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Toxicological Effects of Methyl Parathion and Protection Afforded by Ascorbic Acid in Small Intestine of Swiss Albino Mice: A Histological and Histometric Study

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KEYWORDS Methyl Parathion. Intestine. Crypts. Ascorbic Acid

ABSTRACT Methyl parathion (C8H10NO5PS) is widely used insecticide known to cause fatal intoxication in both human and animals. This study was carried out to investigate the deleterious effects of methyl parathion on small intestine of mice. Animals were injected intraperitoneally an acute dose (3mg/kg bwt) of methyl parathion. Autopsies were done on 7, 14, 21 and 28 days post- treatment. The weight of intestine decreased drastically in methyl parathion treated group as compared to control. Histopathological changes involved degeneration of cryptal and villous region, loss of villi. Histometric analysis showed increase in percentage of pyknotic cells, dead mitotic figures and decrease in number of total cell of crypts. Also, this work was planned to evaluate the ability of ascorbic acid to prevent or reduce the toxic effects of methyl parathion. Mice were administered two doses of ascorbic acid 40mg/kg bwt and 80mg/kg bwt. Results showed that i.p. injection of 80mg/kg bwt of ascorbic acid daily was highly effective in protection against methyl parathion toxicity. Lower dose that is 40mg/kg bwt of ascorbic acid also provided protection but to a lesser extent

INTRODUCTION

Considerable toxicity data of chemicals have been produced in the past few years. The environmental contamination with pesticides is a problem of the regional as well as worldwide importance (US EPA 1999). The toxic chemicals have been found in a variety of environmental samples including water, air and house dust and their presence has also been noted in the tissues of non – occupationally exposed people, particularly in the adipose tissue, blood and urine (Wagner et al. 1991). Recent decline in the use of chlorinated hydrocarbon insecticides, especially DDT and lindane has resulted in a substantial increase in the use of organophosphorus insecticides which are far less resistant to chemical breakdown than the persistent insecticides.

Methyl parathion is an extremely toxic organophosphate insecticide, listed in the World Health Organization's highest category for toxicity, IA. It is widely used for control of chewing and sucking insects and mites in a wide range of crops including cereals, fruits, nuts, vines, vegetables, ornamental plants, cotton and field crops (Kidd and James 1991; US EPA 2003). Methyl parathion induces oxidative stress which results from imbalance between reactive oxygen species (ROS) and antioxidant levels (Lightboy et al. 2001). The main damage induced by ROS results in alteration of cellular macromolecules and change in intracellular calcium and intracellular pH or cell death (Dorval et al. 2003; Fidan and Dundar 2008).

Ascorbic acid is cited as the most abundant and effective antioxidant in the human body (Frei et al. 1989). In view of the intrinsic antioxidant activity of this vitamin, the present study was designed to investigate the protective effects of 40mg/kg bw and 80mg/kg bwt single, daily i.p. administration of ascorbic acid in 3mg/kg bwt methyl parathion – treated mice for 7, 14, 21 and 28 days post- treatment.

MATERIALS AND METHODS

Animals: Adult Swiss albino mice of 20 – 25g were obtained from Animal House of GADVASU, Ludhiana. These were maintained under controlled conditions of temperature (25 ± 2oC) and light (light: dark, 10h: 14h). Six animals were housed in a polypropylene cage containing saw dust as bedding material. They were maintained on standard mice feed (procured from M/S Ashirwad Industries Limited, Punjab University, Chandigarh) and water *ad libitum*. The Animal Ethical Committee of Punjabi University Patiala has approved the present study.

Pesticide and Ascorbic Acid: Methyl Parathion was received from Bayer India Limited,

Mumbai and ascorbic acid was obtained from Loba Chemie Private Limited, Mumbai. Both of them were administered intraperitoneally to mice.

Experimental Protocol: To assess the possible protective role of ascorbic acid on intestine of mice against Methyl Parathion intoxication, animals were divided into following groups:

Group 1: Animals of this group received double distilled water.

Group 2: Animals of this group were given an acute dose of 3mg/kg body weight of Methyl Parathion intraperitoneally.

Group 3: Animals of this group were injected 40mg/kg body weight of ascorbic acid daily after acute administration of 3mg/kg bwt of Methyl Parathion.

Group 4: Animals of this group were injected 80mg/kg body weight of ascorbic acid daily after acute administration of 3mg/kg bwt of Methyl Parathion.

Animals from each group were autopsied by giving an overdose of ether at various post - treatment intervals; viz., 7, 14, 21 and 28 days, and their intestine were removed and processed for histopathological and histometric studies.

Histopathological Study: Intestines were excised out and fixed for 24 hours in alcoholic Bouin's solution, dehydrated and embedded in paraffin wax. It was then processed into 5μ thick sections, stained with haematoxylin – eosin and observed under microscope.

