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Original article

Induced alteration of rat erythrocyte membrane with effect of pyrethroid based compounds

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ABSTRACT

The effects of tetramethrin and prallethrin exposure on plasma total proteins, free amino acids, albumins, urea, urea nitrogen, uric acid, creatinine were tested. Serum SGOT, SGPT and lipid profile, antioxidants super oxide dismutase (SOD), catalase, GSH, G-Px, phospholipids, cholesterol, C/P ratio in membranes of erythrocyte and membrane fluidity were analyzed. The reason of the study were analyzed to examine the possessions of mosquito repellent pyrethroid (MRP) based compounds tetramethrin and prallethrin exposure on plasma profile, antioxidant status of erythrocyte membrane, membrane fluidity in male *Wistar* rats. We tested chronically for three months exposure every day (continuously for 8–10 h per day by inhalation) of tetramethrin and prallethrin markedly available (MRP) repellents treated on male *Wistar* rats. Our results confirmed that tetrarmethrin and prallethrin treatment effect of plasma profile alterations, and lipid homeostasis mechanism in Red Blood cells (RBCs). Tetramethrin and prallethrin treatment significantly increased in erythrocyte membrane phospholipids and decreased levels of cholesterol with no change of protein content, increased C/P ration levels. Inhalation of tetramethrin and prallethrin stimulate plasma biophysical and biochemical modify SGOT, SGPT, erythrocyte membrane cholesterol and phospholipid levels, individual phospholipids and membrane fluidity of exposure rats compared to controls.

1. Introduction

Household pest and agricultural controls are insecticides and synthetic pyrethroids are progressively used more and control the insecticides. It is broadly using other countries and India due to their possible activity on insects, and defense beside mosquitoes and additional insects for various household and farming reason (Narendra et al., 2007; Narendra et al., 2008; Madhubabu and Yenugu, 2012; Mosquera Ortega et al., 2018; Mortuza et al., 2018; Na et al., 2018). Insecticide pyrethroids are used worldwide nearly half of the population and report 25% of market in the manufacturing countries in 90′s are used insecticide pests and their require in present usage countries (Casida and Quistad, 1998), as insects and other mosquito prevalence are more in large parts of the world in endemic condition. Pyrethroids are used continuously concern increase in children and infants might be effect neurological risks are increase use of insecticide in world class (Madhubabu and Yenugu, 2012).

At first, the pyrethroids are less toxic in animals and extra poisonous to insects (Shaw and Chadwick, 1998). A report clearly says that toxic effects and neurotoxicity in various ranging on convulsions, total body tremors and death now induced pyrethroid insecticide (He et al., 1989; Soderlund et al., 2002). There is few published data in pyrethroid insecticides are recorded on the effects based on prallethrin and tetramethrin on mammals, and

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the toxicity gradually coming to the data and their related into light. 12.5–25% of fatality rate is reported in India with effect of pyrethroids (Pankaj and Prahlad, 2004). Poisonoing of Tetramethrin/prallethrin and its sprays of insecticidal, mosquito repellents and easy accessibility etc., and frequently poisoning pyrethroid reports are evident in India (Mishra and Singh, 2003; Ganga and Rajarajeshwari, 2001). Though, in open scientific research available and there is no relevant data on with effects of chronic connected to pyrethroid toxicity in mammals (Pankaj and Prahlad, 2004; Kolaczinski and Curtis, 2004). As pyrethroids are regularly and/or frequently used repellents of mosquito coils or mats gardening/household/ sprays in agricultural continuously revealing to public and inhalation particular chemical compounds for extended period, their expected chronic use stimulate and worry among public, which produced the foundation for the plan of the work, and reason of the work is to identify alter plasma biochemical profile and erythrocyte membrane alterations of *Wistar* rats depiction to usual use of tetramethrin, prallethrin, and to identify with the function and condition of antioxidant and interactions in inhaled rats of prallethrin and tetramethrin.

