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CHRONIC EXPERIMENTAL FEEDING OF IMIDACLOPRID INDUCED OXIDATIVE STRESS AND AMELIORATION WITH VITAMIN C & *Withania somnifera* IN LAYER BIRDS

Sasidhar Babu N, Anand Kumar A*, Gopala Reddy A[1], Amaravathi P and Hemanth I
Department of Veterinary Pathology, College of Veterinary Science, Proddatur – 516360
[1]College of Veterinary Science, Rajendranagar, Hyderabad
E-mail: aakumar7@rediffmail.com (**Corresponding Author*)

Abstract: The present study on effect of imidacloprid induced oxidative stress and its amelioration was carried out at definite time periods in different groups for 90 days on 48 layer birds of above 2 months age that were divided into six groups consisting of 8 in each. The birds were divided into group 1 was control, group 2 was treated with imidacloprid @ 200 ppm in feed, group 3 was treated with vitamin C @ 200 ppm in feed, group 4 was treated with both imidacloprid@200ppm and vitamin C 200ppm, group 5 was treated with *Withania somnifera* @ 500 ppm in feed and group 6 was treated with imidacloprid @ 200 ppm in feed + Vitamin C @ 200 ppm in feed + *Withania somnifera* @ 500 ppm in feed. Liver tissues were collected in liquid nitrogen at the point of slaughter and stored in deep freezer at -20^0 C. The tissue biochemical profile revealed a significant ($P < 0.05$) reduction in GSH concentration and increase in TBARS levels in liver of group 2. Group 3 value was insignificant from control and group 4 showed a significant ($P < 0.05$) increase in comparison to group 2. The alterations in oxidative stress profiles indicated hepatotoxicity and moderate protection was provided to counteract the toxic effects of imidacloprid.

INTRODUCTION

Imidacloprid is an insecticide that belongs to active group nitro guanidine, the action mechanism of which differs not only from that of the non phosphorous compounds and carbomates, but also from that of the pyrethroids (Soloway *et al.,* 1978). Imidacloprid has an excellent insecticide action (Brocksma *et al.,* 1993) and has wide spread use in crop protection and Veterinary medicine. Imidacloprid was registered and marketed for use in more than 120 countries and over 160 crops. Presently, neonicotinoids share about 11-15% of the total global insecticide market (Tomizawa and Casida, 2003). Glutathione (GSH) is one of the most essential non-enzymatic anti-oxidants for detoxification of several exogenous and endogenous intoxicants. It has a direct anti-oxidant function by reacting with

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superoxide radicals, peroxy radicals and singlet oxygen followed by the formation of oxidized glutathione (GS-SG) and other disulfides (Umalaksmi and Devaki, 1992). It acts as an essential co-factor for anti-oxidant enzymes including glutathione peroxidase (GPx) and Glutathione S-transferase (GST) (Hayes *et al.,* 2005). Under oxidative stress, GSH is consumed by GSH related enzymes to detoxify peroxides produced due to increased lipid peroxidation (Cathcart, 1985).

MATERIALS AND METHODS

The present study on effect of imidacloprid induced oxidative stress and its amelioration was carried out at definite time periods in different groups for 90 days on 48 layer birds of above 2 months age that were divided into six groups consisting of 8 in each with prior approval of Institutional animal ethics committee. All the birds were provided with standard diet and deionized water *ad libitum* and allowed to acclimatize for about week days and were observed thrice daily for clinical signs and mortality if any, during the entire period of study.

Following is the experimental design

Group	No. of Birds	Treatment
1	8	Basal diet
2	8	Basal diet + Imidacloprid @ 200 ppm in feed
3	8	Basal diet + Vitamin C @ 200 ppm in feed
4	8	Basal diet + Imidacloprid @ 200 ppm in feed + Vitamin C @ 200 ppm in feed
5	8	Basal diet + *Withania somnifera* @ 500 ppm in feed
6	8	Basal diet + Imidacloprid @ 200 ppm in feed + Vitamin C @ 200 ppm in feed + *Withania somnifera* @ 500 ppm in feed

Liver tissues were collected in liquid nitrogen at the point of slaughter and stored in deep freezer at -20^{0}C to study the organ anti-oxidant profiles.