Histometric Study: Histometric study of cryptal region of intestine was performed. Cells were counted on either side of the crypt starting from the base at a point where the lumen axis touches the epithelium up to cryptal – villus junction. Total cells in crypt region, all dead mitotic figures from stage prophase to telophase and cells showing pyknosis were counted.

Statistical Analysis: Data was analyzed by t – test comparison test, $p<0.05$ was considered as significant and $p>0.05$ as non-significant.

RESULTS

The weight of intestine in toxic group showed a great fall (5.73 ± 0.55gm/100gm body weight) from the control group (10.58 ± 0.24 gm/100gm body weight). In the groups which were given antioxidant protection showed a gradual increase in the weight of intestine. In group 3 the intestinal weight increased to 8.83 ± 0.39 and in group 4 it reached near normal (10.30 ± 0.22) (Fig. 1).

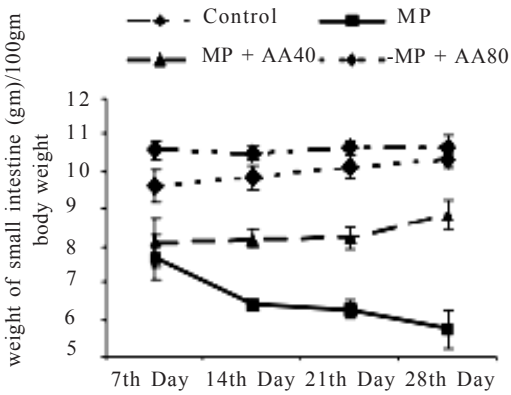

Fig. 1. The variation between the mean weights of intestine (gm)/100gm body weight in control, toxic and antioxidant treated groups

Histological Effect: Figure 2 depicts the structure of normal villi of small intestine. In Group 2 mice progressive damage and degeneration of villi along with loss of basal membrane, pyknotic and dead nuclei were observed in Group 2 mice (Figs. 3 and 4). In Group 3 mice there was observed an increase in number of cryptal cells and intestine showed some recovery in size of villi as well as cryptal region (Figs. 5 and 6). In Group 4 mice the earlier intervals showed although normal structures of villi but few villi with broken tips were also present and in later intervals normal structure of the intestine was observed (Figs. 7, 8 and 9).

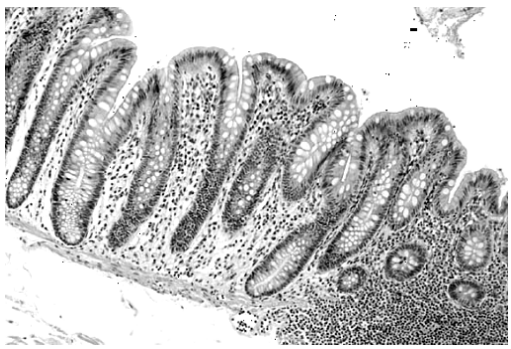

Fig. 2. Photomicrograph showing normal cryptal and villous intestinal structure. X 100

Histometric Effects: Dead mitotic figure count and pyknotic cell count in intestine of toxic group showed statistically significant ($p<0.001$) increase from control group. In Group 3 and 4 a significant decrease in both dead mitotic figures

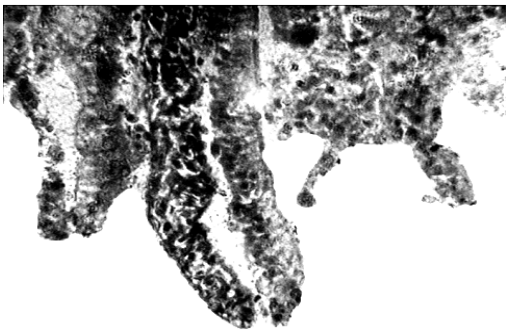

Fig. 3. Photomicrograph of intestine 21 day post treatment of methyl parathion depicting shortening of villi, pyknotic cells and degenerated villous membrane. X 400

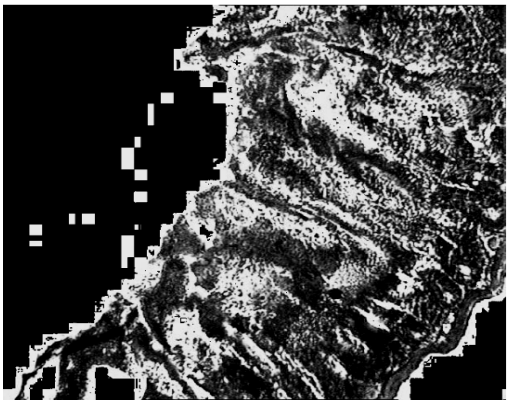

Fig. 4. Photomicrograph of intestine 28 day post treatment of methyl parathion showing complete degeneration of villi. Pyknotic cells and numerous dead mitotic Fig.s are visible. X 100

