DISCUSSION

Observed increase in plasma glucose levels in experimental subjects (Group II allethrin users, Group III prallethrin users) in the present study when compared to normals (controls) who do not use any mosquito repellents suggest an interference of the pyrethroids-allethrin and prallethrin. In general, maintenance of stable levels of blood glucose is a beautiful complex process and is one of the finely regulated homeostatic mechanisms in which various tissues, hormones and enzymes and other factors play a role\(^1\)-\(^3\). Besides the intracellular metabolic adjustments and modulation of the sensitivity of insulin receptor action are responsible for glucose homeostasis. Blood glucose concentration is regulated by the net result of two process glycogenesis and glycogenolysis in liver\(^4\). In addition to this, the blood glucose levels are regulated by hepatic and renal gluconeogenic production of the glucose on one hand and the peripheral glucose utilization on the other\(^5\).

Although the observed increase in plasma glucose in the present study is significant, the glucose level still falls with in the normal range. This reflects a biochemical adaptive role played by allethrin/prallethrin induced biochemical changes which needs further in depth study. The observed hike in plasma glucose may be due to the involvement of one or many of the above mentioned factors\(^6,7\). Besides, increased nitric oxide levels in plasma and red cell lysate and decreased membrane lipid peroxidation suggest a role played by them in regulation of blood glucose to keep glucose concentration in normal range, as NO signaling has been reported to be playing a new role in regulating glucose metabolism in insulin insensitive tissues where it could function in parallel to insulin\(^6,8\). Nitric oxide (NO) appears to protect membrane
integration and function by intercepting lipid derived radicals and thereby antagonized oxidative and photooxidative stress which affect glucose homeostasis. Hence an indirect role of nitric oxide may contribute to the observed change in blood glucose in the present study. Though no data on blood glucose concentration and tissue glucose utilization is available from humans or animals exposed to allethrin and prallethrin, previous studies revealed that an acute administration of Cismethrin (type I) and Deltamethrin (type II) cause an increased rate of blood flow and glucose transport into tissues and also increased glucose utilization by brain and some other tissues resulting in hypoglycemia in rats followed by some toxic symptoms such as tremors and convulsions etc. On the contrary, the data obtained from the present study showed an elevated plasma glucose concentration in humans exposed to chronic inhalation of allethrin or prallethrin suggesting the operation of some mechanism(s) to keep that elevated glucose concentrations to meet the demand of increased utilization of glucose by tissues and also to prevent hypoglycemia which may arise as a result of increased glucose uptake by tissues.

**Plasma profile Vs Pyrethroids**

A significant decrease in plasma proteins and albumins followed by marked increase in plasma free amino acid levels in the present study points to the degradation of proteins in allethrin/prallethrin users when compared to normals (controls). Probably, enhanced activities of one or many proteases/peptidases might have contributed for the same. The transfer of iron and glycolipid moieties from plasma to blood cells and other tissues might have resulted in the observed decrease of these parameters.
Markedly increased concentration of VLDL followed by hike in triglyceride levels with no significant change in cholesterol and HDL suggests some cardiovascular risk in group II and group III when compared to normals that do not use pyrethroid mosquito repellents. However, a decrease in LDL and increased NO levels in plasma and erythrocyte intracellular NO levels suggest some protective measure\textsuperscript{12, 13}. The magic role played by NO is suspected here in lowering plasma LDL levels and in decreasing lipid peroxidative process in erythrocyte membrane. However it is important to note that an enhanced of plasma lipid peroxidation, VLDL and triglycerides indicate cardiovascular risk as well as tissue damage in chronic users of allethrin and prallethrin.

Observed changes in plasma cholesterol, other lipids (triglycerides), and lipoprotein patterns (VLDL and HDL) and plasma SGOT and SGPT and lipid peroxidation in the present study in experimental subjects indicated some coronary and cardiovascular risk, and possibly tissue and liver damage. In addition, the operation of certain rapid protective mechanisms to counteract the above mentioned risk and damage as evidenced from enhanced generation of nitric oxide, reduced LDL and decreased membrane lipid peroxidation in experimental subjects when compared to controls.

