Current Research in Pharmaceutical Sciences
Available online at www.crpsonline.com

ISSN 2250 – 2688

Received: 07/09/2012
Revised: 18/10/2012
Accepted: 31/10/2012

Charushila Jaiswal
CVAS, Udaipur , India.

Y Verma
Department of Veterinary Pathology, Jabalpur, India

A B Shrivastav
Centre for Wild Life Forensic and Health, Jabalpur (India)

Correspondence

Charushila Jaiswal
CVAS, Udaipur (India)

Email: Charushila.jaiswal@gmail.com

α- Tocopherol Protects Wister Rats from Oxidative Stress Induced by Chlorpyrifos and *E.coli* Infection

Charushila Jaiswal, Y Verma, A B Shrivastav

ABSTRACT

72 male albino rats of the Wistar strain were randomly divided into 6 groups. Rats of control group I was fed with standard feed and water through out of experiment. Group II rats were treated with of *E. coli* (0.3 ml) by intra-peritoneal (I/P) inoculation, before 24 hrs of both sacrifices i.e.on 15th and 30th day of experiment. Group III was treated with chlorpyrifos reconstituted with 2 ml soya oil @10.6mg/kg b.wt. orally daily for 30 days. Group IV was treated with chlorpyrifos same as group III and I/P inoculation of *E. coli* (0.3 ml) was same as group II. Group V was treated with chlorpyrifos same as group III with α-tocopherol (VE) @150mg/kg b.wt. 30 minutes before the administration of CPF. Group VI was treated with chlorpyrifos same as group IV with Vitamin E @150mg/kg b.wt. 30 minutes before administration of CPF. This regimen was administered orally for the period of 30 days. At the time of sacrifice blood sample was collected i.e. on day 15th and 30th of the experiment. Oxidative stress was evaluated by measuring erythrocyte osmotic fragility test using different salt concentrations. The study showed that α-tocopherol protects erythrocyte osmotic fragility induced by repeated CPF exposure and *E. coli* infection in albino rats.

Key words: chlorpyrifos, α-tocopherol, E. coli, oxidative stress, erythrocyte osmotic, fragility

1. INTRODUCTION

Studies have demonstrated that repeated exposure to chlorpyrifos (CPF), a chlorinated organophosphate insecticide causes anemia [1]. Anaemia has been observed following chronic acute and subacute exposure to CPF in rats. Although, the molecular mechanism of anaemia has not been fully elucidated, although the main mechanism of CPF toxicity has been attributed to acetylcholinesterase inhibition, the ability of the insecticide to induce oxidative stress in the erythrocyte membranes has been clearly demonstrated[2]. Free radicals (FRs) have been shown to cause adverse effects on tissues and erythrocyte membranes [3]. Naturally, erythrocytes are prone to oxidative stress because of their constant exposure to high oxygen tension, high content of polyunsaturated fatty acid in their membranes, coupled with high amount of hemoglobin-bound iron [4]. However, the erythrocytes are armed with efficient antioxidant machinery in the form of antioxidant enzymes, such as superoxide dismutase, catalase and glutathione-S-transferase, and antioxidant molecules such as vitamins C and E that scavenge reactive oxygen species to maintain cellular integrity[5].

In conditions associated with increased oxidative stress as observed in CPF poisoning, the antioxidant system is overburdened, resulting in lipoperoxidative damage and subsequent alteration in the composition of the erythrocyte membranes. This eventually causes perturbation in the structural integrity of erythrocytes, resulting in oxidative hemolysis.

Erythrocyte osmotic fragility is frequently used as a measure of the strength of the red blood cells [5]. Keeping above facts in mind, present study was conducted to see the effect of CPF on erythrocyte fragility with ameliorative effect of Vitamin E in albino rats.

2. MATERIAL AND METHODS

2.1 Location and place of work

The work was conducted in the Department of Veterinary Pathology, College of Veterinary Science and Animal Husbandry, Madhya Pradesh Pashu Chikitsa Vigyan Vishwavidyalaya, Jabalpur, Madhya Pradesh, India.

