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RESEARCH ARTICLE

SUB ACUTE DERMAL TOXICITY OF METALAXYL WITH SPECIAL REFERENCE TO OXIDATIVE STRESS IN WISTAR RATS

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ABSTRACT

The present study was aimed to evaluate the oxidative stress potential of metalaxyl after its dermal application for a period of 30 days in wistar rats. Rats were divided into two groups with six rats in each group. Group I served as control and were dermally applied with distilled water. Group II received dermal treatment of metalaxyl @ 350mg/Kg.bwt ($1/10^{th}$ LD_{50}) after dissolution in water. Significant increase in Lipid peroxidation was observed on 30th day of treatment in metalaxyl treated group as compared to control. Blood glutathione decreased significantly in metalaxyl treated animals as compared to control ones. There was significant decrease in the activities of SOD, CAT and GPx on 30th day in metalaxyl treated animals as compared to control group. However, no significant change was observed in GST activity which depicted decreasing trend as compared to control. The present study follows that metalaxyl on dermal application can produce inevitable changes in the form of oxidative stress even for short period of 30 days and therefore warns about injudicious use of the pesticide.

INTRODUCTION

Fungicides are dangerous category of pesticides and are sprayed as many as twenty times in a year on fresh fruits and vegetables during humid conditions to prevent growth of mold and fungus affecting productivity (Science News, 1994). Every organ system in the human body is vulnerable to the toxic effects of these synthetic chemicals. It is well understood that the indiscriminate use of agrochemicals under conventional agriculture not only causes severe health hazards for human beings but also has numerous other side effects on the environment including destruction of the biodiversity. Therefore, animal toxicity studies are most important part in the assessment of safety of these chemicals. Metalaxyl, a systemic benzenoid fungicide belongs to chemical group of acylalanine having IUPAC name methyl *N*-(2-methoxyacetyl)-*N*-(2,6-xylyl)-DL-alaninate, with molecular formula $C_{15}H_{21}NO_4$ (FAO, 1995). Metlaxyl is used to control soil-borne diseases caused by *Phytophthora* and *Pythium* on fruits, cotton, soyabean, peanuts, ornamentals and grasses (Sukul and Spiteller, 2000). Few animal studies suggest that the metalaxyl produces hepatotoxicity and various hazardous effects in mammalian animals when given orally for longer period of time (Howard, 1991 and Okdah, 2005). However, there is dearth of studies to evaluate the dermal toxic potential of this commonly used fungicide for different time intervals. Therefore, present study was an attempt to appraise the toxic potential of this chemical after its dermal application for a period of 30 days in wistar rats.

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MATERIALS AND METHODS

Experimental animals

The study on effects of metalaxyl was conducted on healthy wistar rats of either sex weighing 200 to 250 g procured from Indian Institute of Integrative Medicine, CSIR Lab, Jammu. The animals were provided standard pelleted ration and clean drinking water *ad libitum.* All the animals were maintained under standard managemental conditions. A daily cycle of 12 h of light and 12 h of darkness was provided to animals. Prior to start of experiment, the rats were acclimatized in the laboratory conditions for a period of more than 3 weeks. All the experimental animals were kept under constant observation during entire period of study. The experiment was conducted strictly in accordance to the Institutional Animal Ethics committee.

Insecticide used

Metalaxyl (35%WS) was commercially obtained from Jai Shree Rasayan Udyog Limited, Delhi as Srilaxyl 35 in 100 gram pack. The metalaxyl was applied dermally @ 350 mg/kg ($1/10^{th}$ LD_{50}) (US, HSD, 1995) on interscapular region as per method described by Punareewattana *et al*. (2001)

Experimental design and dosage

Rats of either sex were divided into two groups with six rats in each group and were subjected to dermal treatment regimes for 30 days. Group I animals served as control and were applied with distilled water whereas group II received metalaxyl @ 350mg/kg b.wt. (1/10 LD_{50}) dermally after dissolution in water.

Enzyme assay

The rats were anaesthetized with diethyl ether and about 4-5 ml blood were collected from retro-orbital fossa and heart of anaesthetized rats in dry set of test tubes containing heparin @ 5-10 IU/ml of blood on 30^{th} day of treatment. The plasma was immediately separated by centrifugation at 3000 rpm for 15 min and remaining red blood cells were washed with normal saline solution three times, before preparing the RBC lysate. Washing of erythrocytes were undertaken by diluting RBC sediment with normal saline solution in the ratio of 1:1 on v/v basis and centrifuged for 10 minutes after gentle but through mixing. After centrifugation the supernatant was discarded along with buffy coat and again NSS was added to the RBC on v/v basis, mixed gently and centrifuged. This process was repeated for 2-3 times. After final washing 1 per cent hemolysate (100µl washed RBC + 9.9 ml PBS) and 33 per cent hemolysate (330µl washed RBC+ 670µl PBS) in phosphate buffer solution (PBS), pH 7.4 were prepared. The 1 per cent haemolysate was used for the estimation of catalase, superoxide-dismutase, glutathione-peroxidase and glutathione-S-transferase and 33 per cent haemolysate was used for estimation of lipid peroxidation. The activity of lipid peroxidation in erythrocytes was determined according to method described by Shafiq-ur-Rehman (1984). The activity of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione-S-transferase (GST) in erythrocyte lysate were determined by methods of Marklund and Marklund (1974), Aebi (1983), Hafeman *et al.* (1974) and Habig *et al*. (1974) respectively.

