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# Augmentation of hepatic and renal oxidative stress and disrupted glucose homeostasis by monocrotophos in streptozotocin-induced diabetic rats

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ABSTRACT

Several recent studies have demonstrated that organophosphorus insecticides (OPI) possess the potential to disrupt glucose homeostasis leading to hyperglycemia in experimental animals. The propensity of OPI to induce hyperglycemia along with oxidative stress may have far-reaching consequences on diabetic outcomes and associated complications. The primary objective of this study was to assess the potential of monocrotophos (MCP), an extensively used OPI, on hepatic and renal oxidative stress markers and dysregulation of hepatic glucose homeostasis in experimentally induced diabetic rats. Rats rendered diabetic by a single dose of streptozotocin (60 mg/kg b.w) were orally administered MCP (0.9 mg/kg b.w/d for 5 d). Monocrotophos *per se* caused only a marginal increase in blood glucose levels but significantly elevated the blood glucose levels and also disrupted glucose homeostasis by depleting liver glycogen content and increasing the gluconeogenetic enzyme activities in diabetic rats. Experimentally induced diabetes was also associated with alterations in antioxidant enzymes in liver and kidney. MCP markedly enhanced lipid peroxidation in kidney and altered the enzymatic antioxidant defense mechanisms in both liver and kidney of diabetic rats. Collectively our data provides evidence that MCP has the propensity to augment the oxidative stress and further disrupt glucose homeostasis in diabetic rats.



## 1. Introduction

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia and insufficient secretion of endogenous insulin. Although the etiology of this disease is not well-defined, viral infection, autoimmune disease and environmental factors have been implicated [1,2]. Various studies have also shown that diabetes is associated with increased formation of free radicals and decrease in antioxidant potential [3]. The prevalence of diabetes in all age groups is increasing rapidly in all parts of the world. Although obesity, sedentary lifestyles and diets rich in fats are known as risk factors for diabetes, recent evidences point to environmental toxicants as a very important factor to type II diabetic induction, especially, attention has turned to the organophosphorus insecticides (OPI), which represent 50% of all the insecticide use worldwide [4]. Studies have also demonstrated that OPI exposure disrupts glucose homeostasis in animal models and can lead to hyperglycemia after poisoning in humans [5]. However, the impact of chronic exposure to moderate levels of OPI on glucose metabolism and diabetes in humans and the extent to which exposure to other classes of pesticides may contribute to diabetes risk are unclear.

Organophosphorus insecticides (OPI) constitute one of the most widely used classes of insecticides employed for both agricultural and landscape pest control [6] and hence exposures to OPI are virtually ubiquitous. Their wide usage commonly leads to their residues in food crops, soil, or surface water, which become the major route of exposure [7]. There have been increasing concerns about the effects of various OPI in humans and experimental animals. OPI primarily act by inhibiting the enzyme, acetylcholinesterase (AChE), leading to cholinergic stress as a result of accumulation of acetylcholine (ACh). OPI are also demonstrated to cause a variety of physiological aberrations including oxidative stress [8,9], alterations in glucose homeostasis [10,11], immunotoxicity [7] and hyperglycemia [12] in addition to neurotoxicity. Among these, hyperglycemic potential of OPI has been subject to intense scientific scrutiny. A large number of mechanisms, including gluconeogenesis, are believed to mediate OPI-induced hyperglycemia [13]. Recent evidences point to their lasting effects on metabolism and association with increased risk of developing diabetes [5,14].

Monocrotophos (dimethyl (E)-1-methyl-2-(methylcarbamoyl) vinyl phosphate) (MCP), is a broad-spectrum systemic insecticide and acaricide. Because of its widespread use, MCP has been

*Abbreviations:* MCP, monocrotophos; STZ, streptozotocin; OPI, organophosphorus insecticide; LPO, lipid peroxidation; GSH, reduced glutathione; SOD, superoxide dismutase; CAT, catalase.

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detected in ground, surface and rainwater [15]. Monocrotophos is also a major break-down product of Dicrotophos, and these two are considered to be the most avian-toxic substances ever developed [16]. Exposure to MCP is known to cause a variety of biochemical perturbations in mammals and other experimental animals [17] including reproductive [18] and neurobehavioral toxicity [19] in mammals and hyperglycemia in the fish *Clarias gariepinus* [20].

Our earlier study [21] had shown that MCP possesses the potential to alter the lipid profile in normal as well as experimentally induced diabetic rats. In view of the potency of OPI to interfere with factors associated with pathophysiology of diabetes and associated complications, the present investigation aimed to assess the impact of MCP on diabetic outcome as well as its associated complications in a streptozotocin (STZ) diabetic rat model. We have examined the hypothesis that oxidative stress mechanism/s may be responsible in the augmentation of diabetic complications by MCP. The present study was intended to obtain preliminary evidence on the extent of involvement of oxidative stress in two vital organs *viz*., liver and/or kidney of diabetic rats exposed to multiple doses of MCP during the early diabetic condition.

