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IMPACT OF DIMETHOATE ON TOTAL PROTEINS AND FREE AMINO ACIDS OF ALBINO RAT

PENCHALAMMA.R[1], JACOB DOSS.P[2] AND JAYA SUDHA.M[3]

[123]Division of Toxicology, Dept. of Zoology, S.V. University, Tirupati

ABSTRACT

Dimethoate is an Organophosphorous (OP) insect ides and acaricide used for the control of a wide range of insects, including housefly's mites, on a variety of fruits, vegetables, and field and forestry crops. The aim of the current study was to evaluate the subchronic toxicity of orally administered dimethoate in Wistar albino rats, based on the biochemical changes in the Albino rat tissues of total protein and free amino acid levels are estimated by with Dimethoate. In the present investigation the animals were treated with $1/10^{th}$ of LD_{50} of DM via oral gavage (34.5mg/kg body weight). The first group was considered as control animals. Second group of animals were treated with DM for 10 days, third and fourth groups of animals were administered for 20 and 30 days with an interval of 48h respectively. Total proteins decreased and free amino acids as well as increased in Dimethoate – exposed rats. The present study revealed reduction in total protein content in different tissues of albino rats exposed to sublethal dose of Dimethoate.

Key Words: Dimethoate, Albino rat, Total protein and free amino acid.

INTRODUCTION

Dimethoate, O, O-dimethyl S-methylcarbamylmethyl phosphorodithioate with 94% purity was used as the test chemical for the present study. Technical grade Dimethoate was obtained from Hyderabad Chemicals Limited, Hyderabad. A.P., India. Dimethoate has high inefficacy, lesser stability and comparatively low mammalian toxicity. Dimethoate is a commercial insecticide. It is used for control of a wide range of biting and sucking insects, especially aphids, including resistant species in field crops, fruit, vegetables, and in horticulture. It is considered as non-phototoxic on many crop plants. Dimethoate is quickly absorbed, quickly eliminated and has a wide applicability and safety compared to other compounds of its class. Hence, the pesticide Dimethoate is

selected for the present study. The control of insect pests relies heavily on the use of synthetic insecticides. But, their widespread use has led to some serious problems including toxic residues on grass and toxicity to non-target organisms such as mammals, birds and fishes (Zettler and Cuperus 1990; White 1995; and Riebeiro *et al*., 2003). The pollution of the environment plays a crucial role in the occurrence of many diseases affecting plants, animals and man. One of the main factors causing pollution of the environment is the irrational use of organophosphorus insecticides (Al-Haj *et al.*, 2005). Many alterations have been observed in organs of animals due to the organophosphorus insecticides (Betrosian *et al.*, 1995; and Senanayke1998), specially CNS, (Desi *et al.*, 1998; and Lengyl *et al.*, 2005), liver (Gomes *et al.*, 1999), and kidney (Kossmann *et* ***al.*, 1997)**. Dimethoate was introduced in 1956 and has been used as an insecticide in Australia for more than 30 years. At the commencement of this review, dimethoate is the active constituent in 21 registered products in Australia. The approvals of the active constituents and the registrations of products are being reconsidered based on concerns related to toxicology, occupational and health and safety, residues and trade. OPI are primarily recognized for their ability to induce toxicity in mammals through inhibition of acetyl cholinesterase (AChE) and subsequent activation of cholinergic receptors (Costa, 2006).

Proteins are the ubiquitous macromolecules in the biological system and are derivatives of high molecular weight polypeptides (Murray *et al.*, 2007). Proteins involve in cellular architecture, metabolic replications, enzyme mechanism. These are hydrolyzed to amino acids in the body which are further metabolized by incorporation in to proteins or deamination or oxidation of amino acids (Murray *et al.*, 2007). Functionally, proteins exhibit a great diversity and constitute heterogeneous group having diverse physiological functions as structural elements, in contractile systems, for nutrient storage, as vehicles of transport, a hormones, catalysts, toxins and protective agents (Nelson and Cox, 2005). Biological value of proteins is considered on the basis of tissue amino acid composition. The free amino acids content acts as precursors for protein synthesis and gluconeogenesis in all most all tissues (Murray *et al.*, 2007). The physiological state of the cell is dependent upon its free amino acidpool**(**Abidi,1996; Vani, 1991). The amino acids reduced during protein degradation due to activation of proteolysis will once again return the amino acid pool and these free amino acids are the currency through which protein metabolism operates (Munro 1970), showing the interdependence of both amino acids and proteins.

MATERIAL AND METHODS

Test Chemical: Dimethoate Technical (94%) pure in crystalline form was obtained from Hyderabad chemical limited, Hyderabad A.P., India.