**Influence of pyrethroids on membrane compartments**

Decreased red cell membrane phospholipid and cholesterol levels indicated lipid-depleted (phospholipids and cholesterol) cells in group II and group III (experimentals) when compared to normals. A decreased membrane phospholipids, and increased nitric oxide generation causing interception of lipid derived radicals may contribute for the observed decrease in membrane lipid peroxidation in the present study. Besides a direct
free radical scavenging role played by allethrin and prallethrin in hydrophobic region of membrane might also have played a role in the observed decrease in membrane lipid peroxidation.

Increased lipid peroxidation in plasma is an important observation in the present study. Phospholipid and cholesterol depleted cells with altered lipid packing with altered changes in physico-chemical properties, and the interaction of free radicals might have caused increased haemolysis in red cells that are exposed to various concentrations of NaCl ranging from 0.1% to 0.8% suggesting an altered osmotic behaviour of red cells in group II and group III subjects when compared to normals. However all the red cells exhibited normal behaviour with no haemolysis at 0.9% NaCl which is an isotonic concentration.

A prominent decrease in membrane cholesterol followed by decreased phospholipid content with no significant change in membrane protein content was observed in the present study in experimentals when compared to normals. Ultimately there was no consequent change in Chl : PL ratio in experimentals suggested the unchanged membrane fluidity. No alteration in membrane fluidity was observed in the present investigation in spite of cholesterol and phospholipid depletion in the biomembranes. Due to lipophilic nature, pyrethroids are miscible with hydrophobic moiety and probably by forming pyrethroid-phospholipid mixture patches (aggregates/domains) and thereby substituting the depleted lipid content the basic structure might have been (restored) and the physico-chemical properties of the used pyrethroids might have helped in restoration of the physical state of the bilayer without affecting fluidity of the biomembranes. Allethrin/prallethrin bound to bilayers doesn’t
apparently affect the hydrocarbon or polar head group domains of the membrane. Earlier work also suggested that allethrin may lie in an extended orientation in the bilayer with the carbonyl group of the cyclopentanane ring at the lipid water interface. However, this arrangement may be a temporary one, as and when these domains come in contact with degradative enzymes, either membrane damage should occur in that region or the allethrin or prallethrin (pyrethroid) moiety in the affected region gets replaced by new phospholipid and/or cholesterol species. An evidence for the proposed mechanism comes from the DPH and its polar derivative TMA-DPH fluorescence polarization studies of Moya-Quiles et.al\textsuperscript{14} who observed an altered bilayer order without altering the fluid phase and also the incorporation of pyrethroid in model and native membranes with no disordering effect in the bilayer. An increase in the haemolysis was observed in experimentals when compared to normals in the present study when red cells were exposed to different concentrations of NaCl. This suggested that the haemolysis was a secondary effect which arises as a result of changes in the bilayer structure and/or protein conformation induced by the interaction of membrane with the pyrethroids. Though the lipid packing of membranes is affected with low values of PS (phosphotidyl serine), membrane fluidity remains unaltered. Disturbance in the viscoelastic properties and physico-chemical properties caused by some specific or nonspecific actions of pyrethroids on membrane components may account for the increased haemolysis and osmotic fragility observed in present study (Fig. 6 & 7). In general, the release of haemoglobin is facilitated by the incorporation of pyrethroid/pesticides into human erythrocytes. Besides a significant decrease in phosphotidyl serine was observed in the present study with no change in other
phospholipids classes clearly suggesting that PS is an important and sensitive phospholipid species that is influenced by these two type I pyrethroids (allethrin & prallethrin). Phosphotidyl serine is an important membrane phospholipid, several regulatory, structural and other proteins, membrane skeletal proteins such as spectrin are localized within the membrane through their interaction with phosphotidyl serine (PS). On the other hand, disruption of lipid asymmetry leading to exposure of PS on the outer surface of the plasma membrane creates a procoagulate surface on platelets and serves as a trigger for macrophage recognition of apoptic cells\textsuperscript{15}. All the above mentioned changes indicate a pronounced effect of pyrethroids on lipid packing order in cellular membranes.