2.2 Experimental animals

The experiment was approved by the Institutional Animal Ethics Committee of College of Veterinary Science and Animal Husbandry, Madhya Pradesh Pashu Chikitsa Vigyan Vishwayavidyalaya, Jabalpur and all the protocols were followed according to the guidelines given by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).
The study was conducted on 72 male Wistar albino rats of 6-8 weeks age group, weighing around 100 to 120 g procured from Bharat Traders, Bhopal, India. They were housed in poly propylene cages with stainless grill tops steel and were acclimatized for a period of 7 days before the start of the experiment in the laboratory animal house. They were maintained in a 12/12 hours light/dark cycle, with good hygienic conditions and kept on a standard feed and water *ad libitum*. All the experimental animals were kept under constant observation during the entire period of study.
This composition supplied the carbohydrate, crude protein and fat as 3800 Kcal/kg, 18% and 5% respectively.

2.3 Chemicals and substances

Local/Technical grade Chlorpyrifos (20%) procured from Greentech Agrogenesis, Jabalpur, Madhya Pradesh (India) was used in the present study. Evion® caps of 600 mg (Merck Limited, Worli, Mumbai) were used as a source of α-tocopherol (VE).

2.4 Procurement of culture

The culture of *Escherichia coli* (MTCC-723) was procured from Microbial Type Culture Collection and Gene Bank, Chandigarh and maintained on nutrient agar slants.

2.5 Preparation of inoculums

Escherichia coli culture was inoculated into nutrient broth and incubated overnight at 37˚C). Direct smear was prepared to test the purity of the culture (Plate1). To induce infection the concentration of bacteria was kept as 1×10^9 CFU/ml by comparing with brown opacity tube no.7 (Cruikshank *et al*., 1980).

2.6 Design of experiment

72 rats were randomly divided into 6 groups after 7 day of acclimatization. Rats of control group I was fed with standard feed and water through out of experiment. Group II rats were treated with of *E. coli* (0.3 ml) by intra-peritoneal (I/P) inoculation, before 24 hrs of both sacrifices. Group III was treated with chlorpyrifos reconstituted with 2 ml soya oil @10.6mg/kg b.wt. orally daily for 30 days. Group IV was treated with chlorpyrifos same as group III and I/P inoculation of *E. coli* (0.3 ml) was same as group II. Group V was treated with chlorpyrifos same as group III with VE @150mg/kg b.wt. 30 minutes before the administration of CPF. Group VI was treated with chlorpyrifos same as group IV with VE @150mg/kg b.wt. 30 minutes before administration of CPF.

2.7 Evaluation of erythrocyte Osmotic Fragility

In vitro osmotic fragility of erythrocytes was determined according to a method of Cartwright, modified by O'Dell [6] using different saline concentrations from 0.38 to 0.48 g/l and 0.9 g/l (control). Freshly obtained heparinised whole blood was pipetted in saline concentrations followed by careful mixing and incubation for 15 min at room temperature. After centrifugation (5000 rpm at 4°C for 10 min), the concentration of hemoglobin in the supernatant was measured calorimetrically with a spectrophotometer. The percentages of maximal hemolysis were plotted vs. the respective salt concentrations.

$$\text{Percent haemolysis} = \frac{\text{Optical density of test solution}}{\text{Optical density of standard solution}} \text{x100}$$

3. RESULTS AND DISCUSSION

The mean values of % hemolysis in different groups of rats are presented in table 2 and 3 on day 15 and 30 of sacrifice respectively. There was no significant ($P<0.01$) difference in degree of erythrocytes fragility among the rats at various groups at.