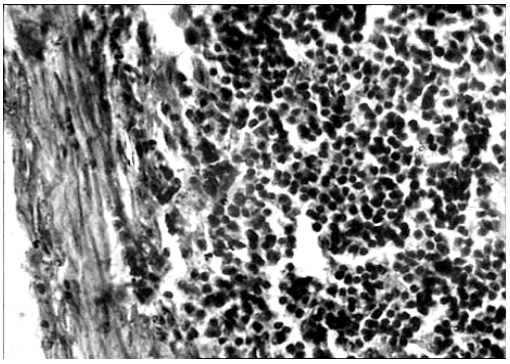

Fig. 5. Photomicrograph of intestine 21 day post treatment of methyl parathion and 40 mg ascorbic acid showing crypt portion with a large number of cells. Dead mitotic cells are also present. X 400

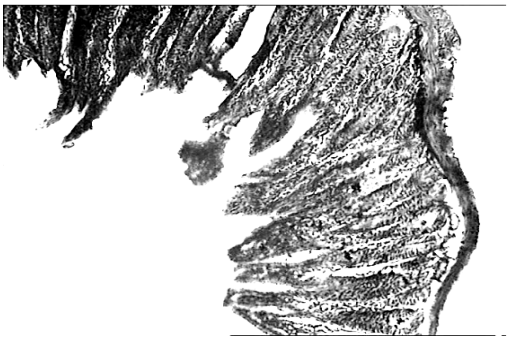

Fig. 6. Photomicrograph of intestine 28 day post treatment of methyl parathion and 40 mg ascorbic acid showing some normalization in the crypt region but the villi have dislodged tips. X 100

Fig. 7. Photomicrograph of intestine 14 day post treatment of methyl parathion and 80 mg ascorbic acid showing normal structure. X 400

Fig. 8. Photomicrograph of intestine 28 day post treatment of methyl parathion and 80 mg ascorbic acid showing normal structure and organization. X 100

Fig. 9. Photomicrograph of intestine 28 day post treatment of methyl parathion and 80 mg ascorbic acid showing normal crypt and villous region. X 100

and pyknotic cells was observed. The mean total cell count in the crypt region was also significantly ($p<0.001$) decreased in Group 2 as compared to Group 1 and a gradual increase in total cell count was observed in Group 3 and 4 but more promising results were observed in Group 4 (Figs. 10, 11and 12).

DISCUSSION

Apart from its role in preparing and absorbing nutrients, the intestine is the first line of

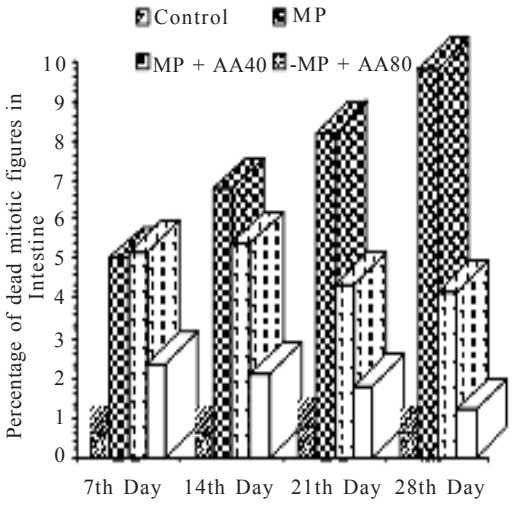

Fig. 10. Average dead mitotic cell count (%age) in intestinal crypt of control, pesticide treated and pesticide + antioxidant treated group

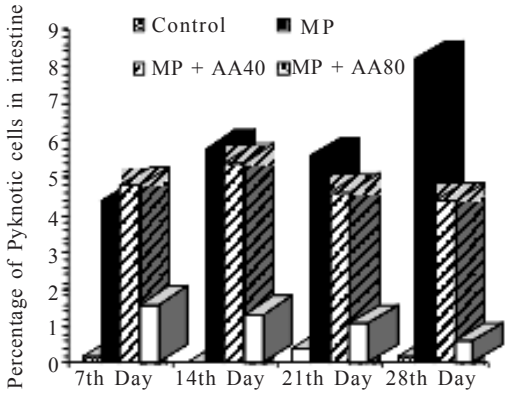

Fig. 11. Average pyknotic cell count (%age) in intestinal crypt among control, pesticide treated and pesticide + antioxidant treated groups

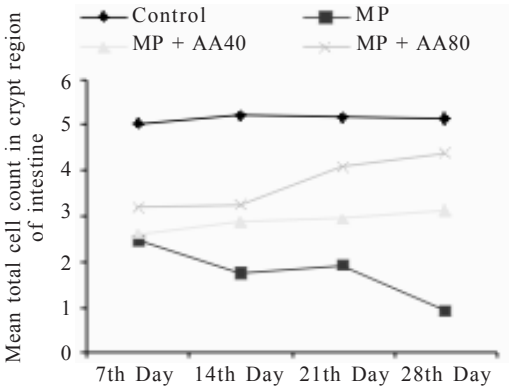

Fig. 12. Mean total cell count (%age) in intestinal crypt among control, pesticide treated and pesticide +

defense against chemical insult through oral route and intraperitoneal administration in abdomen. The circulatory system is closely associated with the intestinal tract, and therefore, entry of substances into the capillaries is rapidly affected. A major factor favoring absorption in the intestine is the presence of microvilli which increase the surface area for absorption.

