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Acephate induced oxidative damage in erythrocytes

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The effect of oral administration of acephate (360 mg/kg body weight), for 15 days, daily, was investigated on the erythrocytes of male rats. Activities of acetyl cholinesterase and glucose-6-phosphate dehydrogenase decreased, while those of glutathione-s-transferase and glutathione reductase increased. Decreased glutathione content and increased lipid peroxidation suggest that there was increased oxidative stress in the erythrocytes of treated animals. Increased cholesterol/phospholipid ratio in the erythrocyte membranes and morphological changes in RBCs (scanning electron microscopy studies) were observed in acephate treated animals. The results clearly suggest that acephate induced oxidative stress in erythrocytes leads to morphological changes.

Acephate belongs to relatively newer class of organophosphate pesticides with low mammalian toxicity[1]. Unlike other organophosphates, it has been shown to be a poor inhibitor of acetylcholinesterase *in vitro* but has a high insect toxicity *in vivo*[2]. The selective toxicity of acephate in insects but not in mammals is due to its conversion to methamidophos exclusively in insects[1]. This property makes it an effective pesticide of choice for crop protection resulting in its indiscriminate use.

However in recent years reports have appeared which show its toxic effects on non-target organisms. It has been found to inhibit human erythrocyte superoxide dismutase and plasma cholinesterase under *in vitro* conditions[3]. Recently, Farag *et al.*[4] have shown decreased number of implantations and live fetuses in acephate treated mice. Poovala *et al.*[5] on the basis of their *in vitro* studies on the role of oxidant stress in acephate induced renal tubular cytotoxicity hypothesized that oxidant stress may play a role in the pathogenesis of acute tubular necrosis and renal dysfunction.

In the present study biochemical evidence to show acephate induced oxidative damage and the consequent morphological changes in rat erythrocytes are reported.

All the chemicals used in the present study were of analytical grade. Most of the biochemicals used were purchased from Sisco Research Laboratories. Acephate (technical grade) was procured from Punjab Pesticides Industrial Cooperative Society, Kharar (Punjab).

Male albino rats (Wistar strain) weighing 180-200g were purchased from the Central Animal House of Panjab University. Animals were divided into two groups: control (Group I) and acephate treated (Group II). There were 6-8 animals in each group. All the animals were kept on laboratory diet (Hindustan Lever Ltd., India) and had free access to water.

Treatment of animals—Animals of Group II were given daily oral (gavage) doses of acephate (360 mg/kg body weight, dissolved in water) for 15 days. The animals of Group I were given only water in the same manner.

Animals were sacrificed on the 16th day after overnight fasting. Blood was drawn from optic sinus of eye.

Erythrocyte hemolysate was prepared by the method of Lohr and Waller[6]. In erthrocyte hemolysate acetylcholinesterase (AChE)[7], glucose-6-phosphate dehydrogenase (G6PD)[6], glutathione-s-tranferase[8] and glutathione reductase[9] were assayed by standard methods. Glutathione (reduced) content was estimated by the method of Beutler and Kelly[10].

Erythrocyte membranes were prepared by hemolysing the erythrocytes in distilled water and then centrifuging at 20,000 *g*. Post hemolytic residue was washed twice with 1 m*M* Tris HCl containing 1 m*M* Tris EDTA (*p*H 7.4). Erythrocyte membranes were kept frozen at --60°C.

In the membrane preparation, lipid peroxidation (LPO) was measured in terms of malonaldehyde (MDA) formation[11]. Protein content was measured by

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Table 1-Effect of acephate administration on the oxidative damage in erythrocytes

[Values are means ± SD of 6 observations. Figures in parentheses are per cent of control]

