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Biochemical and histopathological studies to assess chronic toxicity of triazophos in blood, liver and brain tissue of rats

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ABSTRACT

Triazophos, O,O-diethyl-1-H-1,2,4-triazol-3-yl phosphorothioate, (TZ) is an organophosphorus pesticide which is extensively used in agriculture for controlling insect pests. Except a FAO/WHO report no study has investigated its short-term toxicity with respect to its potential to cause biochemical and histopathological alterations. The present study was designed to identify the effect of TZ at different doses (1.64, 3.2 and 8.2 mg/kg) on the oxidative stress parameters in blood as well as organs involved in xenobiotic metabolism (liver and brain) following chronic exposure for 90 days. Moreover, the study also delineates the effect of TZ on the histo-architecture of these organs. The results indicated a dose dependent induction ($p < 0.001$) of oxidative stress, as evident by increased malondialdehyde (MDA) level and compromised antioxidant defense including glutathione S transferase (GST) activity, glutathione (GSH) content and ferric reducing ability of plasma (FRAP) in blood, and increased MDA level with concomitantly decreased GSH content in tissues, following chronic exposure to TZ. The ratio of MDA: FRAP in blood was found to be increased following chronic exposure to TZ and may serve as a suitable indicator of severity of oxidative damage. Onset of such biochemical alterations is one of the early adaptive responses to TZ exposure which leads to histopathological alterations in terms of diffuse fatty changes expanding from mid-zonal area to whole lobule in liver. However, increased oxidative stress did not bring any morphological alteration in brain. The present study concludes that induction of oxidative stress, leading to subsequent histopathological alterations in liver, is an important mechanism underlying the TZ induced chronic toxicity.

1. Introduction

Widespread application of organophosphate pesticides (OP) due to their low bio-accumulation brings a hazardous risk of exposure to mammalian system because of optimum residual level and steady dissipation rate. Several studies have suggested that OP cause acute as well as chronic health effects in humans [1,2]. Among several OPs, triazophos (TZ), O,O-diethyl O-1-phenyl-1 H-1,2, 4-triazol-3-yl phosphorothioate, is extensively used as a stomach and contact poison against a broad spectrum of pests and flies that damage agricultural, horticultural and forest crops. The main fields of application are cotton, sugarcane, maize, potatoes, vegetables, cereals, fruits, coffee and ornamentals [3,4]. Release of TZ in the environment occurs primarily due to its application as an insecticide and potentially during its formulation, transport and storage [5]. The severity of TZ intoxication may vary with dose, route and extent of exposure.

TZ exerts toxicity by inhibiting the enzymatic activity of mammalian acetyl cholinesterase (AChE) thus leading to the emergence of a 'cholinergic crisis' due to excessive accumulation of acetylcholine (ACh) in synapse [6]. This may be debilitating and possibly fatal. Though the agricultural application of TZ for pest eradication in India is extensive, the biochemical, histological and neurological effects remain unknown. In our preliminary studies we have earlier reported that exposure to TZ for 30 days caused dose dependent induction of oxidative stress in blood and significant histopathological alterations in liver [7]. Therefore, it is pertinent to study the short term/ chronic oral toxicity of TZ at differential doses which may provide an insight into the molecular basis of its action.

2. Materials and methods

2.1. Chemicals and reagents

Nicotinamide adenine dinucleotide phosphate (NADPH), Oxidized and reduced glutathione; 1-chloro-2,4-dinitrobenzene (CDNB); 2,4,6-tripyridyl-s-triazine (TPTZ); 5,5-dithionitrobenzoic

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acid (DTNB) and bovine serum albumin were obtained from Sigma Chemicals Company (St. Louis, MO, USA). 2-thiobarbituric acid (TBA) was obtained from E. Merck (Mumbai, India). All other reagents used were of analytical grade and obtained either from British Drug House (BDH) or Sisco Chemicals (Mumbai, India). Technical grade TZ was received through the courtesy of M/S Hindustan Insecticide Limited (Delhi, India).

2.2. Animals and treatment

Wistar male albino rats weighing 150–200 g were placed in polypropylene cages (17 inch × 11 inch × 6 inch) with 2 rats per cage and kept under standard laboratory conditions of light/dark cycle (12–12 h) and temperature (25 ± 2 °C). They were provided with a nutritionally adequate standard laboratory diet obtained from M/S Hindustan Lever Ltd. (Mumbai, India). Technical grade TZ was dissolved in olive oil (vehicle) and administered orally for 90 days in three doses viz., 1.64, 3.2 and 8.2 mg/kg body weight (bw), respectively. These doses represent 1/50th, 1/25th and 1/10th of oral LD50 of TZ (82 mg/kg) in rats and were selected as per the existing toxicity guidelines [8]. The rats were randomly divided into 4 groups ($n = 8$ per group) as detailed in Table 1. Food and water was given ad libitum. Food consumption, general condition and any other symptoms were observed daily and body weight was recorded weekly. The experiments conducted in the present study were duly approved by Institutional Animal Ethics Committee, University College of Medical Sciences (UCMS) and care of the animals was carried out as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India.