Table No 1. Experimental Design

Group	No. of animals	Treatment	Sacrifice	Studies undertaken
I (Three replicates)	12 (6 rats in eachreplicate)	Standard feed and water + Soya oil @2 ml/kg b. wt. orally once a day throughout the experiment.	First sacrifice at day 15th and second sacrifice at day 30th (2 rats from each replicate i.e. 2x3 =6 rats)	Erythrocyte osmotic fragility test
II	12	Standard feed and water + I/P inoculation of *E. coli* (0.3ml) before 24 hrs of sacrifices	Same as above	Same as above
lll	12	Standard feed and water + chlorpyrifos (10.6mg/kg body wt.) reconstituted with 2 ml soya oil orally once a day throughout the experiment.	Same as above	Same as above
lV	12	As group III + I/P inoculation of *E. coli* (0.3 ml) before 24 hrs of sacrifices	Same as above	Same as above
V	12	Standard feed and water + chlorpyrifos as group III + VE (150mg/ body wt.) 30 min. before administration of CPF orally once a day throughout the experiment	Same as above	Same as above
VI	12	As group V + I/P inoculation of *E. coli* (0.3 ml) before 24 hrs of sacrifices	Same as above	Same as above

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concentration 0.9 g/dl of NaCl. There were highly significant difference in degree of erythrocytes fragility between groups at different concentrations (0.38 - 0.48 and 0.9 g/dl of NaCl) at both intervals. When compared with the control group a significant ($P<0.01$) increase in erythrocytes fragility was recorded in CPF and CPF + *E. coli* groups. Significant improvements in values of % hemolysis were observed in CPF+ VE and CPF+ *E. coli*+VE groups at both sacrifices

The present study has shown the ability of CPF administration to measure erythrocyte fragility due to increased lipoperoxidative damage to erythrocyte membrane. Membrane lipids are vital for the maintenance of cellular integrity and survival [7]. Peroxidation of membrane lipids can result in the inactivation of enzymes and cross-linking of membrane lipids and proteins and in cell death[8]. By-products of lipid peroxidation have been shown to cause profound alterations in the structural organization and functions of the cell membrane including decreased membrane fluidity, increased membrane permeability, inactivation of membrane-bound enzymes and loss of essential fatty acids [9]. This lipoperoxidative alteration in the structural and functional components of the erythrocyte membranes may have caused perturbations in the membrane integrity, resulting in increased erythrocyte fragility observed in the CPF group. In *E. coli* and combined group of CPF increased erythrocyte fragility are in accordance with the findings of certain authors as *E. coli* produced virulence factors such as α-hemolysin leads to hemolysis by forming pores in the erythrocyte membrane which added the effect of CPF leads to increase erythrocyte fragility. Vitamin E has been shown to ameliorate chronic CPF-induced increased erythrocyte fragility in Wistar rats. Vitamin E is a lipid soluble membrane-bound antioxidant that has shown clear evidence for its membrane-stabilization effect. The findings are corroborated with the results of Ambali [10-12] stated that vitamin E, vitamin C and Zn ameliorate the CPF induced erythrocyte fragility. In the group pretreated with vitamin E, agreed with the result of Gultekin *et al*., 2001 indicating that induction of lipid peroxidation in the erythrocyte membrane plays significant role in patho-mechanism of increased erythrocyte fragility following CPF administration. Vitamin E neutralizes the effects of lipid peroxidation through its oxygen scavenging properties[13-14]. Apart from antioxidant function of it also influences the cellular response to oxidative stress through modulation of signal-transduction pathways.

4. CONCLUSION

In conclusion, the present study has demonstrated that chronic CPF exposure results in the increased erythrocyte osmotic fragility, which may have been partly responsible for anaemia. Pretreatment with α-tocopherol has been shown by the present study to ameliorate the erythrocyte membrane damage, which must have been partly responsible for the significant reduction in the erythrocyte fragility.

5. ACKNOWLEDGEMENT

The authors are grateful for Department of Pathology, College of Veterinary Science and A.H., Jabalpur (M.P.), M.P.P.C.V.V. for providing facilities and funds.

