

Imidacloprid induced osmotic fragility in erythrocytes of rats: Protective role of vit.C and tea

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Abstract: The present study was undertaken to evaluate the effect in vitro induction of sub-acute oral dose of imidacloprid and ameliorating role of tea and Vit.C on oxidative stress and osmotic fragility of albino wistar rats. On the basis of imidacloprid having LD₅₀ 450mg/kg body weight in rats, which exert oxidative stress on mammalian system and is moderately toxic via oral route. We observed a significant alteration in osmotic fragility of rat erythrocytes upon imidacloprid treatment shown by mean erythrocyte osmotic fragility at relatively higher NaCl concentration of (0.45%) as compared to mean erythrocyte fragility (MEF) at 0.40%, 0.41%, 0.42%, 0.32% and 0.30% NaCl in control, Vit. C, tea, IP+ tea, IP + Vit.C treated groups, respectively. The results suggest that imidacloprid induced toxicity exerts OF in erythrocytes of rats and pretreatment with Vit. C and tea can mitigate to some extent the toxic effects.

Keywords: albino wistar rats, erythrocytes, Imidacloprid toxicity, osmotic fragility, mean erythrocyte osmotic fragility,.

I. Introduction:

Imidacloprid is a neonicotinoid, which is a class of neuroactive insecticide modelled after nicotine. It is known to exert high toxicity to mammalian systems. It is a neuro-active insecticide marketed as pest control device, for seed treatment and termite control [1]. Imidacloprid are known to cause oxidative stress in erythrocytes. Erythrocytes are prone to oxidative stress due to presence of haemoglobin and polyunsaturated fatty acids. Oxidative stress is associated with increased osmotic fragility of erythrocytes [2]. Several insecticides have been reported to bind the human plasma protein fractions and disturb biochemical and physiological functions within erythrocytes and affect membrane integrity [3]. The erythrocytes serves as the principle vehicle for effective transport of oxygen and carbon dioxide between the lungs and tissues. Erythrocytes are prone to oxidative stress because they exposed to high oxygen tension and have polyunsaturated fatty acid in the membrane and haemoglobin bound iron [4]. Keeping these facts in view the present study was undertaken to evaluate the impact of imidacloprid on the oxidative stress and integrity of mammalian erythrocytes and the protective role of known antioxidants such a Vit.C and green tea from pesticide induced toxicity also needs to be explored. The present study was aimed to further evaluate the efficacy of Vit. C and tea, as an aqueous antioxidants, on alterations induced by the exposure of wistar strain rats to sub-acute dose of imidacloprid.

II. Materials and Methods:

1.1 Chemicals

All chemicals used were of analytical grade.

1.2 Experimental animals

Male albino wistar rats of same age group and body weight between 150-200gm and 8 to 10 weeks of age were selected for all the experiments. Animals obtained from Central Drug research Institute (CDRI), Lucknow, India, were housed in polypropylene cages at an ambient temperature at 25-30°C and 45-55% humidity. Animals were fed standard rat pellets and water. Proper care of animals was strictly followed and study had the approval of Institutional Ethical Committee.

1.3 Experimental Designs and animal treatment: The albino male wistar rats were divided into four groups, each containing three rats. All the treatments were given orally and the live weight was recorded daily and monitored for 24h continued for 30 days. Experimental rats were grouped as follows;

I. Group 1 (Control) received 0.5ml DMSO (C),

- II. Group 2 (Imidacloprid treated Group) received oral dose of 17.8 gm imidacloprid/kg body weight equivalent to 100mg/kg body wt. dissolved in 0.5ml of DMSO. (IP),
- III. Group 3 (Antioxidant treated Group)
 - a) Green Tea treated gp. received 200 mg/kg body wt in 0.5ml distilled water. (T)
 - b) Vit. C treated gp. received 200 mg/kg body wt in 0.5ml distilled water. (Vit. C)
- IV. Group 4 (Antioxidant and Imidacloprid treated Group)
 - a) Animals placed in this group received antioxidants (Tea) of 200 mg/kg body wt. 30min. prior to 17.8gm imidacloprid /kg body wt. imidacloprid treatment denoted as (IP+T).
 - b) Animals placed in this group received antioxidants (Vit.C) of 200 mg/kg body wt. 30min. prior to 17.8gm imidacloprid /kg body wt. imidacloprid treatment denoted as (IP + Vit.C).

After completion of oral administration of 30 days, the rats of each treated group were anesthetized with ether. Five milliliter of blood was collected from each animal by cardiac puncture in heparin containing vials for estimating osmotic fragility of erythrocytes at various percentage levels i.e., 2% to 9% of hypotonic saline solution of 9 % phosphate buffer saline (PBS).

$$\text{Calculation: \% Haemolysis} = \frac{\text{abs. OD}}{\text{Maximum abs. OD}} \times 100$$