## 2. Materials and methods

### 2.1. Animals

Adult male rats (CFT-Wistar strain, 8 weeks old, ~200 ± 10 g) were used for the study. They were housed individually in metallic cages at room temperature (25 ± 2 °C) with relative humidity of 50–60% and on a 12 h light–darkness cycle. They had free access to food and water *ad libitum*. The rats were acclimatized to the commercial diet (Saidurga Feeds and Food, Bangalore, India) for 7 d prior to the start of the experiment. All procedures with animals were conducted strictly in accordance with guidelines approved by the Institute Animal Ethical Committee, regulated by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India. During the experiments, maximum care was taken to minimize animal suffering and in addition, the number of rats used was kept to a minimum.

### 2.2. Chemicals

Streptozotocin was procured from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals used were of analytical grade and were procured from M/s. Sisco Research Lab (Mumbai, India). Technical grade Monocrotophos was a gift from Hyderabad Chemicals Limited, Hyderabad, India.

### 2.3. Induction of diabetes

Animals were administered a single dose of streptozotocin (STZ, 60 mg/kg b.w i.p) dissolved in 100 mM citrate buffer pH-4.5 [22]. Since STZ is capable of producing fatal hypoglycemia as a result of massive pancreatic insulin release, STZ-treated rats were given 5% glucose water for 24 h. After 48 h of STZ injection, blood glucose was monitored in rats by Accucheck Sensor Glucometer and rats showing blood glucose levels above 250 mg/dl were selected as diabetic rats.

### 2.4. Experimental design

The rats were grouped by randomized design into four groups of six animals each as follows: Group A: normal control rats; Group B: MCP control rats; Group C: STZ control rats and Group D: STZ-treated rats were orally administered with MCP dissolved in distilled water (dosage: 0.9 mg/kg b.w/d, 5 d), 48 h after STZ administration and after confirmation of hyperglycemia. The dosage of MCP was equivalent to 1/20 $LD_{50}$ and the criteria of selection of this dosage was based on our preliminary dose-determination study [21]. After 5 d of MCP treatment rats of all the groups were sacrificed, blood was collected by cardiac puncture into tubes and serum separated. Liver and kidney were excised, homogenized in phosphate buffer (0.1 M, pH 7.4 – at 10%) or in trichloroacetic acid (at 20% and 5% for GSH and liver glycogen respectively), centrifuged and the supernatants used for the various biochemical measurements.

### 2.5. Biochemical measurements

#### 2.5.1. Estimation of blood glucose

Blood glucose was estimated using the commercial kit (Span Diagnostics Ltd., Mumbai, India) based on the Glucose Oxidase–Peroxidase (GOD–POD) method and the results were expressed as mg glucose/dl.

#### 2.5.2. Determination of lipid peroxidation

The amount of thiobarbituric acid reactive substances (TBARS) in the tissue homogenate was determined to assess the extent of lipid peroxidation [23]. 500 μl of the liver/kidney homogenate was added to 2 ml of TBA–TCA–HCl mixture (0.374%–15%–0.25 N) and the tubes were placed in a boiling water bath for 15 min. After cooling and centrifugation, the color of the supernatant was read at 535 nm. The amount of thiobarbituric acid reactive substances (TBARS) in the supernatant was calculated using molar extinction coefficient of malondialdehyde (MDA) ($1.56 \times 10^{-5}\ M^{-1}\ cm^{-1}$) and the results were expressed as nmol MDA/min/mg tissue.

#### 2.5.3. Determination of reduced glutathione

Reduced glutathione was measured according to the method of Benke et al. [24]. 50 μl of the deproteinized liver/kidney supernatant was added to 940 μl of phosphate buffer (100 mM pH 8.0) to which 10 μl of DTNB (in phosphate buffer, 100 mM, pH 8.0) was added and mixed. The absorbance of the solution was read at 412 nm. Glutathione concentrations were calculated from standard graph obtained with reduced glutathione and the results were expressed as mg GSH/g tissue.

#### 2.5.4. Determination of superoxide dismutase

Superoxide dismutase (SOD) activity was assayed based on the principle of inhibition of quercetin auto-oxidation by SOD [25]. Autoxidation of quercetin was monitored at 406 nm in a final reaction mixture containing TEMED (0.8 mM), EDTA (0.8 mM) and quercetin (0.014 mM) in phosphate buffer (pH 10.0). Inhibition of quercetin auto-oxidation by SOD was monitored under the same conditions after addition of tissue homogenate. One unit of SOD is the amount of enzyme required to bring about 50% inhibition of quercetin auto-oxidation and the results were expressed as units/mg protein.

#### 2.5.5. Determination of catalase

Catalase activity was measured by the method of Beers and Sizer [26]. 10 μl of supernatant was added to a reaction mixture containing 15 mM $H_2O_2$ with 15μl of $H_2O_2$ (15%) was added to 10 ml phosphate buffer (50 mM, pH 7.4) and the decrease in absorbance due to $H_2O_2$ degradation was monitored at 240 nm for 60 s. The enzyme activity was calculated based on molar extinction coefficient of $H_2O_2$ at 43.6 $M^{-1}\ cm^{-1}$ and the results were expressed as μmol of $H_2O_2$ decomposed/min/mg protein.