Parameters	Control group (Group I)	Acephate treated group (Group II)
Erythrocyte hemolysate		
Acetylcholinesterase (units/mg protein)	0.083 ± 0.015	0.048 ± 0.020[c] (42.16)
Glucose-6-phosphate dehydrogenase (units/mg protein)	0.487 ± 0.073	0.247 ± 0.014[c] (49.28)
Glutathione-S-transferase (units/mg protein)	0.178 ± 0.040	0.200 ± 0.081 (12.35)
Glutathione reductase (units/mg protein)	0.030 ± 0.006	0.078 ± 0.021[c] (160)
Glutathione (reduced) (μmoles/mg protein)	3.260 ± 0.503	2.198 ± 0.322[c] (32.57)
Erythrocyte membrane		
Lipid peroxidation (μmole of MDA /mg protein)	25. 52 ± 1.16	29.35±1.42[b] (15.00)
Cholesterol/phospholipid	0.778	2.421 (211.18)

P values: [a]<0.05; [b]<0.01; [c]<0.001

the method of Lowry *et al*[12]. Lipids were extracted by the method of Folch *et al*[13]. Phospholipid[14] and cholesterol[15] contents were determined by standard methods.

Blood for scanning electron microscopy was fixed immediately in 2.5% glutaraldehyde made in 0.2 *M* phosphate buffer (*p*H 7.2) for 90 min and processed for SEM.

Administration of acephate for 15 days resulted in significant decrease in body weight of rats (unpublished data). There was 25% mortality in the acephate exposed rats. The RBC count of acephate exposed rats was $7.42 \pm 0.42 \times 10^6/mm^3$ of blood, whereas, in the control animals it was $8.10 \pm 0.56 \times 10^6/mm^3$. However, this decrease was not statistically significant.

Effect of *in vivo* administration of acephate on the activities of acetylcholinesterase (AChE), glutathione-S-transferase (GST), glutathione reductase (GR) and glutathione (reduced) content of erythrocytes (hemolysate) has been presented in Table 1.

Acephate treatment resulted in significant decrease (42%) in the erythrocyte acetylcholinesterase activity in rats. Inhibition of AChE by organophosphates is well documented[16,17], but there are not many reports about the inhibition of mammalian acetylcholinesterases by acephate. Spassova *et al.*[2] have reported inhibition of blood and brain AChE activity in rats by 55-75% after 15 min of intraperitoneal injection with 500 mg/kg acephate.

A significant decrease was observed in the activity of erythrocyte G6PD, whereas increases were observed in the activities of glutathione-S-transferase and NADPH specific glutathione reductase. A highly significant decrease was observed in the glutathione content of the erythrocyte hemolysate of the treated animals (32%). The function of G6PD in the mature cells is to generate NADPH which is required for the conversion of oxidized glutathione to reduced glutathione which in turn is necessary for maintaining the critical –SH group(s) of proteins in their functional form. Thus, decreased G6PD activity resulted in decreased glutathione (reduced) content in the erythrocytes. A gradual decrease in blood and liver glutathione in rats after acephate treatment has been reported[18]. Glutathione and glutathione dependent enzymes provide major protection against toxic agents. The observed increase in the glutathione-S-transferase (12%) and glutathione reductase activity (160%) may be due to activation of the natural antioxidant defense system by the pesticide. The observed decrease in the activity of G6PD (49%)during present study may result in the increased fragility of RBC membranes thereby affecting RBC count. Dermal exposure to pesticides (acephate, methamidophos and nicotine) modifies antioxidant enzymes in tissues of rats[19].

Effect of acephate administration on the cholesterol: phospholipid ratio and the level of lipid peroxidation in rat erythrocyte membrane has been presented in Table 1. An increase was observed in the level of lipid peroxidation of the erythrocyte membranes in acephate treated rats. Increased cholesterol/phospholipid ratios were observed in the erythrocyte membranes of the experimental animals which may result in changes in the membrane bound enzymes (e.g. acetylcholinesterase), permeability of the membrane and shape of RBCs. *In vitro* treatment of human erythrocytes with organophosphorus insecticides-phosphamidon and malathion, resulted in changes in the lipid profile and raised lipid peroxidation in erythrocyte membrane[20].