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Table No. 2 Mean values of % erythrocyte hemolysis in different groups of rats on 15th day

Groups	NaCl concentrations (g/dl)							
	0	0.38	0.40	0.42	0.44	0.46	0.48	0.9
Control	100.00 ±0.00	65.33^{e} ±0.83	46.67^{d} ±1.05	44.00^{c} ±1.26	38.33 ^{c}±1.05	31.00^{e} ±0.44	25.83 ^{e}±0.70	0.00^{d}± 0.00
E. coli	100.00 ±0.00	68.33 ^{d}±3.33	47.83^{d} ±0.16	45.33^{bc} ±1.22	39.00 ^{c}±0.44	33.00 ^{d}±0.00	31.17 ^{d}±0.47	0.00^{d}± 0.00
CPF	100.00 ±0.00	74.17^{ab} ±0.83	70.00^{b} ±0.00	69.67^{a} ±0.49	55.00 ^{a}±0.00	41.00^{a} ±0.51	35.00 ^{b}±0.00	3.00^{ab}± 0.00
CPF + *E. coli*	100.00 ±0.00	75.00^{a} ±0.00	71.33^{a} ±0.21	70.84^{a} ±0.65	56.67 ^{a}±1.05	42.67^{a} ±0.21	37.00 ^{a}±0.00	3.66^{a}± 0.49
CPF+ VE	100.00 ±0.00	70.67^{c} ±0.66	60.00^{c} ±0.00	46.50 ^{bc}±0.67	41.50 ^{b}±0.67	37.33^{c} ±0.55	26.40 ^{e}±0.34	2.10^{c}± 0.16
CPF+ *E. coli* + VE	100.00 ±0 .00	72.50^{b} ±0.22	61.00^{c} ±0 .00	47.17^{b} ±1.04	42.50 ^{b}±0.34	39.00^{b} ±0.25	32.50 ^{c}0.34	2.25^{bc}± .22
LSD value (0.05p)		**1.60**	**1.28**	**2.70**	**2.03**	**1.11**	**1.15**	**0.66**

Values in columns with similar superscript indicate non significant difference and those with different superscript indicate significant difference ($p \leq 0.01$).

Table No 3. Mean values of % erythrocyte hemolysis in different groups of rats on 30th day

Groups	NaCl concentrations (g/dl)							
	0	**0.38**	**0.40**	**0.42**	**0.44**	**0.46**	**0.48**	**0.9**
Control	100.00 ±0.00	68.00^{e}±0 .00	40.00^{f} ±0.00	38.00^{e}± 0.00	36.00^{d}±0 .00	32.00^{e}± 0 .00	30.00^{e}± 0.00	0.00^{f} ±0.00
E. coli	100.00± 0.00	74.17 ^{cd}±0.16	43.33^{e}± 1.05	42.00^{d}± 0.34	38.50^{d}±0 .34	34.00^{d}± 0.00	32.57^{d}± 0.22	0.00^{f} ±0.00
CPF	100.00± 0.00	83.33 ^{b}±1.61	75.00^{b}± 0.00	63.30^{b}± 0.33	46.83^{c}±0 .54	36.50^{c}± 0.00	36.50^{b}± 1.64	3.00^{e} ±0.00
CPF+ *E. coli*	100.00± 0.00	85.00 ^{a}±0.00	80.00^{a}± 0 .00	75.00^{a}± 0 .00	65.00^{a}±0 .00	50.00^{a}± 0.00	40.83^{a}± 0.54	9.00^{a} ±0.00
CPF+ VE	100.00± 0.00	73.33 ^{d}±1.05	68.33^{d}± 1.05	58.33^{c}± 1.05	48.33 ^{bc}±1 .05	38.75^{c}± 0.54	33.83^{cd} ±0.60	6.00^{c} ±0.00
CPF+ *E. coli* + VE	100.00± 0.00	75.17 ^{c}±0.16	70.83^{c}± 0 .54	60.58^{c}± 1.66	50.83 ^{b}±2.16	42.60^{b}± 0.21	35.67^{bc} ±0.42	7.00^{b} ±0.00
LSD value (0.05p)		**1.46**	**1.87**	**2.30**	**2.90**	**0.68**	**2.23**	**1.66**

Values in columns with similar superscript indicate non significant difference and those with different superscript indicate significant difference ($p \leq 0.01$)