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## Original Research Article

# Antioxidative effects of aqueous extract of broccoli sprouts against Triazophos induced hepatic and renal toxicity in female Wistar rats

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## ABSTRACT

Oxidative stress (OS) is a major cause of hepatic and renal disorders, so present investigation was designed to evaluate the antioxidative efficacy of aqueous broccoli extract (BE) via three different doses – 10, 20 and 30 mmol – of glucosinolates against toxic effects of triazophos (TZ), an organophosphorous pesticide, in female rats during 30 days experiment. Six groups of rats were made and were orally intubated with TZ and BE as per experimental design. TZ and BE induced OS biomarkers of hepatic and renal toxicity – ALT, AST, urea and creatinine – were noticed in plasma, while catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione-S-transferase (GST), lipid peroxidation (LPO) were estimated in liver and kidney along with histological and apoptotic observation. Plasma ALT, AST, urea and creatinine levels along with organ OS parameters as CAT, SOD, GST and GPx were subtly improved in all BE+TZ treated rats. Decreased LPO and reduced apoptosis along with improved histoarchitecture was observed in all BE+TZ treated rats. Present study suggest that the administration of broccoli extract and TZ combination in rats can prevent severe alterations of hepatic and renal biochemical markers and disruptions of histological structure by antioxidative potential of BE from sprouts.

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## Introduction

Environmental contamination is a major global problem and pesticides form the major component among all contaminants, because of their vast use in agricultural and other domestic practices (Al-Attar, 2015). Human health risk due to exposure to pesticides is constantly increasing, as irrational use of pesticides intended for managed agricultural and other domestic practices, not only produces adverse biological effects in target species, but also has the potential to affect the health of non-target species (Etemadi-Aleagha et al., 2002), through the production of reactive oxygen species (ROS) which ultimately lead to a condition of oxidative stress (OS) (Agrawal and Sharma, 2010). Organophosphorus (OP) pesticides exposure is a major public health issue, as OPs account for more than half of the total world pesticides consumptions due to their easy availability, lower persistence and cost effectiveness (Casida and Quistad, 2004; Jaga and Dharmani, 2003). Triazophos, O,O-diethyl-1-H-1,2,4-triazol-3-yl

phosphorothioate, (TZ) a non-systemic broad spectrum OP is extensively used against a wide range of pests (Li et al., 2008) and has been shown to induce OS, evidenced by the increased level of malondialdehyde (MDA) i.e. end product of lipid peroxidation (LPO) and by differentially modified endogenous antioxidants like catalase (CAT), glutathione-S-transferase (GST), superoxide dismutase (SOD) and glutathione peroxidase (GPx), which can lead to development of moderate to severe pathophysiological hepatic and renal changes (Jain et al., 2011; Sharma and Sangha, 2014).

Consumption of natural foods, such as vegetables, which have a high antioxidant capacity, has a protective effect on human health and people consume vegetables without the fear of their side effects on body physiology (Cekic et al., 2011). Plant extracts from fruits and vegetables with natural antioxidative properties are traditionally used for a long time to strengthen the natural immune defences and thus they slow down the process of oxidative damage caused by ROS (Gao et al., 2003; Lee et al., 2004; Vouldoukis et al., 2004). The glucosinolates (GSLs) in many cruciferous vegetables have been established as potent antioxidants whose breakdown products help to activate endogenous antioxidant defences in *in vitro* and *in vivo* living systems (Plumb et al., 1996). GSLs have been shown to provide protection from OS through the elimination of ROS (Jeffery and Araya, 2009; Traka and Mithen, 2009; Vig et al.,

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2009). In recent years, there has been a strong focus on health benefits of broccoli sprouts and extracts in addition of whole broccoli (Dinkova-Kostova et al., 2007; Fahey et al., 1997; Murashima et al., 2004). Sulforaphane (4-methylsulfinylbutyl isothiocyanate), a metabolite of GSL (glucoraphanin), has been identified as the most potent naturally occurring inducer of phase II enzymes, and protects aerobic cells against xenobiotics and toxic DNA-damaging electrophiles by inducing a network of phase II detoxification and endogenous antioxidant enzymes (Fahey and Talalay, 1999; Guerrero-Beltrán et al., 2012; Juge et al., 2007).

Liver and kidneys are the primary site of detoxification and regarded as indicator organs for toxic effects (Gathwan et al., 2013; Piao et al., 2011). In animal experimental models, number of reports suggest that orally ingested dietary GSLs protect liver and kidney against various xenobiotics and their toxic effects by expressing phase II detoxification enzymes (Baek et al., 2008; Gaona-Gaona et al., 2011; Zhang et al., 2006; Zhao et al., 2010, 2016). However, limited reports are available about antioxidative effects of broccoli extract against toxic chemicals induced toxicity in liver and kidney, while toxicokinetics and oxidative stress induced by TZ in liver and kidneys have been reported in number of studies (Jain et al., 2010; Sharma et al., 2014). Thus, the core idea behind the current study is to demonstrate the antioxidative efficacy of aqueous broccoli extract as a source of natural antioxidants against TZ induced hepatic and renal toxicity in female Wistar rats.