Animal model: Male adult Albino rat of 7 weeks old and aged 200 ± 20 g. were obtained from Indian Institute of Science (II.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*.

EXPERIMENTAL DESIGN

All the male healthy adult male 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 Dimethoate via oral gavage (34.5mg/kg body weight which is $1/10^{th}$ of LD_{50}) for 10 days, third and fourth groups of animals were administered for 20 and 30 days with an interval of 48h respectively.

BIOCHEMICAL E STIMATIONS

Estimation of Total proteins

The total protein content was estimated by the method of Lowry *et al.*, (1951). The 2% homogenates were prepared in 10% TCA and centrifuged at 1000xg for 15 minutes. The supernatant was discarded and the residue was dissolved in a known amount of 1N sodium hydroxide. From this 0.2ml was taken and 4 ml of alkaline copper reagent and 0.4ml of folin phenol reagent (1:1 folin phenol: distilled water) was added. The contents were allowed to stand for 30 minutes at room temperature and the developed colour was read at 600 nm in a Spectrophotometer against a reagent blank. The amount of total proteins present in the sample was calculated by using bovine albumin standard. The values were expressed as mg/g wet weight of the tissue.

Estimation of Free amino acids (FAA)

Free amino acid content was estimated by the method of Moore and Stein (1954) as described by Colowick and Kaplan (1957).

5% homogenates of different tissues were prepared in 10% TCA and centrifuged at 1000xg for 15 minutes. To 0.25ml of the supernatant, 2ml of Ninhydrin reagent was added and kept in boiling water bath for 6.5 minutes and then contents were cooled. The contents were made up to 10ml with distilled water. The intensity of the colour developed was read at 570nm in a Spectrophotometer against the reagent blank.

The free amino acid content was expressed as µ moles of tyrosine equivalents / g wet weight of the 0.5ml of the enzyme source (supernatant).

STATISTICAL TREATMENT

The data was subjected to statistical treatment. One way analysis of variance (ANOVA) and S-N-K tests were performed using SPSS (ver. 12) in the personal computer and $p < 0.05$ was considered as statistically significant.

Table 1 : Changes in the Total protein content in different tissues of *Albino rats* exposed to sub-lethal dose of Dimethoate

Tissues	Control	10 days	20 days	30 days	F ratio
Liver	150.256	126.955	105.699	81.666	23.775*
± SD	15.654	12.555	9.789	7.966	
(% Change)		(-15.51)	(-29.65)	(-45.64)	
Heart	124.888	100.888	85.012	70.623	30.317*
± SD	11.866	10.123	7.236	6.778	
(% Change)		(-19.22)	(-31.93)	(-43.45)	
Kidney	136.339	110.155	90.152	60.222	43.395*
± SD	13.544	10.666	9.222	5.888	
(% Change)		(-19.24)	(-33.91)	(-55.85)	
Pancreas	147.666	120.666	109.667	92.358	40.192*
± SD	13.256	10.222	10.216	8.633	
(% Change)		(-18.28)	(-25.73)	(-37.45)	

Values expressed in mg protein/g. wet weight of the tissue are Mean ± SD of six individual observations. Values in the parenthesis indicate % change over control. Mean values with the same superscript do not differ among themselves through S-N-K test.

$^{*}P < 0.01$, $^{**}P<0.001$

Fig 1: Changes in Total protein content in different tissues of Dimethoate treated *Albino Rat* (mg/gm wet wt of tissue).