Reduced glutathione (GSH)

GSH activity was assessed by the method described by Moron *et al*., 1979. This method is based on reaction of reduced glutathione (GSH) with 5-5' dithiobis-2-nitrobenzoic acid (DTNB) to give a compound that absorbs light at 412 nm.

Thiobarbituric Acid Reactive Substances (TBARS)

TBARS test was carried out by the procedure described by Subramanian *et al*., (1988).

The obtained data was analysed statistically (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

Reduced glutathione (GSH)

The mean concentration of GSH in liver of different groups ranged from 40.32±0.89 to 68.61±0.01 during the present study. There was a significant ($P < 0.05$) reduction of liver GSH concentration in groups 2, 4 and 6 in comparison to group 1. In addition, significant ($P < 0.05$) increase was observed in groups 4 and 6 when compared with group 2 (Table 1). In the present study, there was a significant reduction in GSH concentration in hepatic tissues in groups 2, 4 and 6. This signified the generation of free radicals that induced oxidative stress following imidacloprid treatment and it could be attributed to direct utilization of GSH as an anti-oxidant in terminating the free radical reaction resulting in exhaustion of the GSH during oxidative stress. This depletion in GSH level suggests that there was an increased peroxidation that was evidenced from significant alterations in hepatic and renal biomarkers and corresponding histological alterations in liver and kidney sections of groups 2, 4 and 6. This observation is in accordance with the Duzguner and Erdogen (2010) in rats treated with single i/v administration of imidacloprid at 10 µM, which resulted in depletion of intracellular glutathione in liver and brain. Kapoor *et al*. (2010) reported decreased levels of GSH in liver at 20 mg/Kg b. wt in female rats and Bal *et al*. (2012) documented decreased levels of GSH in testicular tissue at 8 mg/Kg b. wt in adult male rats.

In groups 4 and 6, there was a significant increase in GSH concentration in comparison with group 2. Similar observation was reported by El-Gendy *et al*. (2010) in male mice. This could be due to protective action of vitamin C as it plays primary role in neutralizing free radicals. It can work both inside and outside the cells to combat free radical damage. Since, the free radicals will seek out an electron to regain their stability, vitamin C and *Withania somnifera* are excellent source of electrons so they can donate electrons to free radicals such as hydroxyl and superoxide radicals and quench their reactivity (Bendich, 1990 and Bindhumol *et al.,* 2003).

Thio Barbituric Acid reactive substances (TBARS) (n moles of MDA/g Protein):

The mean values of TBARS in liver of different groups ranged from 0.686±0.01 to 1.436±0.16 during the present study. Significant ($P < 0.05$) increase in TBARS levels was

observed in group 2 when compared to group 1. No significant difference was observed in other groups (Table 1). Significant increase in TBARS concentration in hepatic tissues was observed in groups 2, 4 and 6 that indicated excess free radical production. These findings are in accordance with the reports of Vandana *et al.* (1998), LIU Xiu-fang *et al.* (2008) in birds, Sanjukta Datta *et al.* (2010) and Tarek *et al*. (2012) in rats.

In treatment groups 4 and 6, significant decrease in TBARS levels in comparison to toxic group was noted. This could be attributed due to protective action of vitamin C and *Withania somnifera* as they play primary role in neutralizing free radicals. These findings can be correlated with the observations of Vandana *et al.* (1998), Sanjukta Datta *et al.* (2010) and Al-Olthman *et al.* (2011).

It is concluded that Imidacloprid definitively induced oxidative damage in liver of layer birds and vitamin C, *Withania somnifera* have ameliorative effect.

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Table 1: Mean values of GSH concentration and TBARS values birds of different experimental groups

Groups	GSH concentration (µM/mg protein) Mean ± SE	TBARS values (n Moles of MDA/g Protein) Mean ± SE
Group 1	68.61 ± 0.01^{a}	0.693 ± 0.01^{b}
Group 2	40.32 ± 0.89^{d}	1.436 ± 0.16^{a}
Group 3	67.27 ± 0.30^{abc}	0.686 ± 0.02^{b}
Group 4	65.84 ± 0.88^{bc}	0.84 ± 0.04^{b}
Group 5	67.58 ± 0.03^{ab}	0.733 ± 0.02^{b}
Group 6	65.44 ± 0.92^{c}	0.823 ± 0.02^{b}

S.E (Standard Error) One Way ANOVA Means bearing common superscripts are not significantly different ($P < 0.05$)