Effects of electrolytes, microelements and multiple exposure of Acetamiprid on wistar rats

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ABSTRACT

Subacute toxicity study of acetamiprid was undertaken in 72 female Wistar rats randomly divided into four groups (each consisting of 18 individuals). Acetamiprid was administered orally at 0, 25, 100 and 200 mg/kg of body weight to rats of Groups 1, 2, 3 and 4 respectively. Group 1 served as control. Calcium, sodium, potassium, chloride, zinc, copper, iron and cobalt were significantly increased in acetamiprid administered groups. However, no alterations were observed in plasma phosphorus and manganese concentration in acetamiprid treated rats. The repeated oral toxicity study on acetamiprid suggested that it has toxic potential and is a high risk insecticide.

Keywords: Acetamiprid, Electrolyte, Subacute, Wistar rat.

Introduction

The frequent and continuous use of pesticide has resulted in widespread distribution in environment. These pesticides are toxic not only to insects and pests, but at different levels to animals and man. Indiscriminate use of acetamiprid (pesticide) to manage the insects by the farmers is causing pesticide entry into food chain induces biological magnification, which in turn instigating residue related toxicity to man and animal [1]. These agrochemicals, if not properly used, may pose serious hazards to human and animal health. Therefore, the present day concern is with regard to their judicious and proper use, so that they can be applied safely with proper instructions and guidance to have minimum risk to human and animal health [2].

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Materials and methods

The present study was conducted on six weeks old healthy Wistar female rats. The rats were procured, and housed in cages at Animal house of the Institute. Experimental protocol was approved by institutional animal ethics committee before starting of experiment.
Animals were allowed to acclimatize for a period of 7 days prior to experiment, and provided with standard feed and ad libitum water (purified by reverse osmosis followed by UV treatment). Acetamiprid was procured from Department of Veterinary Pharmacology and Toxicology Center of the Institute. Acetamiprid was formulated using normal saline as a vehicle. Acetamiprid suspension was administered directly in stomach by oral gavages with dose volume of 10 ml/kg. Body weights were recorded before administration of acetamiprid. The daily oral administration was continued for 28 days.

**Experimental design**

A preliminary dose range study was done to determine the dose of acetamiprid in female Wistar rats for subacute toxicity study. For subacute toxicity study 72 female Wistar rats were randomly divided into four groups (Groups 1, 2, 3 and 4) each having 18 individuals. Based on the preliminary dose range study, Group 4 which was administered with the highest dose @ 200 mg/kg body weight showed tremors and excessive salivation and hyperaesthesia, whereas Group 2 administered with the low dose i.e. @ 25 mg/kg body weight exhibited very mild salivation compared to control group which was administered to daily for 28 days. Group 3 which was administered with medium dose @ 100 mg/kg body weight showed watery salivation and tremors in the pilot study. At the end of the experiment on day 28 plasma samples were collected before final culling of rats for the estimation of electrolytes and microelements. For collection of plasma, blood was collected by retro orbital bleeding method [4]. Heparin (sodium salt) was used as an anticoagulant (10 IU/ml). Rats were kept in desiccators and anaesthetized by using absorbent cotton soaked with isoflurane.

**Estimation of plasma calcium level**

Calcium level in plasma was estimated by standard kit in spectrophotometer by Cresolphthalein complexone method following the manufacturers’ protocol. Calcium concentration was expressed in mg/dl.

**Estimation of plasma phosphorus level**

Phosphorus level in blood was measured in terms of measuring the inorganic phosphorus level. Inorganic phosphorus level in plasma was estimated by standard kit in UV spectrophotometer by UV endpoint method. Phosphorus concentration was expressed in mg/dl.

**Estimation of plasma sodium level**

Sodium level in plasma was estimated by standard kit in spectrophotometer by Trinder’s method- End point colorimetric assay. Sodium concentration was expressed in mmol/l.

**Estimation of plasma potassium level**

Potassium level in plasma was estimated by standard kit in spectrophotometer by Tetraphenyl boron method- End Point Turbidometric Assay. Plasma potassium concentration was expressed in mmol/l.

**Estimation of plasma chloride concentration**

Chloride level in plasma was estimated by standard kit in spectrophotometer by Mercurus (II) thiocyanate method. Plasma potassium concentration was expressed in mmol/l.

