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Influence of Green Tea on Haematological and Lung Histological Disorders Induced by Malathion in Rats

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Abstract: The aim of the present study was investigation of antitoxin effects of green tea on malathion toxicity in rats. Forty eight rats were divided into 4 groups, control, extract green tea (1% w/v, daily), malathion (135 mg kg^{-1} i.p., daily) and rats injected with malathion and treated with crude green tea. After 5 weeks, the animals were weighed and autopsied for detailed histopathological and haematological studies on the last day after final dosing. Insignificant changes in blood hemoglobin was shown when compared to control except increase in WBC count ($p \leq 0.05$) and significant decrease in RBC count ($p \leq 0.05$). Histopathological findings of lung tissue samples were significantly different in comparison of controls and the experimental rats, where the normal alveolar structure in group 1, 2 and 4, but increased alveolar macrophages and interstitial neutrophil infiltration and minimal fibrosis were detected in malathion group. The repair and recover of lung tissue injuries occur in group 4 may due to the antioxidant, antitoxin and chemopretective properties of different components of green tea. So, the present study recommends that the using crude green tea or active biological components in prevention the toxicity of pesticides such as malathion.

Key words: Malathion, lung, toxicity, pesticides, histopathology, haematology

INTRODUCTION

Pesticides have been used in agriculture to enhance food production by eradicating unwanted insects and controlling disease vectors. Environmental pollution from organophosphate pesticides is an important issue that attracts widespread public concern. Residual amounts of organophosphate pesticides (OPs) have been detected in the soil, water bodies, vegetables, grains and other food products (John *et al*., 2001; Galloway and Handy, 2003). Malathion [O, O-dimethyl-S-(1,2-dicarbethoxyethyl) phosphorodithioate] is one of the most widely used organophosphate pesticides for agriculture and public health programs (Ahmed *et al*., 2000). As a result of the low persistence of OPs pesticides, they were introduced as replacements for the highly persistent organochlorine pesticides in the 1970s (Galloway and Handy, 2003). Malathion is known to induce excitotoxicity through its bioactivated analog, malaoxon (Hazarika *et al*., 2003).

The metabolism of malathion in bodies of vertebrates and invertebrates is complex. As a result of metabolic changes, with the contribution of phosphatases and carboxyesterases, many metabolites are produced (malaoxon) of varied toxicity (Marrs and Dewhurst, 2000).

An unusual effect has been observed from exposure to malathion that has not been observed with other pesticides. A single oral dose of the chemical trimethyl-phosphorothioate which is present in all malathion, at levels of 20 mg kg^{-1} body weight, was found to cause a reduction in lung cells in the bronchiolar epithelium of rat lungs. While the number of lung cells in a given area of lung tissue in

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normal rats was over 50, there were only approximately 20 cells found in the same area of lung tissue for rats exposed to the one dose of the malathion impurity Respiratory failure is the most common cause of death due to malathion poisoning. Narrowing of the bronchi and markedly increased bronchial secretions can occur. Respiratory failure results from respiratory depression coupled with paralysis of the respiratory muscles and progressive airway obstruction from bronchorrhea. In addition, pulmonary aspiration of the hydrocarbon solvents found in many commercial preparations can cause inflammation of the lungs. Children may be more vulnerable because of relatively higher minute ventilation per kg and failure to evacuate an area promptly when exposed (Fox, 1983).

Nowadays, there is considerable emphasis on identifying the potential of natural plant products as chemopreventive agents present in food consumed by the human population (Wattenberg, 1990; Kelloff *et al*., 1994). Many plant products exert antioxidative effects and some of these are widely used in food in different parts of the world (Sheela and Augusti, 1995; Khan *et al*., 1997).

The tea is one of the most popular beverages consumed worldwide. About 3 billion kg of tea is produced and consumed yearly. Tea, brewed from the plant *Camellia sinensis* is consumed in different parts of the world as green, black or Oolong tea. Green tea is favored in Japan and China and initial research on the benefits of green tea was carried out in these countries because of the local customs. Brewed tea contains many compounds, especially polyphenols and several studies show that polyphenolic compounds present in tea reduce the risk of a variety of diseases (Mukhtar and Ahmad, 1999, 2000). The possible beneficial health effects of tea are being extensively investigated and have received a great deal of attention in recent times. This study examines the effects of crud green tea on the changes in haematology and lung histology induced by malathion in rats.

MATERIALS AND METHODS

Forty eight healthy adult male rats of Wistar strain, weighing 150-180 g were used for experimentation in four groups 12 animal for each. Animals were maintained in groups of 5 or 6 per cage with food and water *ad libitum* and artificially lighted 12 h day^{-1} and temperature (25±2°C). The rats were randomly divided into four groups and treated for 5 weeks as follows. