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Ameliorating Potential of *Panax Ginseng* on Oxidative Stress following Subacute Exposure to Acetamiprid in Rats

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ABSTRACT

Oxidative stress is common when the cellular antioxidant capacity is overwhelmed by the reactive oxygen species generated by the metabolism of pesticides which either inhibit or modulate the expression of antioxidant enzymes. The present study was made with objective to evaluate the ameliorating potential of *Panax ginseng* on oxidative stress following subacute exposure to Acetamiprid in rats. Twenty four adult male Wistar rats were divided in four groups comprising six each. Group I served as control and was administered with groundnut oil (1ml/100gm), group II was administered with Acetamiprid (52.5 mg/kg body weight), group III served as *Panax ginseng* (50 mg/kg body weight) control and in group IV possible ameliorative effect of *Panax ginseng* was examined against Acetamiprid. Vehicle, Acetamiprid and *Panax ginseng* were administered daily to the rats orally by gavage for 28 days and animals were sacrificed at the end. Liver, kidney, spleen and brain were processed for determination of oxidative stress related parameters viz: Lipid peroxidation (LPO), reduced glutathione (GSH), Superoxide dismutase (SOD), catalase and glutathione reductase (GR) levels. Acetamiprid produced toxicity which was evident in the form of enhanced lipid peroxidation and decrease in activities of GSH, SOD, catalase and glutathione reductase levels in all the organs examined. *Panax ginseng* was significantly effective in restoration of these parameters towards normal. Thus, it was concluded that *Panax ginseng* has ameliorating potential on oxidative stress following subacute exposure to acetamiprid in rats

Keywords: oxidative stress, acetamiprid, ginseng, ameliorating potential, rat model

Oxidative stress is an imbalance between free radicals production and the ability of the body to counteract their harmful effects through neutralization by antioxidants. The free radicals (FR) are defined in terms of the atoms, or molecules which contain one or more unpaired electrons that makes them several folds more reactive than their corresponding ions. The reactive oxygen species (ROS) and FR have the ability to cause peroxidation of unsaturated lipids constituting the membrane of cells and depletion of cellular reserves of reducing elements (enzymatic and non-enzymatic; jointly called as antioxidants defense system), which the body can produce indigenously (Nita and Grzybowski, 2016). The pesticides have been shown to induce production of ROS by altering the balance between the oxidants and antioxidants through promoting lipid peroxidation (LPO) and depleting the antioxidative cellular reserves (both the enzymatic and non-enzymatic) leading to a condition of oxidative stress (Singh and Sharma, 2018). Alterations in the components of the antioxidant system can be used as biomarkers for pesticide poisoning (Lushchak *et al.,* 2018). Neonicotinoid insecticides such as acetamiprid has large-scale applications ranging from plant protection (crops, vegetables, fruits), veterinary products, and biocides to invertebrate pest control in fish farming due to its long term retention, high efficiency and low poisoning (Simon-Delso *et al.,* 2015). The current global usage figures and their persistence in the environment suggest that the neonicotinoids could have adverse effects on human health (Wood and Goulson, 2017). Doltade *et al.* (2019) cited that acetamiprid exposure has toxic

effect on liver, kidney and brain in terms of induction of oxidative damage through LPO and altered antioxidant defence system.

The antioxidant effect of ginseng may be responsible for its wide pharmacological actions in clinical practice by a free radical reaction-inhibition mechanism. The protective effect of extract from *Panax ginseng* against oxidative stress is attributed to its free radical scavenging activity. It was reported that the non-saponin components of red ginseng suppressed the harmful effects of free oxygen radicals (O_2, H_2O_2, and OH_2), which play an important role in tissue degeneration. Ginseng extract scavenges hydroxyl radical and protects unsaturated fatty acids from decomposition caused by iron mediated lipid peroxidation (Seoul Hee Nam *et al.,* 2018). Protective effects of phenolic rich ginger extracts against toxicity induced oxidative stress was recently reported by Vipin *et al.*(2017). In view of wide application it seems of great utility to strengthen the safety aspects of acetamiprid by detailed scientific study. Accordingly, the present study was envisaged to study the oxidative distress protective effects of ginseng against acetamiprid induced toxicity.

MATERIALS AND METHODS

Preparation of Herbal Extract

The dried root of *Panax ginseng* was powdered and soaked in distilled water for 24 hours with continuous stirring at 40ºC. The mixture was filtered through muslin cloth and Whatmann filter paper No. 42 and concentrated in a rotatory vacuum evaporator at 40-50ºC. The final extract was produced after drying the filtrate in incubator with fan (40ºC) and lyophilized. The percent yield (w/w) of aqueous extract of *Panax ginseng* was 11.5%.