**Estimation of microelement levels**

The concentration of microelements (Zn, Cu, Fe, Co, Mo and Mn) in the plasma was determined by Atomic Absorption Spectrophotometer. 1 ml of plasma was digested by Tri-acid solution (nitric acid : sulfuric acid : perchloric acid ratio of 9:2:1) and the final volume was made upto 100 ml by adding distill water to the digested solution. The values obtained were expressed in ppm.

**Statistical analysis**

The data were statistically analysed using complete randomized design single factor analysis of variance by Snedecor and Cochran [5]. The mean values between treatment and control group were tested for critical difference.

**Results**

Mean values of plasma concentration of calcium, phosphorus, sodium, potassium and chloride for each experimental group are represented in **Table 1**. Mean values of calcium were significantly (P≤0.05) lower in all the acetamiprid treated rats. There was no significant difference in plasma phosphorus concentration of acetamiprid administered groups as compared to control group but a dose dependent increase in sodium, potassium and chloride were observed in the acetamiprid administered rats as compared to control rats. Mean values of plasma concentration of Zinc (Zn), Copper (Cu), Iron (Fe), Cobalt (Co), Molybdenum (Mo) and Manganese (Mn) for each experimental group are given in **Table 2**. There was significant (P≤0.05) increase in the plasma concentrations of Zn, Cu, Fe, Co and Mo in rats of Groups 2, 3 and 4 as compared to rats of control group. Interestingly the increments were clearly dose dependent. There was no significant difference in plasma concentration of Mn in any of the acetamiprid treated rats as compared to the rats of control (Group 1).
Table 1: Effect of daily administration of acetamiprid for 28 days on plasma electrolyte concentrations

<table>
<thead>
<tr>
<th>Electrolyte</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium (mg/dl)</td>
<td>12.06±0.218a</td>
<td>9.99±0.354b**</td>
<td>8.89±0.527b**</td>
<td>7.69±0.202b**</td>
</tr>
<tr>
<td>Phosphorus (mg/dl)</td>
<td>6.37±1.305</td>
<td>6.96±0.920</td>
<td>7.18±0.365</td>
<td>9.96±1.977</td>
</tr>
<tr>
<td>Sodium (mmol/l)</td>
<td>153.33±5.577c</td>
<td>208.33±12.758b**</td>
<td>230.00±8.563b**</td>
<td>315.00±8.465b**</td>
</tr>
<tr>
<td>Potassium (mmol/l)</td>
<td>6.64±0.113b</td>
<td>6.53±0.457b</td>
<td>6.79±0.293b</td>
<td>8.97±0.369b**</td>
</tr>
<tr>
<td>Chloride (mmol/l)</td>
<td>97.74±1.768c</td>
<td>102.91±2.278bc</td>
<td>107.12±1.225b**</td>
<td>116.63±1.412b**</td>
</tr>
</tbody>
</table>

Values are expressed in Mean ± S.E. Values with same superscripts in a row did not differ significantly.