2. Study methodology

2.1. Experimental design and procuring animals

60 days old twenty four albino *Wistar* strain of male rats, body weight ranging from l20 to 140 grms, were taken from NCLAS, Hyderabad, Telangana, India and quarters in cage separately AC room (25 + 1 °C) during 7.00 a.m to 7.00 p.m. Trial animals divided into three groups in each one 8 rats. Using rats were both repellents mosquito spray/mats/liquid vaporiser from available in local market available. Sprays are containing of (w/w) tetramethrin (Group II) 0.55%, and the liquid vaporiser contain (w/w) 98.4% appropriate ingredients as signify by the company 1.6% *d-trans* prallethrin (Group III). Pyrethroid insecticide is release both by flaming the liquid vaporizer/spraying or insertion the mat in the commercially presented devices of electric. Present studies were: I Group controls; II Group Exposed tetramethrin rats; III Group exposed prallethrin rats (each group 8 rats). All the rats were recorded daily weight and intake of food and water was change on alternate days. Finish investigational phase, in every group of rats were fasted during the night and next using of cervical displacement rats be sacrificed. Collected tissues and without delay for additional study for processed. The animal study, we are followed all procedures of including the whole surgical, feeding, and raising process were based on approved methods by the committee of ethical clearance, treatment and approved study procedure was obtain subsequent guiding principle. Current study was also accepted by institutional ethical committee-King Saud University, Riyadh, Saudi Arabia.

2.2. Blood collection and sample preparation

Total proteins estimation in plasma (Reinhold, 1953), Wootton method by albumin (1974), urea by Natelson (1957) urea nitrogen and uric acid Uricase/POD method, creatinine by Varley (1988) free amino acid by Moore and Stein (1948) plasma transaminases, glutamate oxalo acetate trasminase (SGOT) and glutamate pyruvate trasminase (SGPT) by Reitman and Frankel (1957) methods. Antioxidants superoxide dismutase (SOD) by Mishra and Fridovich (1972) and Concetti et al. (1976) Catalase (CAT) and red cell reduced glutathione (GSH) by Habig et al. (1974) glutathione peroxidase (G-Px) by Paglia and Valentine (1967) Hexokinase by King (1965). Na^+-K^+ATPase by Ismail and Edelman (1985) Glycosylated hemoglobin by Eross et al. (1984) Hexose by Niebes (1972). Hexosamine by Wagner (1979). Sialic acid by Winzler (1955) estimated proteins were using Lowry et al. method in erythrocytes (1951) by Zlatkis method by cholesterol were estimated (1953) phospholipids by Connerty et al. (1961) and used Skipski et al. method individual phospholipid compositions were tested (1964) fluidity of membrane by Shinitzky & Barenholz (1978) and Folch et al. (1957).

A sample blood of 5 ml is taken from the rat models by heart puncture and every 5 ml composed in tubes enclose heparin. On centrifuged for 15 min at 1273 g (3000 rpm) erythrocytes are separated from one blood sample. And with the help of physiological saline, the erythrocytes washed for the three times and the buffy coat was removed. Except the catalase assay samples aliquots of erythrocytes are set at −20 °C erythrocyte membrane parameters study is done with 5 mi of other blood sample. These samples should in ice case to the laboratory and within 2 h it has be processed.

2.3. Erythrocytes isolation

By the method of Beutler (1975) the erythrocytes are isolated. To remove the lymphocytes the filtrates are collected by passing the anticoagulated blood through the cellulose column. The saline is used to dilute the filtrate and the centrifugation is done to the collected erythrocytes for 10 min at 1000 rpm. Till the erythrocytes are obtained for the study the washing step/process is repeated.

2.4. Preparation of membrane erythrocytes

Dodge et al Method (1963) is applied in preparing erythrocyte membrane and spun at 15,000 × g among 5 mM phosphate buffer (pH 8.0) the cells are lysed for 30 min after erythrocyte suspension is washed with the help of saline buffer with phosphate (pH 7.2) with the help of the equivalent buffer the later tread is repetitive to supernatant carefully and for obtain haemoglobin-free ghosts for further study.

