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INFLUENCE OF PROFENOFOS ON SELECTED BIOCHEMICAL PARAMETERS IN THE LIVER OF *ALBINO RAT*

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ABSTRACT

As new applications for many organophosphate (OP) compounds are discovered, the use of OP compounds has gained importance and this trend is like to continue in the years ahead. In many developing countries, particularly in India, pesticides are being indiscriminately used at every stage to kill a variety of pests that cause harm to agricultural crops. Unfortunately these pesticides are causing lot of metabolic disturbances to the non-target organisms and human beings in particular. Profenofos, an OP insecticide is used on a variety of crops Lepidopterans and mites. It is especially used to control various white flies' effect on vegetables. It is also used extensively in household applications. Hence the objective of the present study is to examine the effect of $1/10^{th}$ of LD_{50} of Profenofos (*i.e* 39.5mg/kg body weight) was studied in the liver with reference to the important metabolites and enzymes of protein metabolism in *Albino rat* after exposure to 10, 20 and 30 days respectively. All the experimental male *Albino rats* were administered with 39.5mg/kg body weight orally with an interval of 48h. The experiment was carried out for 30 days. Animals were randomly divided into four groups. The first group served as control. Second group of animals were exposed to Profenofos for 10 days, third and fourth groups of animals were exposed for 20 days and 30 days respectively. From the results it is clear that continuous exposure to Profenofos alters important parameters of protein metabolism. Total proteins showed a decrement in Profenofos exposed groups while the rest of the parameters showed an increase and this increase was more in 30 days when compared to 20 days and 10 days administered groups. All the parameters studied in the present investigation were severely affected in Profenofos exposed *Albino rats*.

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INTRODUCTION

OP compounds have been used widely for several decades in agriculture for crop protection and pest control, thousands of these compounds have been screened and more than one hundred of them have been marketed for these purposes[1]. OP compounds are arguably one of the most common causes of insecticide poisoning worldwide. In developing countries like India, OP compounds are not only cheap but also easily available and hence these compounds are a constant source of both intentional and unintentional poisoning. Of late the use of OP compounds has gained more importance than before and this trend is likely to continue in the years ahead, because new applications for these compounds have been discovered [2]. Indiscriminate and excessive use of these pesticides is a major concern to non-target organisms, thus the use of pesticides has become a great concern worldwide [3]. The majority of OP insecticides can be grouped according to their chemical structure as a dimethoxy OP [with two $O–C_2H_5$ groups attached to the phosphorus that binds to and inhibits acetylcholinesterase] or a dimethoxy OP (with two $O–CH_3$ groups). The identity of these alkyl groups has fundamental effects on the pharmacodynamics of poisoning and treatment, determining to a large extent whether oximes effectively reactivate OP-inhibited AChE [4]. However, a few OP insecticides do not fit into these categories, including profenofos and prothiofos. Both are highly lipid soluble, moderately toxic OP insecticides. They have an S-alkyl ($S–C_3H_7$) group attached to the phosphorus, in addition to the more typical $O–C_2H_5$ group. The consequences of this structure are not clearly understood. We therefore studied the sub-lethal effect of Profenos on important protein metabolites and enzymes in the liver of *Albino rats* exposed for 30 days. Profenofos (O-(4-bromo-2-chlorophenyl) O-ethyl S-propyl phosphorothioate) is a moderately hazardous (class II) pesticide by WHO broad spectrum insecticide extensively used for the control of various caterpillars, white fly and mites on cotton and vegetable crops[5]. It is used in household applications and causes severe environmental pollution[6]. Effect of Profenofos on animal occurs through food and water. Profenofos is a potentially ground water contaminating insecticide, slightly soluble in water and readily miscible in organic solvents. The substance is hydrolyzed with increasing pH and moreover the half-life of Profenofos in soil is about one week. This property of Profenofos makes it a better choice for spray as compared to organochlorines, which are more persistent. Profenofos is highly toxic to aquatic organisms, zooplankton, crustaceans and insects. It is moderately toxic to birds and less toxic for mammals[7].The toxicity caused by Profenofos appears fatal even at a fairly low plasma concentration as recorded in a case of fatal human poisoning where low concentrations of metabolites were detected suggesting that Profenofos is rapidly metabolized[8]. Profenofos induces apoptosis and necrosis in cultured human peripheral blood lymphocytes in *in vitro* conditions [9]. Profenofos can induce oxidative stress which may be used as a diagnostic index in Profenofos poisoning [10]. The effect of thiobarbituric acid reactive substances, scavenging enzymes and glutathione in the brain of the *Albino rats* were also studied [11]. Although there are many clinical reports on the effect of OP compounds, yet information pertaining to the effect of Profenofos poisoning is limited. Hence, in the present investigation of various parameters pertaining to the protein metabolism were evaluated in the liver of Profenofos exposed *Albino rats*. The present study clearly indicates that continuous exposure to Profenofos seriously impairs protein metabolism in the liver of *Albino rats.*