**Nitric oxide Vs Pyrethroids**

An increased nitric oxide levels in plasma and erythrocyte compartments of experimentals are evident from measurements of nitrite and nitrate concentrations in plasma and erythrocytes. Nitric oxide is endogenously produced chiefly by endothelial cell Nitric oxidesynthase (NOS) and is released into blood stream where it is quickly scavenged by Hb in erythrocyte or oxidized to nitrite and further oxidized to nitrate\textsuperscript{16}. In addition a significant of nitric oxide (NO) production occurs in other tissues as different isoforms of NOS exist. Nitric oxide plays a principle role in basal blood flow regulation and vascular homeostasis. Besides many other physiological processes are mediated and regulated by a direct or indirect actions/of NO and/or NO derived species. NO is capable of diffusion to great distances at physiological oxygen tension in tissue maintaining a teleological balance. This has been observed in erythrocytic red cell mass and plasma mass\textsuperscript{17}. During hyphoxic or metabolic stress intravascular NO stabilizing
species such as SNO, albumin, nitrite, nitrate, nitrosamines, iron, nitrosoles and recently identified nitrated lipids may participate in nitric oxide (NO) homeostasis\textsuperscript{17}. Nitric oxide would readily diffuse across the cell membrane with its frequent interactions with other molecules and ions and thereby causing various physiological changes and altered functions of several molecules including enzymes. Simultaneously, nitrite which is formed from NO, appears to cross the erythrocyte membrane by more than one mechanism such as simple diffusion and/or protein dependent route and or co-transport mechanism(s). The ability of ascorbate to reduce nitrite to nitric oxide (NO) is well established\textsuperscript{16, 18, 19} and this has prompted speculation and recycling of nitrite to nitric oxide (NO) by ascorbate in brain could be of physiological importance \textsuperscript{20}. Haemoglobin very actively interacts with nitric oxide and nitrite. Nitric oxide is known to enhance the affinity of haemoglobin to oxygen. Unlike many radical species, nitric oxide displays highly relative activity with long biological half life with a capability of transmembrane diffusion. The role of NO as endogenous biological oxidant is well established \textsuperscript{21, 22}. The effectiveness of nitric oxide in preventing peroxidation of lipid by serving as potent scavenger of alkoxyl (LO\textsuperscript{•} and peroxyl LOO\textsuperscript{•} radicals). Besides, NO scavenges some other radicals such as Tyr. Trp\textsuperscript{23}.

Besides nitrite, erythrocytes contain several vasodilators among which are nitros(yl)ated proteins, nitrated lipids, and adenosine triphosphosphate (ATP). Increased levels of nitrite and nitrate in plasma and erythrocyte lysate in chronic mosquito repellent inhaled volunteers in the present study suggest the important roles played by nitric oxide and its derivatives include
1) in decreased membrane lipid peroxidation.

2) conferring tolerance to erythrocyte membrane by preventing change in fluidity and other physico-chemical properties such as tonicity, osmotolerance, viscoelastic properties, against pyrethroid induced toxicity or toxic effects.

3) in the insertion of allethrin in the erythrocyte bilayer, and in formation of lipid pyrethroid aggregates which substitute depleted membrane cholesterol and phospholipid content.

4) and, in displacement of phosphotidyl serine.

Evidences also suggest that NOS interacts with anionic phospholipid vesicles but not with neutral ones. NOS was found to associate with PS or phosphotidic acid (PA) but not with phosphotidyl ethanolamine or phosphotidyl choline indicating that NOS-phospholipid binding requires an electrostatic interaction with Lys732LysLeu is essential for nNOS-PS and nNOS-CaM (Calmodulin) interactions. Studies using NOS inhibitors such as Calmodulin have shown significant protection against the Quinolphos (QP)-induced increase in the BBB permeability, suggesting possible involvement of NO in the barrier disruption. Nitrite rapidly enters erythrocytes and reacts with Hb but doesn’t exert a strong oxidant stress in human erythrocyte.
References