Intestine constitutes the most sensitive tissue in the gastrointestinal tract because its cell population in the villi is dynamic and under normal conditions it is in a steady state. The cells are produced in the crypts, migrate up the villi and finally are sloughed off from the tips of the villi. The cells in the active mitosis are sensitive to stress. The outer covering of villi and inner lining of crypts of lieberkuhn are covered by one cell thick constant renewing epithelium. Accord-

ing to the Unitarian theory of the origin of epithelial cells of gastrointestinal tract, all the four main types of cells of intestinal epithelium originate from the stem pool (Cheng and Leblond 1974). There is a steady and constant flow of new cells from proliferative compartment into maturation zone, where they differentiate as they move up and out of the crypt to villi. This constant flow of cells is essential to provide a steady state relationship between proliferative and non – proliferative cells and to ensure an unbroken mucosal covering of the villi (Lesher 1967).

The present findings revealed that the intraperitoneal administration of methyl parathion produced various histopathological changes which include mitotic inhibition, dead mitotic figures, loss of villi, decrease in number of cells in crypt region, vacuolated and pyknotic nuclei in crypts and presence of notches in villi. Similar results were also reported by Sakr and Allail (2005), Khogali et al. (2005), Dede et al. (2007), Gera et al. (2009) and Rady (2009).

Similar histopathological changes lymphocytic infiltration, desquamation, hemorrhage and necrosis of epithelial cells of the stomach and intestine were also noticed by Manna et al. (2004) in rats treated with á – cypermethrin insecticide. *In vitro* study showed that the cultured intestinal and colonic cell proliferation was decreased by diazinon insecticide (Greenman et al. 1997). The infiltration of lymphocytes and cell lining proliferation in the intestine were earlier also reported by many investigators (Singh and Srivastava 1994; Abou Rabou 1996).

In the present study, mice treated with ascorbic acid showed a marked normalization in structure of small intestine. The group IV (80 mg/kg body weight of ascorbic acid treated) mice presented almost normal histology of intestine quite comparable to control mice (Group I). These results are in agreement with the studies of Higa et al. (2007). Many researchers have correlated morphological injury of intestine to the pathogenesis (Collard and Gelman 2001) due to deleterious effects of free oxygen radicals in ischemia / reperfusion injuries (Mecord 1985; Granger et al. 1986; Hirata et al. 1996; Collard and Gelman 2001). Thus, a group of antioxidants has been targeted as potential useful protective agents against morphological changes identified after small intestine injuries (Finkelstein et al. 1988). Cathcart (1985) and Nakamura et al. (1997) suggested that ascorbic acid washes out free radicals produced during ischemia and scavenges reactive oxidants produced immediately after reperfusion.

This is a well known fact that the sensitivity of cells varies with the phase of division. It has been suggested that cells in G phase (7 – 10%), M phase (35 – 40%) and early S phase (50 – 55%) are comparatively less sensitive, whereas the cells in mid and late S phase are most resistant (Lesher 1967). Cells of the villi never undergo DNA synthesis and it is believed that these lack a characteristic DNA content of G phase (Chen and Withers 1972).

In the present study, histometric counts of intestine showed a huge and highly significant (pd"0.001) increase in dead mitotic cell count in crypt region of Methyl Parathion treated mice (Group II) as compared to the control.

Thus, in the present research work, early cell death measured as the number of dead cells in the crypt region confirms the well known fact that the cell belonging to the dividing population are more prone to the oxidative damage. The similar rise in dead mitotic figure count was also observed by Sharma (1996) in irradiated intestinal tissue of mice.

Further, a gradual and continuous decrease in dead cell count and pyknotic cell count was observed in both Groups (III and IV) of mice treated with ascorbic acid, but more encouraging results were observed in Group IV mice provided 80mg/kg body weight of ascorbic acid. A decrease in total cell count in the crypt region of intestine was observed in the methyl parathion treated mice in comparison to the control. It can be accounted for the observed cell death and inhibited division of cells, and also to the continuous migration of the cells out of the villi (Sharma 1996, 2005).An increase in the total cell count was observed in the antioxidant treated mice and it touched near normal values in mice treated with (80mg/kg body weight) of ascorbic acid.

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