3.63±0.05** | 3.43±0.04** | 3.22±0.03* |
| Brain AChE (U/lit) | 865.54±7.27 | 860.95±6.63 | 690.45±6.67* | 714.45±6.68* | 735.67±5.23* | 744.43±5.43** |
| Plasma AChE (U/lit) | 842.54±4.67 | 850.75±6.23 | 603.45±7.78* | 654.45±7.78** | 692.43±5.23** | 699.67±7.43** |

Units: Proteins (mg/100 mg tissue), CAT (µmole of H₂O₂ 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), LPO (nM MDA/100 mg tissue); Values expressed as Mean ± SE (n=8 for plasma and n=5 for brain); *Significant difference (P ≤ 0.05) compared to control
**Significant difference (P ≤ 0.05) as compared to TZ
BE : Broccoli extract; TZ: Triazophos; BE1: 10 µmol of broccoli extract and Triazophos; BE2: 20 µmol of broccoli extract and Triazophos; BE3: 30 µmol of broccoli extract and Triazophos.

Table 5. Histopathological changes in spleen after treatment with TZ and aqueous extract of broccoli sprouts in female Wistar rats.

| S. No. | Feature                  | Control | BE | TZ | BE1 | BE2 | BE3 |
|--------|--------------------------|---------|----|----|-----|-----|-----|
| 1      | Red pulp abnormality     | -       | -  | -  | -   | -   | -   |
| 2      | Degenerated Germinal centers | -     | -  | +++| ++  | ++  | ++  |
| 3      | Distorted PALS           | -       | -  | ++ | ++  | +   | +   |
| 4      | Necrosis                 | -       | -  | +  | +   | +   | +   |
| 5      | Thickness of capsule     | N       | N  | ↑ed,+++| ↑ed,++| ↑ed,++| +   |
| 6      | Marginal Sinus thickness | -       | -  | ++ | ++  | +   | +   |

- nil; + minimal (<10%); ++ mild (<25%); +++ moderate (<40%); N: normal; ↑ed: increased; BE : Broccoli extract; TZ: Triazophos; BE1: 10 µmol of broccoli extract and Triazophos; BE2: 20 µmol of broccoli extract and Triazophos; BE3: 30 µmol of broccoli extract and Triazophos.

maintaining the healthy state of the body against free radicals mediated tissue or cellular damage (Chaudiere and Ferrari-iliou 1999). CAT, GST, GPx and GR activity levels were significantly reduced in the spleen of TZ treated rats, and were restored in all BE+TZ group rats. SOD, CAT, GPx, GST and other endogenous antioxidants play an important role in maintaining the redox potential of the cell by regulating the production and clearance of free radicals (Hu et al., 2014).

Broccoli extract coordinates various phase II detoxification enzyme systems, thereby having protective effects on oxidative stress (Guerrero-Beltrán et al., 2012) and has protective efficacy against pesticide induced toxicity. Major cytoprotective effects of sulforaphane, a type of GSL, are driven by the increased expression of Kelch-like ECH-associated protein 1 (Keap1), Nuclear factor erythroid 2 (Nrf2) pathway, anti-inflammatory mechanisms including inhibition of the NF-κB pathway etc (Baird and Dinkova-Kostova 2011; Gerhauser 2013). Nrf2 play a critical role in protecting and maintaining spleen (Zhan et al., 2017) and brain (Ren et al., 2011) health from toxic chemicals and other stress-inducing factors. Elevated LPO levels, in terms of MDA, in TZ-treated rats was reversed in all BE+TZ group rats, suggest that BE can reduce lipid peroxidation by increasing the activity of the antioxidant enzyme and improving the free radical clearance ability of the spleen. These observations are in agreement with a number of studies on rats (Mohamadin et al., 2010; Singh et al., 2013; Hu et al., 2014) where natural antioxidants have resulted in reduced MDA levels with restored organ homeostasis, thus consolidates present
findings. Xenobiotics metabolism and xenobiotic-related signaling pathways are key factors in activating xenobiotic toxicity (Wu et al., 2017a). The neuroprotective role of glucosinolate in the brain has been evidenced in the upregulation of defense mechanisms (Giacoppo et al., 2014). BE restored AChE levels in all BE+TZ group rats and similar observations have been observed for AChE in the brain of rats treated with chlorpyrifos and grape seed extract rich in natural antioxidants (Singh et al., 2013), in trichlorfon plus hydroxycinnamic acid treated rats (Sharma and Singh 2012), revealing the protective effect of broccoli extract. CAT activity level was significantly increased and SOD activity was reduced with TZ treatment, while BE + TZ group rats restored these levels compared to TZ group rats. SOD has been reported with same activity behaviour in brain tissue of rats treated with propoxur, a widely used carbamate insecticide.