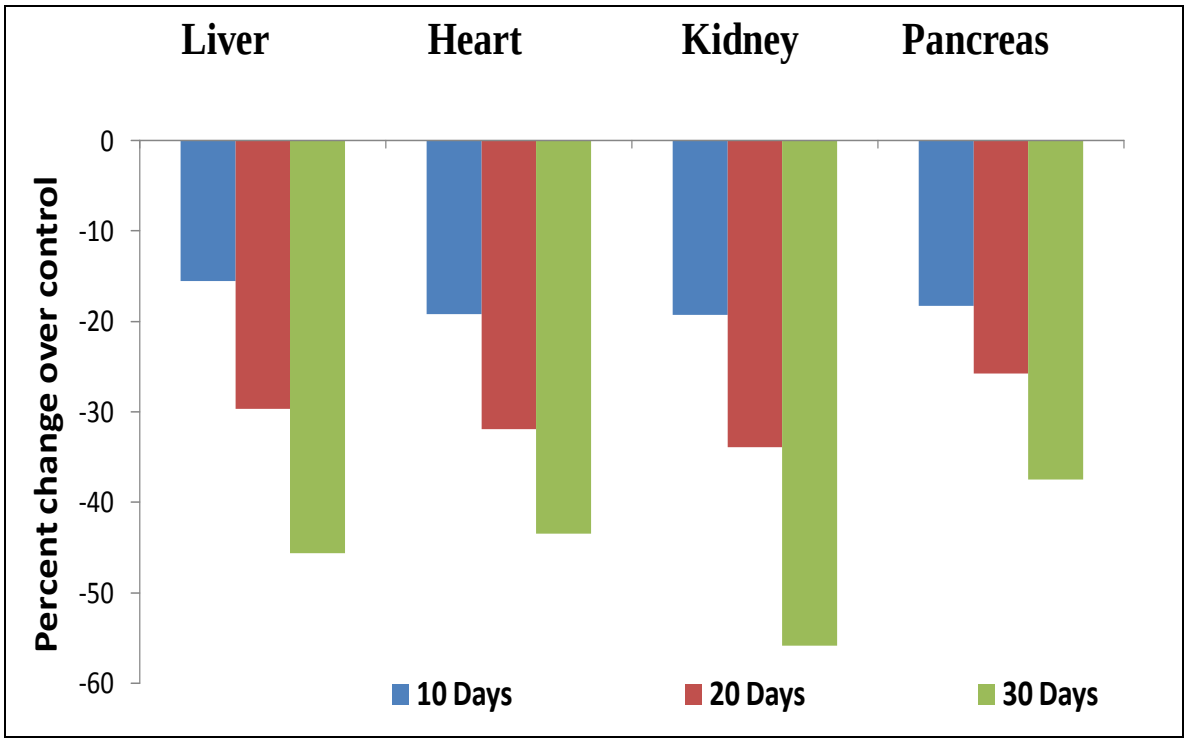


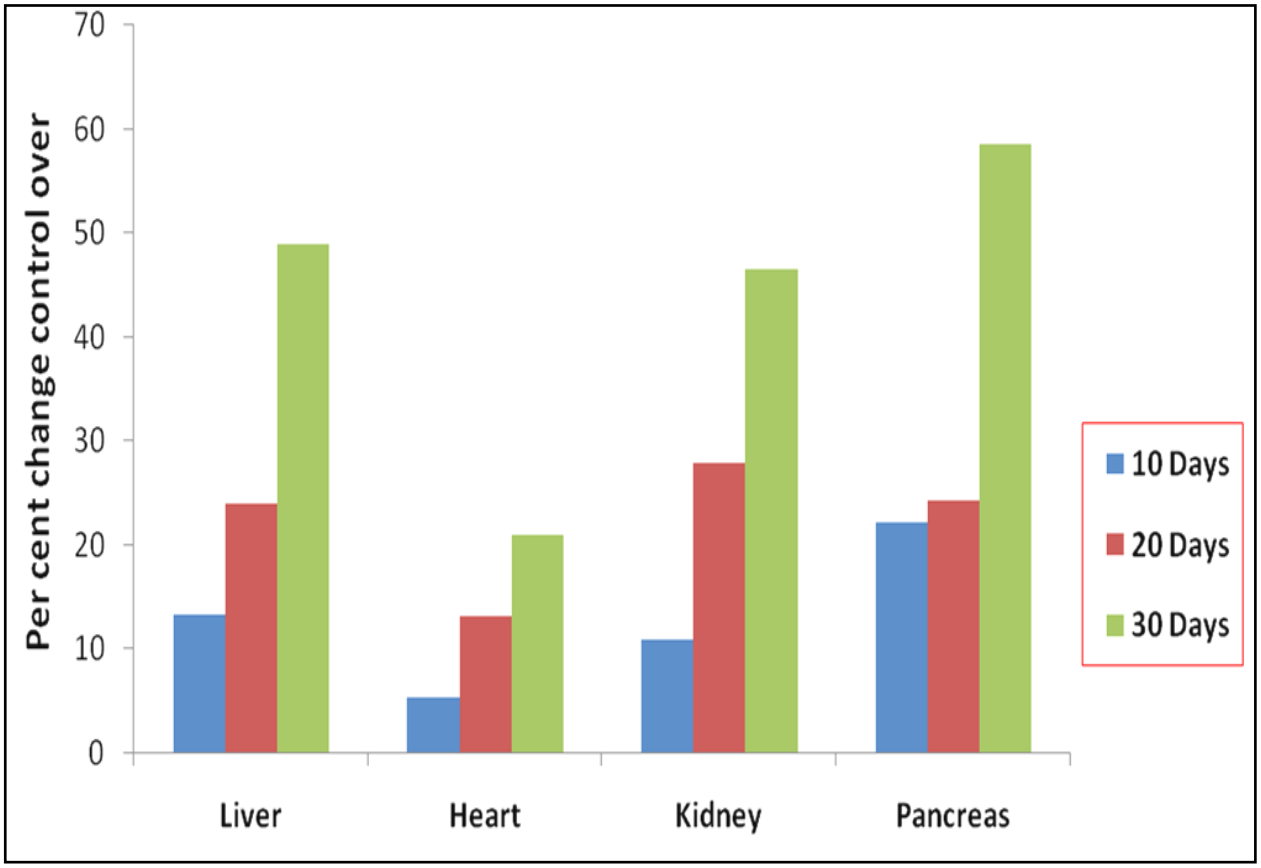
Table 2 : Changes in the Free amino acid content in different tissues of *Albino rats* exposed to sub-lethal dose of Dimethoate