Animal Model

Wistar rats of 2 to 2.5 month old age, weighing between 150 to 250 gm, were used in this study. The animals were kept in polypropylene cages and acclimatized for two weeks in the animal shed under standard managemental conditions. Standard rat feed and water was provided ad libitum throughout the experimental period. All the experimental animals were kept under constant observation during entire period of study. All studies were performed in accordance with the guidelines on regulation of scientific experiments as approved by the Institutional Animal Ethics Committee.

Toxicological and Pharmacological Agents

Technical grade Acetamiprid (96.8% pure) was a kind gift. The desired concentration of Acetamiprid was made in groundnut oil while the extract of *Panax ginseng* was dissolved in water.

Experimental Design

Sub-acute oxidative stress was conducted in adult male Wistar rats. Rats were divided in four groups comprising six animals each. Group I served as control and was administered with groundnut oil (1ml/100gm), group II was administered with Acetamiprid, group III served as *Panax ginseng* control and in group IV possible ameliorative effect of *Panax ginseng* (50 mg/kg body weight) was examined against Acetamiprid (52.5 mg/kg body weight). Vehicle, Acetamiprid and *Panax ginseng* were administered daily to the rats orally by gavage for 28 days. The dose of Acetamiprid was selected on the basis of LD_{50} in rats (525mg/kg).

Assessment of Oxidative Stress

Estimation of different oxidative stress-related biochemical parameters in liver, kidney, spleen and brain was carried out. A double beam UV-VIS spectrophotometer was used for recording the absorbance of the test samples.

Preparation of Liver, Kidney and Brain Homogenates

Liver, kidney and brain tissues (500 mg) were weighed and put in 5 ml of ice-cold PBS (pH 7.4). Another 200 mg of sample from each tissue was weighed separately and taken in 2 ml of 0.02 M ethylenediamine tetra acetic acid (EDTA) solution for reduced glutathione estimation. The homogenates (10%) prepared by IKA homogenizer under ice-cold condition were centrifuged for 10 min at 3000 rpm. The supernatant was stored at -20ºC until assay of different oxidative stress-related parameters.

Assays for Measuring the Activities of Antioxidant Enzymes

Lipid peroxidation (LPO) was evaluated in terms

of malondialdehyde (MDA) production by using thiobarbituric acid-reactive substances (TBARS) test (Fernanda *et al*., 2005). Reduced glutathione (GSH) was determined by estimating free-SH groups, using 5-5' dithiobis 2- nitrobenzoic acid (DTNB) method of Tipple and Rogers, 2012. For estimation of GSH, 10% homogenate was made in 0.02 M EDTA. Superoxide dismutase (SOD) was estimated as per the method described by Spitz and Oberley, 2001. The catalase activity in tissue supernatant was measured spectrophotometrically at 240 nm by calculating the rate of degradation of H_2O_2, the substrate of the enzyme (Christine *et al.*, 2016). Glutathione reductase (GR) activity was assayed by the method of de Menezes and Augusto, 2001**.**

RESULTS AND DISCUSSION

Lipid Peroxidation

LPO in terms of malondialdehyde (MDA) estimated in different tissues of rats after exposure to Acetamiprid, Panax and their combination for 28 days are presented in Fig.1a. The MDA level in liver, kidney, spleen and brain were significantly ($p<0.05$) higher in acetamiprid treated animals as compared to all other groups. Panax when given along with acetamiprid caused significant reduction of LPO as compared to acetamiprid treated animals. LPO in various organs of control and Panax group were comparable to each other. An increase in the level of MDA indicative of lipid peroxidation in Acetamiprid treated group in the present study is indicative of prooxidative effect of Acetamiprid. Increase in lipid peroxidation because of Acetamiprid has been reported by Mondal *et al*. (2014) in wistar rats. In Acetamiprid plus Panax group, lipid peroxidation was drastically reduced which may be attributed to the protective action exhibited by Panax. Qi *et al.* (2014) demonstrated that G-Rb_1 could reduce lipid peroxidation and oxidative damage and reported the detoxifying actions of *Panax ginseng* extract with improved MDA level for lipid peroxidation, against the oxidative damage following exhausting exercise. Ramesh *et al.* (2012) also found that fermented Panax ginseng extract (GINST) minimized the oxidative stress.

Reduced Glutathione

Reduced glutathione levels were estimated in liver, kidney, spleen and brain after 28 days exposure to acetamiprid and acetamiprid with Panax in rats and are presented in Fig. 1b. GSH levels in various organs in acetamiprid group were significantly ($P<0.05$) lower than the control and Panax group. Panax co-treatment with acetamiprid partially restored altered GSH levels but even remained significantly ($P<0.05$) lower than control and Panax group. However, in combination acetamiprid and Panax group, except liver where the GSH level were comparable to acetamiprid group, in all other organs viz: kidney, spleen and brain GSH levels were significantly higher ($P<0.05$) than acetamiprid group. The level of GSH measured in various organs of control and Panax group were comparable to each other.