2.5. Quantity of fluorescence

The extraction of lipids and measurement of fluorescence in erythrocyte membrane is carried on spectrofluoro meter. By use emission 430 nm and excitation at 360 nm we obtain stable condition anisotropy (r) fluorescence dimensions intended for pyrene, DPH. According to Shinitzky and Barenholz (1978) the level of anisotropy (r) fluorescence equation is:

$$r = (I|| - I\_g)$$

$$(I|| + 2I\_G)$$

With the polarization flat similar are at right angles to the stimulating ray, the intensities measured. Finally in the assay the concentration of protein was 0.4 mg/ml whereas the absorption of probe was 10^{-6} M. Folch et al. (1957) the measurements of fluorescence are carry out on extracts the lipid and are standardize to similar substance of protein (0.4 mg/ml). At Tris 10 mM pH 7.4 the samples are suspended at 25 °C the measurements were performed.

3. Results

Information obtainable in Table 1 suggests to activities of antioxidants increase significantly and antioxidant enzymes like SOD both pyrethroid exposed rat's tetramethrin and prallethrin, catalase levels were significant increase in prallethrin exposed rats but decreased in tetramethrin rats surprisingly when compared to

Table 1
Effect of antioxidant enzymes inhalation of pyrethroid insecticide tetramethrin and prallethrin rats.

Parameter	Groups		
	Controls	Tetramethrin exposed rats	Prallethrin exposed rats
Superoxide Dismutase (SOD) (Units/min/mg Hb)	5.8 ± 0.62[a]	7.5 ± 0.76[b]	7.3. ± 0.56[b]
Catalase (CAT) (IU/10^4/gm Hb)	8.5 ± 0.44[a]	7.7 ± 0.82[a]	12.8 ± 0.66[b]
Red Cell Reduced glutathione (GSH) (μ moles/gm Hb)	3.4 ± 0.18[a]	4.3 ± 0.08[b]	4.4 ± 0.08[b]
Glutathione peroxidase (G-Px) (IU/gm Hb)	16.8 ± 1.04[a]	25.5 ± 1.25[b]	24.8 ± 1.22[b]

Data report as Mean ± SEM, ($P \leq 0.05$) from each added according to DMR, n = 8.

Table 2
Result of mosquito repellents on the activities of serum enzymes, Na^+-K^+- ATPase activities and glycolated enzymes of erythrocyte rats.

Parameter	Groups		
	Controls	Tetramethrin exposed rats	Prallethrin exposed rats
Glutamate Oxalo acetate Transaminase (GOT) (IU/L)	52.5 ± 2.62[a]	56.4 ± 2.28[b]	58.8 ± 2.44[b]
Glutamate Pyruvate Trasminase) (GPT) (IU/L)	25.9 ± 1.66[a]	23.2 ± 1.54[a]	24.6 ± 1.38[a]
Hexokinase (IU/gm Hb)	0.95 ± 0.01[a]	0.98 ± 0.28[a]	0.94 ± 0.34[a]
Na^+-K^+-ATPase (μg pi liberated/ min/mg/protein)	1.34 ± 0.03[a]	1.42 ± 0.05[b*]	1.40 ± 0.04[b*]

Data report as Mean ± SEM, ($P \leq 0.05$) from each added according to DMR, n = 8. * ($P \leq 0.01$).

Table 3
Pyrethroid cause on glycosylated hemoglobin, glycoproteins of plasma in rats.

Parameter	Groups		
	Controls	Tetramethrin exposed rats	Prallethrin exposed rats
Glycosylated hemoglobin (Hb A_1C %)	2.4 ± 0.22[a]	3.5 ± 0.10[b*]	3.6 ± 0.14[b*]
Hexose (mg/dl)	124 ± 0.60[a]	214 ± 0.84[b]	218 ± 0.65[b]
Hexosamine (mg/dl)	60 ± 0.94[a]	115 ± 0.74[b]	116 ± 0.88[b]
Sialic acid (mg/dl)	82.4 ± 0.64[a]	122 ± 0.52[b]	124 ± 0.64[b]