The observed biochemical changes also resulted in significant morphological changes in the erythrocytes of acephate treated rats (Fig. 1). The prominent changes were the distortions in normal discocyte shape, appearance of central and peripheral protuberances and formation of acanthocytes. Changes in membrane lipid composition lead to morphological

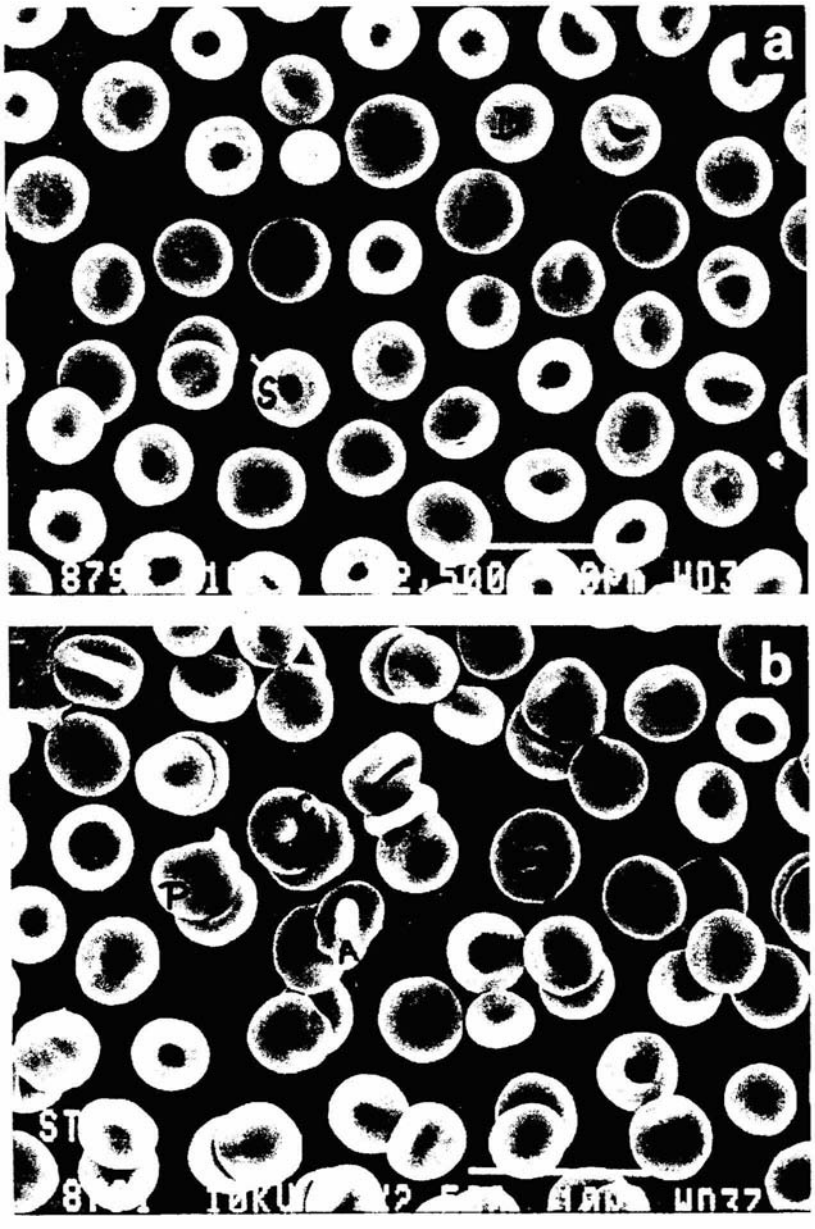

Fig. 1—(a) Erythrocytes from control rat showing numerous biconcave discocytes (D) and a few cup shaped stomatocytes (S) (b) Erythrocytes from acephate treated rat showing central (C) and peripheral (P) protuberances and erythrocyte of atypical shape (A).

changes in blood cells in response to various chemical treatments[21,22]. Thus, the transformations of normal erythrocytes into crenated (spiculed) red cells observed during the present investigation may be at least partly due to disturbed lipid microenvironment of the membrane.

Based on the results from the present study, it is clear that acephate administration results in increased oxidative stress leading to membrane damage.