2.3. Samples

After 90 days, all the rats were put on overnight fasting and the blood samples were collected through retero-orbital plexus in heparinized vials for erythrocyte GSH estimation and non-heparinized or plain vials for obtaining serum. Biochemical investigations using serum were carried out on the same day. All the rats were sacrificed by cervical dislocation and organs (liver and brain) were excised for biochemical and histopathological studies.

2.4. Biochemical estimation of oxidative stress parameters

2.4.1. In blood/serum

The total protein content in serum was estimated by Lowry's method [9]. Serum GST activity was measured as described previously [10]. Briefly, the assay mixture contained in a total volume of 3 ml, 1 mM CDNB in ethanol (final concentration of ethanol not less than 4%), 0.001 M GSH, 0.1 M potassium phosphate buffer (pH 6.5) and serum sample. The formation of the resulting adduct (CDNB-GSH, S-2,4 dinitrophenyl glutathione) was monitored by noting the net increase in the absorbance at 340 nm against reagent blank for 0–5 min. The enzyme activity was determined by using the extinction coefficient 9.6 mM/cm and the result is expressed as μmoles of product formed/min/mg protein or units/mg protein.

Table 1
Animal groups and their respective treatment.

S.No	Experimental groups	Treatment (p.o.)
1.	Control	Vehicle (olive oil)
2.	Group I	TZ (1.64 mg/kg)
3.	Group II	TZ (3.2 mg/kg)
4.	Group III	TZ (8.2 mg/kg)

Lipid peroxidation (LPO) in serum was measured as malondialdehyde (MDA) levels as described previously [11]. Briefly, 0.5 ml serum was precipitated with 20% trichloroacetic acid (TCA). The precipitate was suspended in 0.05 N sulphuric acid and TBA (0.07% in 1 M sodium sulfate) and incubated in boiling water bath for 30 min. The malondialdehyde (MDA)-TBA adduct thus formed was extracted with butanol and measured at 532 nm. The results are expressed as μmoles/ml.

Total glutathione (GSH) content in whole blood was estimated as described previously [12]. Briefly, the reaction mixture (1 ml) contained 25 μl of hemolysate, NADPH [0.2 μmoles/ml in 0.01 M/0.005 M phosphate EDTA buffer(pH7.5)] and glutathione reductase (1 unit).On addition of DTNB, the resulting chromophoric product i.e. 2-nitro,5-thio benzoic acid was measured at 412 nm. The result is expressed as μg/ml blood.

Ferric reducing ability of plasma (FRAP) was determined as described previously [13]. Briefly, the working FRAP reagent was prepared by adding 300 mmol/l acetate buffer (pH 3.6), 10 mmol/l TPTZ in 40 mmol/l HCl and 20 mmol/l $FeCl_3.6H_2O$ in ratio of 10:1:1 respectively. Sample was added, thoroughly mixed and the absorbance was monitored at 593 nm for 4 min. The values are expressed as μmol/ml.

2.4.2. In tissues

Brain and liver, immediately after excision, were washed with ice-cold saline, weighed and processed on the same day. Briefly, the tissue was homogenized with 50 mM sodium phosphate buffer (pH 7.4) containing 50 mM Tris hydrochloride and 1.15% potassium chloride at 4 °C using a motor driven Teflon Potter homogenizer. The homogenate was centrifuged at 7000×g for 15 min at 4 °C and the supernatant was used for assessment of oxidative stress parameters.

LPO was assayed by measurement of malondialdehyde (MDA) levels as described previously [14]. Formation of MDA-TBA adduct was measured at 532 nm following extraction with Butanol:Pyridine (15:1 v/v) and the results are expressed as μmoles/g wet tissue.

For estimation of GSH content in tissues, 5% trichloroacetic acid was added to the supernatant and centrifuged at 5000 rpm for 15 min at 4 °C to precipitate the proteins. The resulting supernatant was added to the assay mixture containing 0.3 M sodium phosphate (pH 8.4), 0.4% w/v DTNB in 1% tri-sodium citrate. After thorough mixing, the absorbance of the mixture was immediately measured at 412 nm. The results are expressed as μg/g wet tissue [15].

2.5. Histopathogical analysis

Liver and brain were excised from all rats and fixed in 10% neutral formalin buffer. Tissue sections (5 μm thick) were cut and stained with hematoxylin and eosin for histopathological studies.

2.6. Statistical analysis

Results are expressed as mean ± SD. Differences in oxidative stress parameters between different treatment groups were analyzed using one-way ANOVA with post hoc Tukey's SD multiple comparison procedure at $p < 0.05$. All statistical analyses were carried out using the SPSS v15.0 software, in consultation with the Department of Biostatistics and Medical Informatics, University College of Medical Sciences, Delhi.