Data report as Mean ± SEM, ($P \leq 0.05$) from each added according to DMR, n = 8. * ($P \leq 0.01$).

control rats. Levels of Reduced Glutsthione (GSH) are increased in both the experimental rats (tetramethrin and prallethrin) with controls and Glutathione Peroxidase (GPx) is increased in erythrocytes of experimental subjects to pyrethroids, II and III groups while balance to I group controls. Information presented in Table 2 suggests that with an increase glutamate oxalo acetate trasminase (SGOT) serum levels significantly, glutamate pyruvate trasminase (SGPT) levels were decreased significantly in prallethrin exposed rats. erythrocyte Na^+-K^+-ATPase activities increased significantly the II, III groups investigational rats while balance to I group Control rats, erythrocyte glycolated enzymes and no change of hexokinase in both groups (group II and group III). Table 3 reported plasma glycosylated hemoglobin Hb A_1C is a good and reliable indicator of diabetes increased significantly pyrethroid exposed rats be experiential in group III & II with evaluate I group controls, plasma hexose, hexosamine, sialic acid are components of glycoproteins increase significantly in experimental groups (II & III) with controls group I, The glycoproteins formation are irregular stage central in the kidney diseases and pathogenesis of liver in experimental issue (II, III groups) with match up to I group. Fig. 1a and b shows that there was significant increase plasma total proteins, decreased levels of albumins, increased significantly urea and urea nitrogen concentration in pyrethroid groups (tetramethrin and prallethrin) with compared to controls. Fig. 2a and b shows observed free amino acids, uric acid and creatinine levels were significant increase, Fig. 3a and b erythrocyte cell membrane cholesterol levels were decrease and phospholipids were increased with C/P ratio were significantly increases in experimental II, III groups with I group. Fig. 4a and b confirmed erythrocyte membrane fluidity was significant decreased with no change of red blood cell protein content. Fig. 5a–c reported individual phospholipids compositions decrease SM, PS, PE, PI and lyso PA and PA but PC was increased in both the subjects (tetramethrin and prallethrin) when compared to controls.

4. Discussion

These compounds chiefly affect selective membrane transport processes and cellular metabolism causing toxicity in targeted insects and non-target mammalians. Though acute pyrethroid toxicity is common and well known, limited information is available on chronic toxicity of these pyrethroids in rats and humans and experimental animals (Na et al., 2018). Actual effects/events related to pyrethroid toxicity and/or their precise mechanisms related to chronic use of these compounds are not clear. Upon

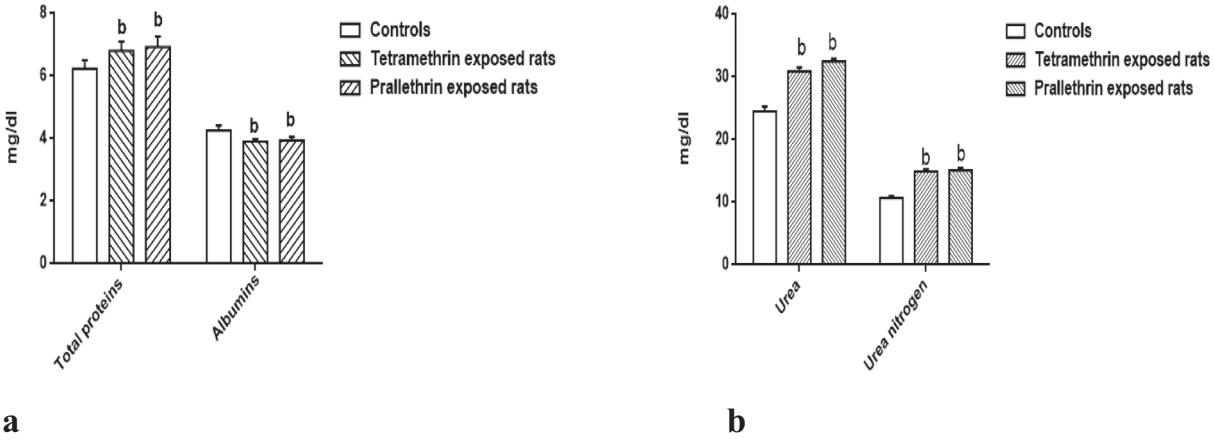

Fig. 1. a and b. Mosquito repellent pyrethroids effect on plasma total proteins, albumins, urea and urea nitrogen. Data report as Mean ± SEM, ($P \leq 0.05$) from each added according to DMR, n = 8.