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References

1 Mahajna M, Quistad G B & Casida J E, Acephate insecticide toxicity: safety conferred by inhibition of the bioactivating carboxyamidase by the metabolite methamidophos, *Chem Res Toxicol*, 10 (1997) 64.

2 Spassova D P & Singh A K, QSAR for acetylcholinesterase inhibition and toxicity of two classes of phosphoramidothioates, *SAR QSAR Environ Res*, 11 (2001) 453.

3 Dowla H A, Panemangalore M & Byers M E, Comparative inhibition of enzymes of human erythrocytes and plasma *in vitro* by agricultural chemicals, *Arch Environ Contam Toxicol*, 31 (1996) 14.

4 Farag A T, Eweidah M H & El-Okazy A M, Reproductive toxicology of acephate in male mice, *Reprod Toxicol*, 14 (2000) 457.
5 Poovala V S, Kanji V K, Tachikawa H & Salahudeen A K, Role of oxidant stress and antioxidant protection in acephate-induced renal tubular cytotoxicity, *Toxicol Sci,* 46 (1998) 403.
6 Lohr G W & Waller H D, Glucose-6-phosphate dehydrogenase, in *Methods of enzymatic analysis*, edited by H U Bergmeyer (Academic Press, New York) 1963, 744.
7 Ellman G L, Courtney K D, Andress V & Featherstone R M, A new and rapid colorimetric determination of acetylcholinesterase activity, *Biochem Pharmacol*, 7 (1961) 88.
8 Habig W H, Pabst M J & Jakoby W B, Glutathione-S-transferase—The first enzymatic step in mercapturic acid formation, *J Biol Chem*, 249(1974) 7139.
9 Horn H D, Glutathione reductase. in *Methods of enzymatic analysis*, edited by H U Bergmeyer (Academic Press, New York) 1971, 875.
10 Beutler E D & Kelly B M, Estimation of reduced glutathione, *J Lab Clin Med*, 61 (1963) 882.
11 Wills E D, Mechanisms of lipid peroxide formation in animal tissues, *Biochem J*, 99 (1996) 667.
12 Lowry O H, Rosebrough N J, Farr A L & Randall R J, Protein measurement with the folin phenol reagent, *J Biol Chem*, 193 (1951) 265.
13 Folch J, Lees M & Stanley G H S, Simple method for the isolation and purification of total lipid from animal tissues, *J Biol Chem*, 226 (1957) 497.
14 Marinetti G V, Chromatographic separation, identification and analysis of phospholipids, *J Lipid Res*, 3 (1962) 1.
15 Sperry W M & Brand F C, The colorimetric determination of cholesterol, *J Biol Chem*, 150 (1943) 315.
16 Timofeeva O A & Gordon C J, Changes in EEG power spectra and behavioral states in rats exposed to the acetylcholinesterase inhibitor chlorpyrifos and muscarinic agonist oxotremorine, *Brain Res*, 893 (2001) 165 .
17 Storm J E, Rozman K K & Doull J, Occupational exposure limits for 30 organophosphate pesticides based on inhibition of red blood cell acetylcholinesterase, *Toxicology*, 150 (2000) 1.
18 Patel P B & Charkrabarti C H, Changes in the activity of some hepatic enzymes during organophosphorus insecticide-acephate (orthene) treatment in albino rats, *Indian J Physiol Pharmacol* , 26 (1982) 311.
19 Panemangalore M & Bebe F N, Dermal exposure to pesticides modifies antioxidant enzymes in tissues of rats, *J Environ Sci Health B* , 35 (2000) 399.
20 Datta C, Gupta (Dasgupta) J & Sengupta D, Interaction of organophosphorus insecticidesphosphamidon & malathion on lipid profile & acetylcholinesterase activity in human erythrocyte membrane, *Indian J Med Res*, 100 (1994) 87.
21 Brecher G & Bessis M, Present status of spiculed red blood cells and their relationship of the discocyte-echinocyte transformation: a critical review, *Blood* ,40 (1972) 333.
22 Sherman I W, Biochemistry of plasmodium (malarial parasites), *Microbiol Rev*, 43 (1979) 453.