MATERIALS AND METHODS

Test Chemical

Profenofos (94.0% purity) was obtained from Nagarjuna Agrichem. Limited,Hyderabad, A.P., India

Animal and Experimental Design

The protocol was approved by Institutional Animal Ethics Committee, S.V. University (Resolution No. 8a/2012-2013/(i)a/CPCSEA/IAEC/SVU/PJD-MRS/dt. 1-2-2012). Male adult *Albino rats* of 7 weeks old and weighing 200 ± 10 g. were obtained from Indian Institute of Science (I.I.Sc), Bangalore. They were housed at an ambient temperature 28 ± 2°C in a 12-h light/dark cycle and a minimum humidity of 40%. The animals had free access to commercial pellet diet supplied by Sai Durga Feeds and Foods, Bangalore, India and water *ad libitium*. All the male healthy adult *Albino rats* were randomly divided into four groups having with six rats per group. The first group animals were considered as control animals. Second group of animals were treated with Profenofos via oral gavage (39.5 mg/Kg body weight) for 10 days, third and fourth groups of animals were administered for 20 and 30 days with an interval of 48h respectively.

Biochemical estimations

The total protein content was estimated by the method of Lowry *et al.* [12]. Free amino acid content was estimated by the method described by Colowick and Kaplan [13]. Protease activity was estimated by the method of Moore and Stein [14] considering the amount of free amino acids liberated from the protein substances as a measure of proteolytic activity. The activity of aspartate aminotransferase (AST) and alanine amino transferase (ALAT) was assayed by the method described by Bergmeyer [15]. The activity of glutamate dehydrogenase (GDH) was assayed by the method of Lee and Lardy [16]. Ammonia was estimated by the method of Bergmeyer [15]. Urea was estimated by the diacetylmonoxime method as described by Natelson [17].

Statistical treatment

The data was subjected to One way Analysis of Variance (ANOVA) and post ANOVA tests (S-N-K test) using SPSS (ver. 20) in the personal computer and $p < 0.01$ was considered as statistically significant.

RESULTS

Biochemical changes

The results are presented in the Table 1. From the results it is clear that Profenofos exposed *Albino rats* showed a decrease in the protein content. The decrease was more in 30 days exposed animals. Free amino acids, Protease activity, AAT, ALAT and GDH activities showed a steady increase when compared to the control. Similarly Ammonia and Urea content also showed an increase when compared to the control.The increase was more in the animals which were exposed to longer duration *i.e.* for 30 days.

Table 1
Biochemical and enzymatic changes in the liver of Profenofos intoxicated Albino rats

Liver	Control	10 days	20 days	30 days	F value
Total Proteins (mg/g. wet wt. of tissue)	120.826 8.899	101.836 11.480 (-15.71)	89.491 8.966 (-25.93)	68.135 10.476 (-43.61)	29.232*
Free amino acids (µmoles of tyrosine/g. wet wt. of tissue)	61.055 5.399	67.415 5.708 (10.42)	81.524 3.708 (33.53)	86.597 8.376 (41.83)	23.407*
Protease (µmoles of tyrosine/mg protein/h)	1.283 0.098	1.430 0.138 (11.44)	1.658 0.137 (29.26)	1.870 0.144 (45.78)	23.431*
Aspartate Amino transferase (µmoles of pyruvate/mg protein/h)	1.262 0.103	1.413 0.185 (11.97)	1.596 0.098 (26.42)	1.805 0.141 (43.03)	17.805*
Alanine Amino transferase (µmoles of pyruvate/mg protein/h)	6.655 0.506	7.718 0.789 (15.98)	8.420 0.685 (26.53)	10.590 0.371 (59.13)	44.648*
Glutamate dehydrogenase (µmoles of formazon/mg protein/h)	0.534 0.014	0.597 0.059 (11.67)	0.687 0.067 (28.63)	0.765 0.079 (43.24)	17.023*
Ammonia (µmoles of ammonia/g. wet weight of tissue)	8.004 0.389	8.922 0.732 (11.467)	10.079 0.715 (25.92)	11.916 1.151 (48.87)	27.049*
Urea (µmoles of urea/g. wet weight of tissue)	2.933 0.374	3.379 0.248 (15.20)	3.895 0.238 (32.76)	4.588 0.285 (56.41)	35.757*