III. Result and discussion

	GP.1	C	GP.2	IP	GP.3 (a)	TEA	GP.3(b)	Vit.C	GP.4(a)	IP +TEA	GP.5(b)	IP +Vit.C
% PBS	OD (540 nm)	% Hemolysis	OD(540nm)	% Hemolysis	OD(540nm)	% Hemolysis	OD(540 nm)	% Hemolysis	OD(540 nm)	% Hemolysis	OD(540nm)	% Hemolysis
0.9	Ref.	-	Ref.	-	Ref.	-	Ref.	-	Ref.	-	Ref.	-
0.8	0.016	2.73	0.002	0.189	0.002	0.2012	0.005	0.651	0.025	4.167	0.005	0.651
0.7	0.028	4.78	0.008	0.754	0.007	0.704	0.007	0.911	0.034	5.667	0.007	0.911
0.6	0.075	12.80	0.013	1.2252	0.013	1.3078	0.012	1.56	0.045	8.333	0.012	1.56
0.5	0.084	14.33	0.015	1.414	0.02	2.012	0.019	2.47	0.017	2.833	0.019	2.47
0.4	0.298	50.85	0.945	89.07	8.38	84.3	0.546	71.09	0.083	13.833	0.546	71.09
0.2	0.485	82.76	1.01	95.19	0.943	94.869	0.676	88.02	0.502	83.67	0.676	88.02
DDW	0.586	-	1.061	-	0.994	-	0.768	-	0.6	-	0.768	-

Table.1.1 The above table shows MEOF (mean erythrocyte osmotic fragility) in erythrocytes of phosphate buffer saline (PBS) in the Gp.1(control), Gp.2(IP), GP.3 Antioxidants(tea and vit.C) and GP.4 Imidacloprid and antioxidants (IP+ Tea)and (IP+ vit.C) groups of rats.

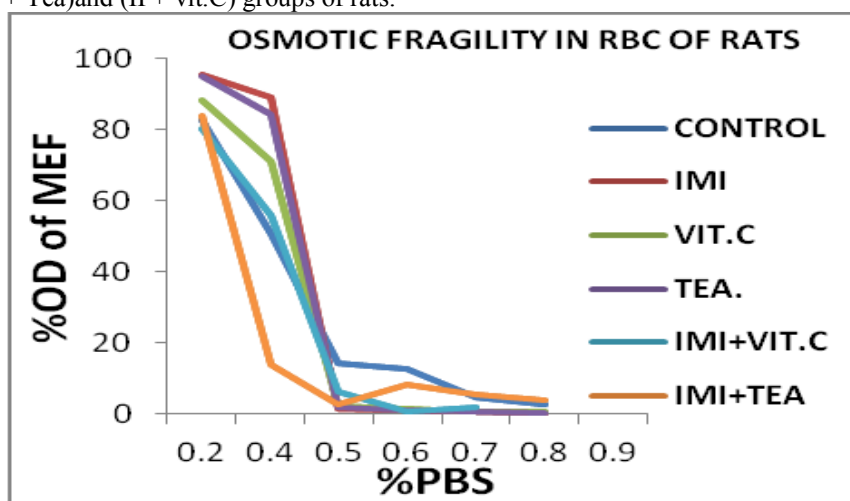


Fig.1.1 The above figure shows the effect of the imidacloprid (IP), Vit.C, tea, imidacloprid with antioxidant tea and vit.C over the control on mean erythrocyte osmotic fragility(MEOF) in erythrocyte of rats in phosphate buffer saline(PBS).

Osmotic fragility is the ease with which RBCs undergo lysis, when kept in hypotonic solution. When the red cells are placed in hypotonic saline, water is drawn in by the higher intracellular osmotic pressure. Consequently the cells become spherical and ultimately the red cell membrane gives way with liberation of haemoglobin into the surrounding fluid. The process of liberation of haemoglobin from the red cells is known as haemolysis. The importance of osmotic fragility estimation lies in the fact that it gives the information about the total status of red cell metabolism and membrane stability. In the present study a significant increase in osmotic fragility of rats erythrocytes upon imidacloprid treatment was observed. The same results have been observed by [5], [6], [7], and [8]. In addition, Vit.C and tea have been reported to protect erythrocytes against oxidative stress induced hemolysis. The pretreatment of rats with Vit.C and tea protect the erythrocytes from imidacloprid induced osmotic fragility.

The study confirmed the toxicity induced by the imidacloprid causes increase in osmotic fragility of rat erythrocytes upon imidacloprid treatment shown by mean erythrocyte osmotic fragility at relatively higher NaCl concentration of (0.45%) as compared to mean erythrocyte fragility(MEF) at 0.40%,0.41% 42%,0.32% and 0.30% NaCl in control, Vit. C, tea, IP+ tea, IP + Vit.C treated groups, respectively. Hence, from the present study it reflects that oxidative stress is a common feature of imidacloprid toxicity whether in prolonged or single exposures. The supplementation of tea and Vit.C indicated its protective role in protection against imidacloprid induced toxicity[6].The supplementation of antioxidants like Vit. C, tea to rats resulted in decreased oxidative stress in erythrocytes. The Vit.C and tea supplementation indicated its protective role in protection against imidacloprid induced toxicity. The main role of antioxidants is their ability to trap free radicals. Hydrophilic scavengers are found in cytosolic, mitochondrial and nuclear compartment. Osmotic fragility is the ease with which RBC undergo lysis, when kept in hypotonic solution. When the red cell is placed in hypotonic saline, water is drawn in by the higher intracellular osmotic pressure. Consequently the cells become spherical and ultimately the red cell membrane gives way with liberation of haemoglobin into the surrounding fluid. The process of liberation of haemoglobin from the red cells is known as haemolysis.

IV. Conclusion

On the basis of the present results it can be concluded that imidacloprid having LD₅₀ 450 mg/kg body weight in rats, exert oxidative stress in mammalian system and is moderately toxic via oral route. It has been reported that imidacloprid induced toxicity can be protected through the oral supplementation of tea and Vit.C and the role of intracellular antioxidants to scavenge the free radicals produced during the oxidative stress.

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