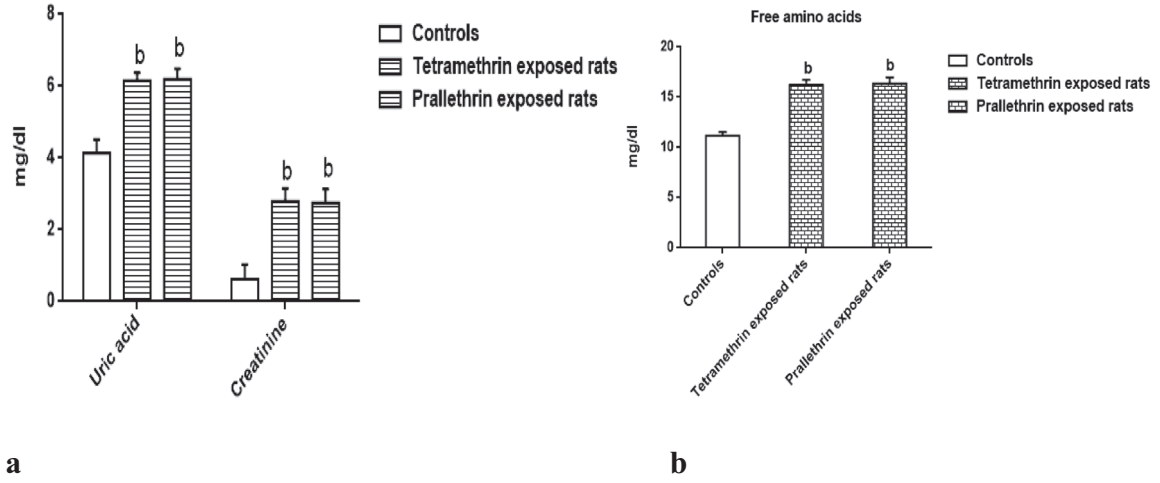

Fig. 2. a and b. Mosquito repellent pyrethroids effect on plasma uric acid, creatinine and free amino acids. Data report as Mean ± SEM, ($P \leq 0.05$) from each added according to DMR, n = 8.