One of the indices of oxidative stress is the depletion of the antioxidant Glutathione reduced (GSH). GSH, with the enzymes GSH peroxidase and GSSG reductase, serve to detoxify H_2O_2 to water and molecular oxygen and help to maintain the cysteinyl-thiols ($R\text{-}CH_2\text{–}SH$) groups of proteins in the reduced state, which is often necessary for their functional integrity. Decreased level of GSH in Acetamiprid treated group reflects oxidative stress. Sub chronic oral toxicity of acetamiprid has been reported to decrease the level of GSH (Shakthi Devan *et al.,* 2015) in wistar rats. The observation of increased GSH level in Acetamiprid plus Panax group was in accordance with the findings of Shahin (2018) who reported that *Panax ginseng* increased GSH levels in rats.

Superoxide Dismutase

Effect on Superoxide dismutase (SOD) (U/mg protein) in liver, kidney, spleen and brain in rats following 28 days exposure to Acetamiprid (ACE), *Panax ginseng* and their combination is presented in Fig. 1c. Administration of acetamiprid in rats caused significant ($P<0.05$) decrease in SOD activity in liver, kidney, spleen and brain as compared to all other groups viz: control, combination acetamiprid and Panax, and Panax alone group animals. A significant ($p<0.05$) restoration of SOD activities in liver, kidney and spleen were observed after Panax co-treatment as compared to acetamiprid alone treated animals, but only partial restoration in brain where SOD activities was still comparable to acetamiprid group. The restorations of SOD activities in amelioration groups were not enough to that of controls and Panax groups and were