## Material and methods

### Chemicals

Apoptosis and Necrosis Quantification Kit were purchased from Biotium, Acridine orange and Ethidium bromide were purchased from Sigma-Aldrich. All other chemicals were purchased from Sigma-Aldrich, SDFCL (SD Fine-Chem Ltd), SRL (Sissco Research Laboratories Pvt., Ltd.) or were either of analytical grade or the highest purity commercially available. Triazophos as Truzo 40 EC was obtained from Meghmani Organics Limited, Chharodi, India. Standard rat feed was purchased from Ashirwad Industries, Mohali, India.

### Plant sample preparation

Punjab Broccoli 1 (*Brassica oleraceae* var. *italica*) seeds were obtained from Department of Vegetable Sciences, Punjab Agricultural University, Ludhiana. Seeds were surface-sterilized by rinse in 70% ethanol, followed by rinsing with distilled water and sprouts were produced. Sprouts were grown without added nutrients in inclined perforated trays (35 × 40 cm), watered gently along with 16 h light and 8 h dark photoperiod and temperature from 25 °C to 27 °C. Sprouts were harvested gently on 1, 3, 5, 8 and 10 days and were dried in oven for 1 h at 90 °C. Dried sprouts were plunged into 5 vol of boiling water (90 °C) and boiling was continued for 30 min. The mixture was then cooled, filtered, and the filtrate was lyophilized to provide a dry broccoli extract (BE) powder.

For quantification of glucosinolates (GSLs), 0.3 ml of distilled water was added to 200 mg of powder and 3 ml of 2 mM palladium (II) chloride and mixed. After incubation at 25 °C for 1 h, absorbance at 425 nm was measured using a spectrophotometer. Absorbance was shown by an average of three measurements and was used for estimation of total GSL contents (Moller et al., 1985). Among all, 5 days old sprouts were found to contain the highest concentrations of GSLs and were analyzed for biochemical profiling through standardized methods for total antioxidants: vitamin C ( $16.57 \pm 1.45 \mu\text{mol/g}$  dry BE powder), total polyphenols ( $23.82 \pm 2.33 \text{ mg GAE (gallic acid equivalents)/g dry BE powder}$ ),

total glucosinolates ( $108.80 \pm 3.13 \mu\text{mol/g}$  dry BE powder) and total flavonoids ( $45.67 \pm 3.33 \mu\text{g QE (quercetin equivalents)/g dry BE powder}$ ).

For BE doses preparation, total glucosinolates (GSLs) concentration was considered as base and GSLs contents were inferred as the average for 200 mg of dried samples. Subsequently three doses – 10, 20 and 30  $\mu\text{mol}$  of GSLs were prepared from dry BE powder through known volume of distilled water, and were used for further dosing through oral intubation against the 1/10th of  $\text{LD}_{50}$  (i.e. 8.2 mg/kg b.w.) TZ induced toxicity in female rats.

### Animals

Female Wistar rats 9–12 weeks old and 140–170 g of weight were procured from the Department of Livestock Production and Management, GADVASU, Ludhiana. Rats were housed in polypropylene cages (two rats in each cage) using paddy husk bedding in laboratory, where the humidity ( $55 \pm 5\%$ ), temperature ( $25 \pm 2^\circ\text{C}$ ) and normal photoperiod of 12–12 h light-dark cycle were environmentally controlled. Rats were provided with laboratory standard rat feed and water *ad libitum*. All methods and procedures of animal handling during research were conducted in accordance with the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India and experiments conducted in the present study were duly approved by Institutional Animal Ethics Committee (IAEC), Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana vide letter no. 3901-35 dated 06.08.2012.

### Experimental design

Female rats were acclimatized for ten days in laboratory conditions prior to experimentation and were divided into six groups with eight rats per group.

**Group I** (Control) animals received olive oil + distilled water and served as control;

**Group II** (BE) animals received 10  $\mu\text{mol}$  glucosinolates (GSLs) of BE;

**Group III** (TZ) animals received TZ at the dose of 1/10th of  $\text{LD}_{50}$  (i.e. 8.2 mg/kg b.w.) in olive oil;

**Group IV** (BE1) animals received 8.2 mg/kg b.w. TZ in olive oil + 10  $\mu\text{mol}$  GSLs of BE;

**Group V** (BE2) animals received 8.2 mg/kg b.w. TZ in olive oil + 20  $\mu\text{mol}$  GSLs of BE;

**Group VI** (BE3) animals received 8.2 mg/kg b.w. TZ in olive oil + 30  $\mu\text{mol}$  GSLs of BE.

All the BE and TZ treated rats collectively named as BE + TZ rats. All treatments were carried out for 30 days by oral intubation.

### Organs weight and blood plasma preparation

After 30 days of treatment female rats were fasted overnight and were then mildly anaesthetized using chloroform and blood sample from each rat was collected directly from heart in heparinised vials. Blood was centrifuged at 2300 r.p.m. for 15 min and supernatant was obtained as plasma for biochemical analysis. After dissection liver and kidneys were excised, cleared off the adhering tissue and weighed.

### Sample preparation for biochemical studies

After dissection, a small portion (0.5 g) of liver and a whole kidney was homogenised in 0.1 M phosphate buffer saline (PBS) (pH 7.4) solution, centrifuged and supernatant was used for the biochemical parameters, which were assayed by standard methods. ALT and AST from plasma were estimated by Reitman and

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