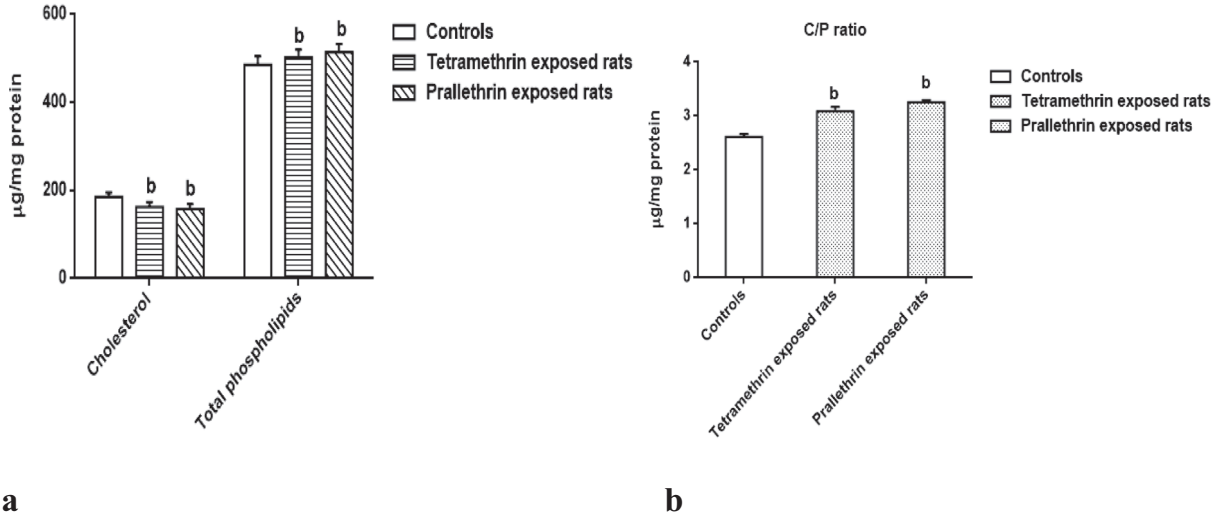

Fig. 3. a and b. Pyrethroid induce alterations of membrane phospholipids, cholesterol, C/P ratio. Data report as Mean ± SEM, ($P \leq 0.05$) from each added according to DMR, n = 8.

prolonged exposure due to inhalation tetramethrin and prallethrin are released as active ingredients. When these repellent-containing formulations are used, they enter into circulation continuously and are dispersed to every tissue, accumulate the several tissues causing effective damage. Pyrethroids are interacts with lipophilic biomembranes and well-known targets (Sinha et al., 2004; Theeraphap et al, 2003 Narendra et al., 2007). Enhanced oxidative stress, increased antioxidant status and involvement of trasaminases and lipid profile have been implicated in chronic toxicity of pyrethroids. Membrane interactions with pyrethroids are responsible for several characteristic effects. Therefore the present study is designed to investigate erythrocyte membrane proteins and cholesterol levels were increased significantly with decreased phospholipids and increased C/P ration (Cholesterol/Phospholipid), erythrocyte membrane fluidity increased the biochemical events and mechanisms associated with the membranes of RBCs and other relevant plasma, red cell parameters in rat exposed to tetramethrin and prallethrin inhalation.

Pyrethroid based tetramethrin and prallethrin are enter easily into rats exposed continuously 8–10 h a day; these compounds enter into circulation with maximal accumulation in all biomembranes and in specific adipose tissues, nerve, blood, additional tissues with an increased uptake due to their lipophilic nature (Narendra et al., 2008, Ganga and Rajarajeshwari, 2001). In the work erythrocyte membrane antioxidants increase significant, superoxide dismutase (SOD), catalase levels were in prallethrin exposed rats increased significantly no alter in tetramethrin exposed group match up to with controls. Previous report (Narendra et al., 2008) There was an boost superoxide dismutase (SOD), catalase (CAT) enzyme activity of antioxidants in group III exposed prallethrin rats and no change in tetramethrin exposed rats, increase significantly glutathione (GSH) in erythrocytes and increased concentration of glutathione peroxidase (GPx) in erythrocytes of II, III groups exposed rats with I group rats.

In metabolic oxidation hydrogen peroxide (H_2O_2), Superoxide radicals (O_2^{0-}) and hydroxyl radicals (OH) are involved. CAT, SOD, and GPx antioxidant enzymes injure at the cell stage by oxidation. Xenobiotics are producing oxidative stress; stimulate turbulence in antioxidant enzyme coordination. Some information shows pyrethroid insecticides cypermethrin, fenvalerate treatment of mice and rats induced improve action of superoxide dismutase and catalase, production of free radical (Gabbianelli et al., 2002, Franco