Statistical analysis

The difference between two means based on individual observations was determined by unpaired Student's t-test. The significance was assayed at $P < 0.05$ and $P < 0.01$ levels (Snedecor and Cochran, 1967).

RESULTS AND DISCUSSION

Oxidative stress parameters were evaluated and the data obtained have been given in table 1. Perusal of the table revealed significant ($P<0.01$) increase in MDA value in metalaxyl treated animals as compared to control after 30 days of dermal application. Blood glutathione decreased significantly ($P<0.01$) in metalaxyl treated animals as compared to control. The activity of Catalase (CAT) decreased significantly ($P<0.01$) after 30^{th} day of dermal treatment with metalaxyl. Similarly the activities of Superoxide Dismutase (SOD) and Glutathione Peroxidase (GPx) significantly ($P<0.05$) in treated animals as compared to control. No significant alteration was observed in concentration of Glutathione-S-transferase (GST) in metalaxyl treated animals as compared to control. Malondialdehyde (MDA) is the end point of lipid peroxidation process which may be defined as an oxidative deterioration of polyunsaturated lipids (Dar *et al.,* 2013). Glutathione (GSH) an important antioxidant, protecting the membrane from oxidative insult, is thus considered as a critical determinant for the threshold of tissue injury caused by environmental chemicals (Machlin and Bandlich, 1987). Several enzymatic antioxidant defences designed to scavenge reactive oxygen species (ROS) in the eukaryotic cells protect them from oxidative injury. A fine balance between several antioxidant species and ROS appears to be more important for the overall protection of cells. Repeated dermal application of metalaxyl in rats caused a significant increase in lipid peroxidation on 30^{th} day of experimentation as compared to the control group of rats. These findings are in agreement with studies on metalaxyl by Lamfon (2011) and Hashem (2012). According to Calviello *et al*. (2006**)** fungicide-induced damage is closely associated with increase in lipid peroxidation and the decrease in the antioxidant enzymes.

Table 1. Effect of repeated dermal application of metalaxyl on oxidative stress parameters in rats

Parameters/Units	Control	Treatment
Lipid peroxidation (n mol MDA formed/ml erythrocytes)	7.12±0.35	13.14±1.27[b]
Blood glutathione	75.14±5.36	50.84±4.78[b]
SOD (Units/mg protein)	59.20±3.71	40.82±5.69[a]
CAT (µmole H_2O_2 decomposed /min/mg protein)	60.05±5.59	35.57±3.77[b]
GPx (Units/mg protein)	18.82±2.02	11.83±1.33[a]
GST (µmole of conjugate of GSH-CDNB/min /mg plasma protein)	0.064±0.012	0.054±0.011

Values given are mean ± SE of the results obtained from 6 animals unless otherwise stated. [a,b] significantly different as compared to control values at 5% ($P<0.05$) and 1% ($P<0.01$) level of significance respectively.

Glutathione is a tripeptide comprising of glutamic acid, glycine and cystine, found in all tissues and occurs in approximately 2 mM concentrations in red blood cells (Beutler, 1975). GSH is an important naturally occurring antioxidant, which prevents free radical damage and helps detoxification by conjugating with chemicals. In addition, GSH is pivotal to the cellular antioxidant defenses by acting as an essential cofactor for antioxidant enzymes including glutathione peroxidase (GPx) and glutathione-s-transferase (GST) (Mascio *et al*., 1991 and Hayes *et al*., 2005). Under oxidative stress, GSH is depleted by GSH related enzymes to detoxify the peroxides produced due to increased lipid peroxidation (Cathcart, 1985). Decreased blood glutathione level has been observed due to metalaxyl oral treatment in rats (Hashem, 2012). A significant decrease in glutathione levels in rats exposed to benomyl has also been observed by Banks and Soliman (1997). SOD levels in metalaxyl-treated group of rats decreased significantly after after 30^{th} day of exposure as compared to their control group. These findings are in consonance with the studies of Lamfon (2011) and Saber *et al*.

(2011) in metalaxyl-treated albino mice. Sakr and Abel-Samie (2008) has also found that mancozeb fungicides induce a significant decrease in the serum antioxidant superoxide dismutase. Catalase is a haeme-containing enzyme that catalyzes the dismutation of hydrogen peroxide into water and oxygen. The enzyme is found in all aerobic eukaryotes and is important for the removal of hydrogen peroxide generated in peroxisomes (microbodies) by oxidases, involved in ß-oxidation of fatty acids, the glyoxylate cycle (photo-respiration) and purine catabolism. Stress conditions in which there is a large free radical generation also result in the depletion in catalase activity (Hertwig and Feirabend, 1992), thus justifying the decreased activity of this enzyme. Glutathione peroxidase is a selenium containing enzyme which reduces hydrogen peroxide forming GSSG and thereby serves as an alternative means of detoxifying activated oxygen. The activity of GPx is dependent upon glutathione level. Decreased glutathione activity in present study might be the reason for decreased activity of GPx. Repeated oral administration of metalaxyl in rats caused non significant decrease in GST levels on 30th day of experimentation. However findings of Hashem (2012), Calviello *et al*. (2006) and Sakr and Abel-samie (2008) revealed significant decrease in GST after oral treatment with metalaxyl.

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