Table 6. Histopathological changes in brain of TZ and broccoli extract treated Wistar rats.

| S. No. | Feature        | Control | BE   | TZ  | BE1 | BE2 | BE3 |
|-------|----------------|---------|------|-----|-----|-----|-----|
| 1     | Congestion     | -       | -    | +   | +   | +   | -   |
| 2     | Pyknosis/ Necrosis | +     | +    | +++ | ++  | ++  | ++  |
| 3     | Neuronal vacuolation | -    | -    | +++ | ++  | +   | +   |
| 4     | Neuronal degeneration | -    | -    | ++  | +   | +   | +   |
| 5     | Edema          | -       | -    | -   | -   | -   | -   |
| 6     | Haemorrhage    | -       | -    | +   | -   | -   | -   |

- nil; + minimal (<10%); ++ mild (<25%); +++ moderate (<40%); BE : Broccoli extract; TZ: Triazophos; BE1: 10 µmol of broccoli extract and Triazophos; BE2: 20 µmol of broccoli extract and Triazophos; BE3: 30 µmol of broccoli extract and Triazophos.

Fig. 1. Effect of broccoli extract on spleen histology against TZ induced toxicity (Magnification: 400x, Stain: Eosin and hematoxylin; Control: Control group rats; TZ: Triazophos; BE1: 10 µmol of broccoli extract and Triazophos). F: follicle; T: trabeculus; TV: trabecular vein; OC: outer cortex; DOC: disturbed outer cortex; RP: red pulp; Yellow arrow showing the variation in thickness of capsule.

Fig. 2. Effect of broccoli extract on spleen histoarchitecture against TZ induced toxicity (Magnification: 400x, Stain: Eosin and hematoxylin). F: follicles; Fh: follicles showing hypocellularity; P: PALS sparsely populated by T-cells in TZ rats; marginal zone disturbed in TZ rats (black arrow).
against the *Nigella sativa* oil supplementation having antioxidative properties (Rezvanfar et al., 2010). Decreased SOD levels in the brain undoubtedly resulted in the number of ROSs. More SOD is required to capture free radicals and BE administration lead to overexpression of SOD, GPx, and CAT their elevated levels helped in detoxification of intermediate metabolites of TZ and ROSs from the body. Enzymatic antioxidants such as SOD, GPx, and CAT levels are increased in the tissues due to the antioxidative property of broccoli which helped to initiate the adaptive response to the increased oxidative stress caused by TZ. LPO is a good indicator to assess the magnitude of the oxidative damage produced by ROS in the brain. Increased brain LPO may be linked to higher production of free radicals as seen after TZ treatment, suggesting that the brain may be more prone to TZ-induced oxidative damage and increased LPO in TZ treated rats confirm a relationship between the degrees of toxicity in the brain to the altered endogenous antioxidants. Similar observations with chlorpyrifos treatment have also been observed in the brain of

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**Fig. 3.** Effect of broccoli extract on cerebral histoarchitecture against TZ induced toxicity (Magnification: 100x, Stain: Eosin and hematoxylin) Arrows showing microglial cells found in association with normal looking neurons due to TZ toxicity