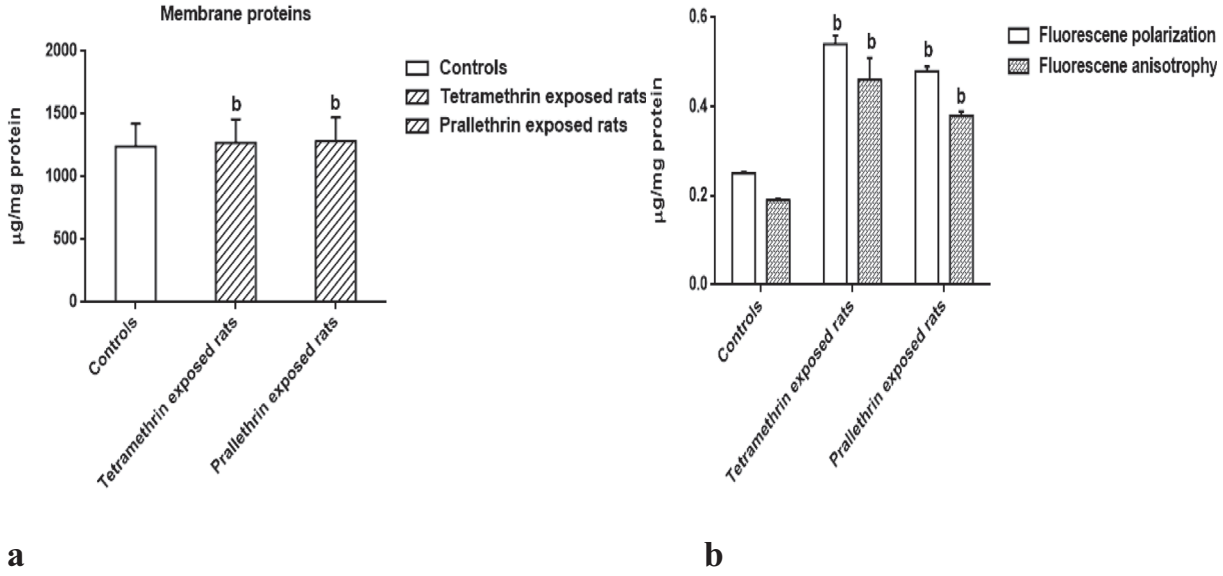

Fig. 4. a and b. Pyrethroid induced changes on erythrocyte membrane proteins, membrane fluidity. Data report as Mean ± SEM, ($P \leq 0.05$) from each added according to DMR, n = 8.