Tissues	Control	10 days	20 days	30 days	F ratio
Liver	65.415	74.125	87.256	105.123	12.769*
± SD	5.685	6.885	8.125	10.635	
(% Change)		(12.11)	(25.34)	(38.03)	
Heart	40.325	42.125	50.136	56.985	13.072*
± SD	3.542	4.333	5.125	5.233	
(% Change)		(4.27)	(19.57)	(29.24)	
Kidney	53.368	60.048	78.369	81.333	30.224*
± SD	5.368	6.222	6.898	7.985	
(% Change)		(11.12)	(31.90)	(34.38)	
Pancreas	60.652	72.236	74.269	90.126	6.711*
± SD	6.111	7.644	7.105	9.224	
(% Change)		(16.04)	(18.33)	(32.70)	

Values expressed in mg protein/g. wet weight of the tissue are Mean ± SD of six individual observations. Values in the parenthesis indicate % change over control. Mean values with the same superscript do not differ among themselves through S-N-K test.

*P < 0.01, **P<0.001

Fig 2 : Changes in Free amino acid content in different tissues of Dimethoate treated *Albino Rat* (µ moles of tyrosine /gm wet wt of tissue).



RESULTS AND DISCUSSION

Administration of DM induced typical signs of OP toxicity in different biochemical parameters studied in the present investigation and the effect was more pronounced after 30 days when compared to 10 days. During the last six decades, the extensive use of OP compounds in agriculture and for public health purposes, has led to drastic effects on non-target animals. Most of these chemicals are unfortunately not highly selective and therefore have been proved highly toxic to non-target animals including man and other desirable forms of life that co-inhabit the environment. Therefore, the improper application of these pesticides may result in serious illness and

even death. During the chronic period of stress, proteins are the source of energy. During these stress conditions the animal requires more energy to detoxify the toxicants and to overcome stress. Increment in the free amino acid level in the present investigation indicates that it was the result of the breakdown of protein for energy requirement and impairment of amino acid synthesis (Singh *et al.* 1996). It is well known that stress conditions induce elevation in the transamination pathway. Any abnormality or stress in the amino acid metabolism will has its own consequences by elevating the catabolic products like ammonia and urea. This causes a serious disruption in the norm al amino acid metabolism.

The Dimethoate administration resulted in a significant decrease of total proteins in selected tissues. The protein level suppression may be due to loss of protein either by reduce in protein synthesis or increased Proteolytic activity or degradation. This may be due to catabolism of proteins make a major contribution to the total energy production. The decrease level of total proteins or elevated level of free amino acids was progressive in all the doses of study. The decrease in protein content indicates proteolysis leading to elevation in total free amino acids content. Total protein content also decreased in non-target vertebrate fauna after pesticide treatment indicating pesticide produced changes in the biochemical system of non–target organisms. Proteins play an important role in the life of all living organisms. Pesticides disturb protein synthesis by proteolysis or protein hydrolysis (Nagarjuna, 2007; Rajeswari, 2008).

Proteins are the chief organic macromolecule for all aspects of cellular structure and function, expected to react first upon pesticide exposure. Pesticides alter the buffering system of the intracellular environment very rapidly. Pesticides impair protein metabolism leading perhaps into disarray of functional and structural status of the cell (Shukla *et al.*, 1998). The literature pertaining to "pesticide protein interaction" contributes immensely to the field of pesticide biochemistry. The pesticides are found to be alter the structural and soluble proteins by causing histopathological and biochemical changes in the cell (Shakoori *et al.*, 1976). Total protein content decreased in non-target vertebrate fauna after pesticide treatment indicates pesticide-produced changes in the biochemical systems of non-target organisms. Proteins play an important role in the life of all living organisms. Pesticides disturb protein synthesis (Sivaiah, 2006; Madhava Rao, 2007; Begum**,** 2007).

The present study has revealed significant variation in protein metabolism in different tissues of albino rats following Dimethoate administration. The fall in protein levels was most probably due to its hydrolysis as evident

by increase in free amino acid content in different tissues indicates the metabolic adaptation to overcome the effect of the Dimethoate toxicity.

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