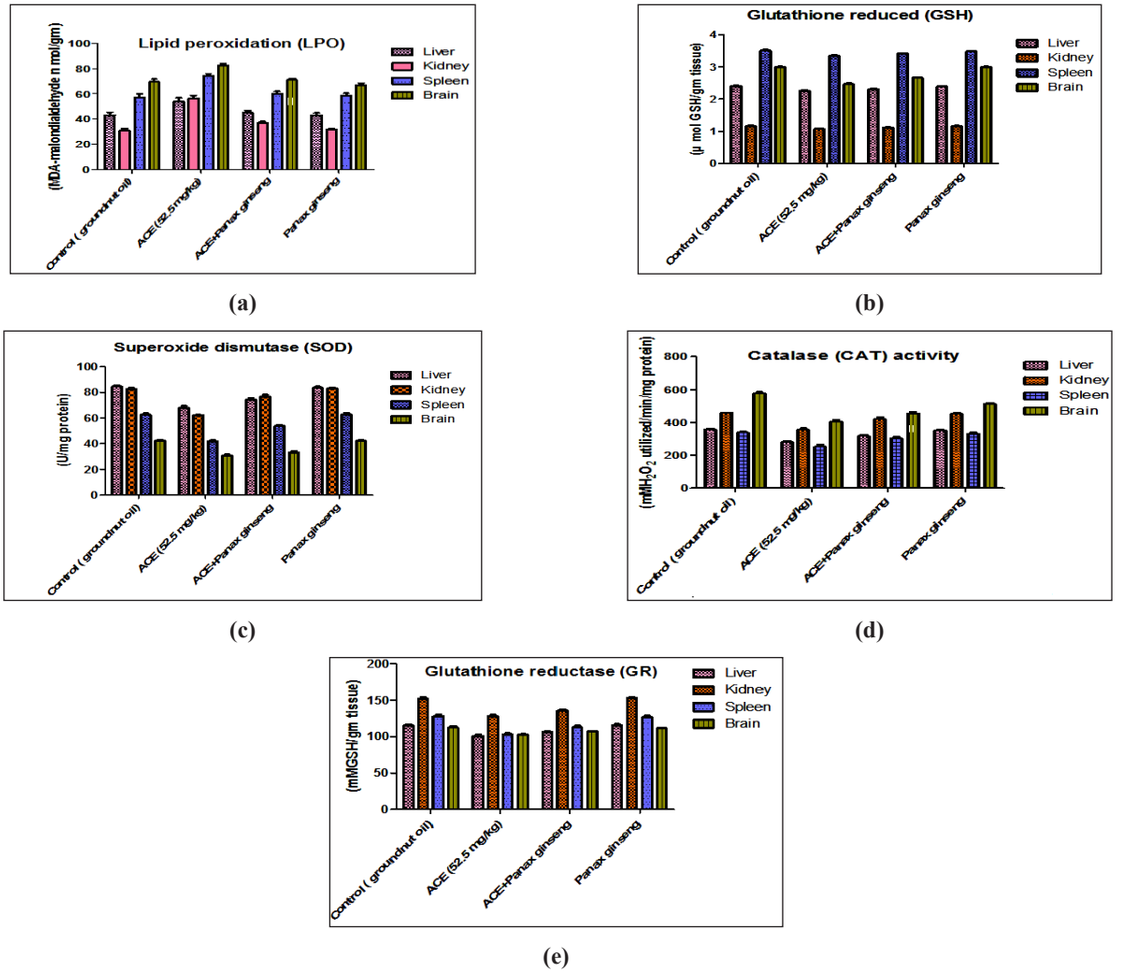

Fig. 1 (a+b+c+d+e): Effect of *Panax ginseng* on oxidative stress related parameters in rats following 28 days exposure to Acetamiprid (ACE)

significantly (P<0.05) less than either of these. The SOD activities in various organs of control and Panax group were comparable to each other.

Because of Acetamiprid induced oxidative stress in rats exposed to it, significantly lower Superoxide dismutase (SOD) activity as compared to control was obvious in the present study. Quintana *et al.* (2018) have also reported that Acetamiprid induces prooxidative changes viz: decrease of superoxide dismutase activity, reduced glutathione content, and increase of content of lipid and proteins peroxidation products. Superoxide dismutase (SOD) activity in Acetamiprid plus Panax group was restored towards normal which is in accordance to the findings of Lee *et al.* (2017). An iron SOD was purified as a homodimer from *Panax ginseng* by employing neutral pH buffer extraction, ammonium sulfate precipitation, isoelectric point precipitation and ion exchange methods by Ding *et al.* (2014) indicative of Panax as source of SOD itself.

Catalase

Catalase (CAT) activities in different organs are expressed in terms of nM H_2O_2 utilized/min/mg protein and are presented in Fig. 1d. CAT activity was found to be a significantly (p<0.05) decreased in acetamiprid treated

groups in liver, kidney, spleen and brain tissues. Panax co-treatment caused significant ($p<0.05$) increase in the CAT activities in all the tissues as compared to acetamiprid alone treated groups. Although, improved, the restored CAT activities in liver, kidney, spleen and brain tissues from Panax co-treatment groups were still significantly ($P<0.05$) lower than either control or Panax group. The CAT activity in various organs of control group were comparable to Panax group.

Hydrogen peroxide generated by dismutation of superoxide anion radical by SOD, is removed by either several isoenzymes of glutathione peroxidase or by catalase. Catalase (CAT) activity becomes important at higher concentrations of hydrogen peroxide, at which the enzyme decomposes most of this compound. In our findings, significantly lower catalase activity of Acetamiprid treated rats reflects oxidative stress because of Acetamiprid. Oxidative stress due to Acetamiprid and lower CAT activity has been reported also by Gasmi *et al.* (2017). Soo *et al.* (2016) in their study found that Catalase (CAT) and glutathione peroxidase (GPx) activities were significantly ($p<0.05$) increased by ginseng and concluded that new ginseng product may be useful as a functional food with strong antioxidant potential.

Glutathione reductase (GR)

Glutathione reductase level in liver, kidney, spleen and brain following 28-days exposure to acetamiprid, Panax and their combination in rats are shown in Fig. 1e. Significantly ($P<0.05$) decreased levels of glutathione reductase were noticed in acetamiprid treated groups as compared to all other groups viz: control, combination acetamiprid and Panax, and Panax alone group animals. Significant ($P<0.05$) restoration of GR was observed in liver, kidney, spleen and brain in rats co-treated with acetamiprid and Panax as compared to acetamiprid alone treated groups. The restorations of glutathione reductase level in amelioration groups were not enough to that of controls and Panax groups and were significantly ($P<0.05$) less than either of these. The glutathione reductase levels in various organs of control and Panax group were comparable to each other.

Glutathione reductase, also known as GSR or GR, is an enzyme that reduces glutathione disulfide (GSSG) to the sulfhydryl form GSH. The activity of glutathione reductase is used as indicator for oxidative stress. In our study, level of Glutathione reductase was significantly lower in Acetamiprid treated group again substantiating its pro-oxidative effect. In Acetamiprid plus Panax group the readings were in between control and Acetamiprid treated group which indicated restoration because of administration of extract of *Panax ginseng*. Youssef (2015) has reported an increased level of GR in rats and significant reduction in oxidative damage following treatment with *Panax ginseng*. They concluded that consumption of *Panax ginseng* reduces lipid peroxidation and restores antioxidant capacity by suppressing oxidative stress in rats.

CONCLUSION

Thus it was concluded that *Panax ginseng* has ameliorating potential on oxidative stress following subacute exposure to acetamiprid in rats.

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