**Fig. 4.** Effect of broccoli extract on Cerebral cortex of brain against TZ induced toxicity (Magnification: 400x, Stain: Eosin and hematoxylin) Arrows showing neuron swelling and vacuolation; P: Pyknotic degenerating neurons; M: microglial neurons.
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The addition of *Nigella sativa* oil against propoxur has led to a reduction in MDA levels in the rat brain (Mohamadin *et al*., 2010) further confirms our current findings. Chronic exposure to monocrotophos has been shown to cause significant changes in brain and spleen, including affected granular cell neurons in the cerebrum region, distorted cerebral cortex with neuronal loss, and spleen of rats were observed with severe congestion and hypocellularity in white pulp follicles (Yaduvanshi *et al*., 2010). Spleen showed congestion, hypocellularity and mild to moderate distortions in germinal centers of white pulp follicles after TZ treatment, which were minimized and improved by BE treatment. Histopathological effects of maternal 4-tert octylphenol, an OP, exposure on spleen also showed distorted white pulp and increased destruction in the spleen regions (Barlas and Aydofan, 2009). Similarly, saffron (*Crocus sativus*) petal ethanolic extract, rich in natural antioxidants, has been found beneficial and supplementation improved the spleen histology in rats (Babaei *et al*., 2015) further consolidates present findings. Histological changes in brain tissues of TZ treated rats showed mild congestion and hemorrhage in internal regions of the cerebral cortex. These observations are in agreement with Yaduvanshi *et al*., (2010) findings monocrotophos, induced number of histological alterations in brain tissues of rats. TZ treatment induced neurons pyknosis, vacuolation and swelling, which were restored with BE supplementation due to the neuroprotective activity of the BE and a significant recovery of neuronal damage, and decreased necrosis was observed. Circumventricular organs, such as subcommissural organ, lack the normal blood-brain barrier and are vulnerable to certain pharmaceutical and toxic compounds that normally cannot penetrate through an effective blood-brain barrier. Because of a lack of blood-brain barrier in these regions, their vulnerability makes their evaluation in toxicity studies particularly important (Yaduvanshi *et al*., 2010; Garman 2011). TZ induced damage in the subcommissural organ was ameliorated by BE supplementation. Further, isothiocyanates (glucosinolates) have a neuroprotective

**Fig. 5.** Effect of broccoli extract on monomorphic pattern of neurons within the pyramidal layer of hippocampus region of brain (Magnification: 400x, Stain: Eosin and hematoxylin) Arrows showing monomorphic pattern of neurons within the pyramidal layer of hippocampus.

**Fig. 6.** A. and B. Effect of TZ on subcommissural organ (SO) and C. cerebral cortex of brain histoarchitecture (Magnification: 100x, 200x and 400x; Stain: Eosin and hematoxylin) Black Arrows showing distorted ependymal cells (DEC); Yellow Arrow showing neuron swelling and vacuolation; CA: cerebral aqueduct; PC: posterior commissure; P: Pyknotic neurons; M: microglial neurons.
potential for the treatment of neurodegenerative diseases, as reported by Giacoppo et al., (2015) also supports present findings.
OPs increase free radicals production in the brain and lead to the activation of endogenous antioxidants of target cells, which is the most prominent defense mechanism against free radicals-induced damage (Rezvanfar et al., 2010). Further studies also showed that the protective effect of plant-derived natural antioxidants was associated with the amelioration of oxidative stress and the preservation of antioxidant enzymes balance induced by pesticides in the brain (Hussien et al., 2013). From the above discussion, it can be inferred that antioxidant enzymes clear free radicals, lowers lipid peroxidation by decreased release of MDA, and then reduces the degree of membrane damage. Reduced MDA levels, both in the brain and spleen, due to broccoli extract, has been attributed to a minimum loss of membrane lipids due to scavenging of free radicals by up-regulated endogenous antioxidants as well as by natural dietary antioxidants on their own. Thus cellular integrity has been restored and histoarchitecture of the brain and spleen was improved in all the BE+TZ treated rats.

Conclusion
The present study showed that TZ induced oxidative damage and neuro-splenic toxicity in rats through altered stress biomarkers and histopathological conditions. Aqueous extract of broccoli sprouts had a strong antioxidant affinity to reverse the TZ toxic effects and helped in maintaining the structural integrity of the cell and histoarchitecture of both the brain and spleen. It is a preliminary study about the neuroprotective role of broccoli sprouts against OP pesticide; however, further studies are needed to explore the molecular mechanism underlying the role of extract components and their induced protection against pesticides associated neuro-splenic toxicity.

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Conflict of interest
The authors declare that they have no conflict of interest.

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