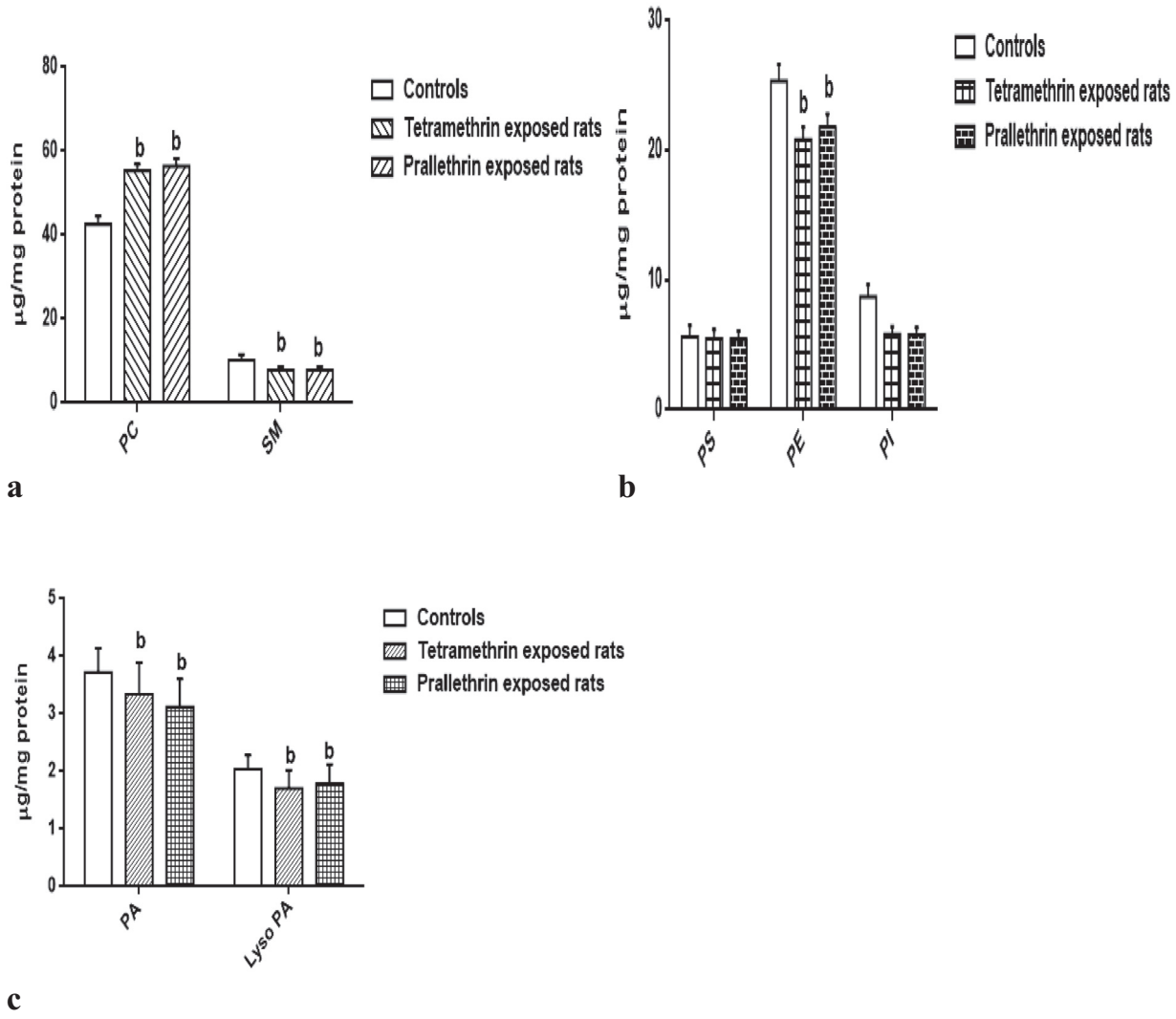

Fig. 5. a, b and c. Altered Phospholipid composition of erythrocyte membrane effect of pyrethroid exposed rats. Data report as Mean ± SEM, ($P \leq 0.05$) from each added according to DMR, n = 8.

et al., 2006). Pyrethroids contain probable to mode of actions are two: it might oxidative induce stress; it is a membrane structure disturb, accumulate in membrane cell hydrophobic compound (Gabbianelli et al., 2002). The cytosol antioxidant enzymes activities are two, CAT, SOD, were changed in I table, and their esters of pyrethroids, are decay to cyanides and aldehydes from cyanohydrins. Thiocyanate and CO_2 are converted from Cyanide ions, oxidative stress in pyrethroid toxicity are produce by aldehydes and additional lipophilic conjugates.

Tetramethrin and prallethrin produced oxidative stress which increased serum urea and creatinine in both groups in comparison with control our results are stand earlier studies. Present results alterations of individual phospholipids composition decrease SM, PS, PE, PI and lyso PA and PA but PC was increased in both the subjects (tetramethrin and prallethrin) when compared to controls. These results supported the Cengiz et al. (2016) the composition of fatty acid, subclasses of phospholipid (phosphatidylserine (PS), phosphatidylethanolamine (PE), phosphatidylchlonine (PC), phosphatidylinositole (PI), Oreochromis niloticus (*Perciformes: Cichlidae*) gill tissue, result of deltamethrin. Although the observed significant modify in plasma constituents and erythrocyte membrane, membrane fluidity show fall and smaller near to usual range, clinically this modification cannot be overlooked completely. In sight of the disorder in metabolism in experimental rats showing to pyrethroids is essential seeing as extended expression incidence of little disorder.

5. Conclusions

Increase in erythrocyte membrane antioxidant enzymes such as SOD, Catalase in prallethrin exposed rats with no change in tetramethrin rats, GSH and GPx activity were increased in experimental groups and it shows biochemical adaptive changes in membranes of erythrocyte. Increased production of plasma SGOT and decrease SGPT levels were observed. Further Na^+-K^+-ATPases increases in both groups and long-standing apply of prallethrin, tetramethrin observed toxic effects on rat membranes of erythrocyte.

Statistical examination

Data report as Mean ± SEM, ($P \leq 0.05$) from each added according to DMR, n = 8.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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