Values are expressed in Mean ± SD of six individual observations. Values in parenthesis indicate % change cover control. Mean values with the same superscript do not significantly differ among themselves through S-N-K test. *P < 0.01

DISCUSSION

Typical signs of OP toxicity were observed in Profenofos exposed animals. All the parameters were significantly altered in experimental rats and the effect of Profenofos was more in 30 days indicating that continuous exposure is harmful to non-target species. Extensive use of OP compounds over the last several decades has led to drastic effects on non-target animals. OP compounds in India are not only cheap but also easily available and hence these compounds are a constant source of both intentional and unintentional poisoning.Of late the use of OP compounds has gained more importance than before and this trend is likely to continue in the years ahead, because new applications for these compounds have been discovered[2]. Indiscriminate and excessive use of these pesticides is a major concern to non-target organisms, thus the use of pesticides has become a great concern worldwide[3]. OP stress leads to the damage of the membranes of vital organs which result in the secretion of majority of the enzymes like AST, ALAT and alkaline phosphatase are secreted into the blood [18]. There are reports which indicate that OP compounds raise ALT and AST levels in rats [19, 20]. In the present investigation, we have also found that Profenofos administration increases the ALT and AST levels in the liver of rats. Yahya *et al.* [21] reported that morphology of the various organs are damaged under OP toxicity. During chronic period of stress proteins are the source of energy and the animals require more energy to detoxify the toxicants and to overcome stress. An increase in the amino acids observed in experimental rats indicates that it is the result of the breakdown of protein for energy requirement and impairment of amino acid synthesis [22]. Stress conditions induces elevation in the transamination pathway and any abnormality or stress in the

amino acid metabolism has its own consequences by elevating the catabolic products like ammonia and urea which causes a serious disruption in the normal metabolism. The rapid rise in the free amino acid contents is attributed to step up proteolysis or increased synthesis of free amino acids by transaminase action. The increase in free amino acid pool may also be useful to the rats to overcome stress under Profenofos exposure. The GDH activity in the Profenofos exposed *Albino rats* showed a significant increase which indicates an increased oxidation of glutamate. GDH catalyzes the key reactions which provide substrates for either protein synthesis or carbohydrate metabolism. The increased GDH activity in the present investigation might have led to an increased oxidation of glutamate with a consequent production of ammonia. This is augmented by the changes in the transaminase activity. An increase in the GDH activity may also be due to the mitochondrial permeability or lysosomal damage and since GDH is a mitochondrial enzyme, any alteration in the organization of mitochondria may lead to the alteration in the enzyme activity. Significant increase in the GDH activity in Profenofos exposed *Albino rats* indicates an increased oxidation of glutamate. The increased GDH activity in the experimental animals might have led to an increased oxidation of glutamate with a consequent production of ammonia. This is augmented by the changes in the transaminase activity. Increase in the GDH activity may also be due to the mitochondrial permeability or lysosomal damage. Any alteration in the organization of mitochondria may lead to the alteration in the enzyme activity as GDH is a mitochondrial enzyme.Both AAT and ALAT activities showed a significant increase in Profenofos exposed rats. Transaminase activity is reported to increase during pathological conditions. It can be suggested that liver has been significantly damaged under Profenofos exposure. Ammonia and urea are waste products of protein metabolism that needs to be excreted. Therefore, marked increase in ammonia and urea indicates that Profenofos has profound effects on non-target animals. An increase in the urea and creatinine in OP exposed animals were reported by many authors. [17.] The OP toxicity leads to a significant increase in the activities of superoxide dismutase, catalase and glutathione peroxidase and glutathione reductase in liver was reported [18]. The results of the present study suggest that Profenofos adversely effect liver functions leading to its physiological impairment. Profenofos might have affected protein metabolism and detoxification system in the liver and this effect seems to be more when the animals are continuously exposed for a longer period of time. The present study suggests that sublethal doses of Profenofos exert its toxic effect by altering all the parameters of protein metabolism in the liver of *Albino rat* and usage of Profenofos should be restricted to the maximum possible extent.

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