"P≤0.05

Discussion

Effects of acetamiprid on pancreas and kidney may be responsible for hypocalcaemia [6]. An increase in serum calcium concentration was observed in rats treated with 31.80 mg/kg fenvalerate [7]. The increase in phosphorus level in plasma may be due to the severe dehydration [8]. Oral administration of acetamiprid suspended in normal saline solution might have caused refusal of animals to drink water leading to dehydration.
Calcium and phosphate metabolism are markedly altered during uremia and are involved in development of renal secondary hyperparathyroidism in monogastrics [9]. Although all the details are not known it seems that in course of renal failure there is an analytically imperceptible hyperphosphatemia and hypocalcaemia. Hyperphosphatemia observed in rats of all treated groups might also be due to rhabdomyolysis as seen in the histopathology of muscle in author’s another study. The hypocalcemia may be a direct biochemical effect of hyperphosphatemia or, a deficiency of active forms of vitamin D or both. The hypocalcemia stimulates parathyroid hormone release, which enhances the calcium level in the blood. The increase sodium concentration is correlated with the water loss, which is more prominent in high dose group. Due to kidney damage excretion of ammonia through urine is hampered which in turn cause uremia, leading to anoxic condition in the tissues. Decreased oxygen pressure in the tissue affects ATP production, which is necessary for energy dependent Na-K ATPase pump in cell membrane. As a result potassium comes out of the cell and sodium go inside the cell along with water leading to hydropic degeneration and subsequently there is rupture of the cells resulting in hypernatremia and hyperkalemia [6]. There were hyperchloremia in all the acetamiprid treated rats in a dose dependent manner. This may be due to dehydration and compensatory respiratory alkalosis. Most of the chloride in the body comes from table salt (sodium chloride) in the diet. Chloride is absorbed by the intestine during food digestion. Any excess chloride is passed out of the body through the urine. Chloride levels in the blood generally rise and fall along with sodium levels in the blood. The amount of chloride in the blood is indirectly regulated by the hormone aldosterone, which also regulates the amount of sodium in the blood. Elevations in chloride may be associated with diarrhea, certain kidney diseases and over activity of the parathyroid gland [6]. Zinc has a direct stabilizing effect on cellular membrane and might alter membrane fluidity and there by affects ion gating, cytoskeletal activity and membrane bound enzyme activation [10]. Elevation in plasma Zn level (Group 4) caused abnormality in superoxide dismutase activity, alteration in serum lipoprotein profile and depressed immune response [11] by impaired cell mediated immune response to allogenic tumor cell inoculation and reduced delayed type hypersensitivity reaction to DNFB [12, 13]. Similarly, the humoral immune response to SRBC was reduced, predominantly through interference with T-helper cell proliferation and function, leading to reduced B-cell and plasmacyte development [14-16]. Widespread damage of tissues and subsequently Zn level alteration has likewise been linked to immune dysfunction in human, including impaired T-cell mitogenic responses and decreased natural killer cell activity [17, 18]. Elevated level of Cu in plasma was found in acetamiprid treated rats. Absorbed Cu first is bound to circulating plasma protein like albumin and is subsequently stored in specific tissues in association with Cu binding proteins - cerebrocuperin for brain, erythrocuprein for erythrocyte and hepatoxerepin for liver [19]. These storage proteins are predominantly metallothioneins and superoxide dismutases [20]. Ceruloplasmin is a α2-macroglobulin produced by the liver, which is responsible for binding up to 95% of the Cu in plasma in most species [20]. Interleukin-1 (IL-1), released during inflammatory disorders and particularly during bacterial infection or endotoxemia, induce hepatic ceruloplasmin synthesis, leading to mobilization of Cu from storage tissues, including liver and kidneys, and induction of hypercupremia [21]. While this response may enhance the oxygen free radical related bactericidal functions of phagocytic cells, it may also increase the auto oxidative damage done to the host itself. Some authors have reported that higher serum copper level is a specific indicator of liver damage [22]. In cirrhosis cases, serum copper levels were higher in patients compared with control [23-24]. In the present study, copper level significantly increased in all the treated groups compared to control rats of group I indicating severe liver damage in female rats of all acetamiprid treated groups. Copper plays an important role in transporting Fe across membranes [25]. Most of the circulating Cu in plasma is attached to the serum glycoprotein ceruloplasmin. Ceruloplasmin has ferroxidase activity and might be required to deliver Fe into circulation. In the present study increase plasma level of Fe might be due to increase plasma level of ceruloplasmin, which directly increases Fe in the circulation by ferroxidase activity. The principle function of Co is as a component of vitamin B12 (cyanocobalamin). As vital component of biologically active vitamin B12, Co is required for metabolic function of erythropoiesis (as cofactor for purine and pyrimidine synthesis); histidine, methionine and choline metabolism (as a cofactor for transfer of methyl group) and conversion of propionic acid to succinyl CoA (as a cofactor for methylemanonyl CoA isomerase) [11]. In the present study, increase level of Co in all the groups may be due to overloading of vitamin B12 in the plasma. Increased serum vitamin
B₂ values have been noted in liver cell necrosis, presumably due to release from the damage hepatic stores [26].

Conclusion

There was significant (P≤0.05) increase in levels of plasma Mo in a dose dependent manner in acetamiprid treated rats. Molybdenum containing metalloenzyme includes aldehyde oxidase, sulphide oxidase and xanthine dehydrogenase/ oxidase. Xanthine oxidase is perhaps the most prominent biochemical role for Mo. Xanthine oxidase catalyses the conversion of purines to uric acid for excretion and is responsible in part, for generation of oxygen free radicals in tissues during inflammation or ischemia. Over activity of xanthine oxidase caused tissue damage was also evident from histopathological findings in the present study. Increased level of Mo might be due to increase level of xanthine oxidase. It may be concluded that sublethal dose of acetamiprid causes alteration in the electrolyte and micromineral balance in mammals. Indiscriminate introduction of acetamiprid into food chain through agro industry may affect electrolyte system of animal and human causing high susceptibility to various stresses and secondary infections.

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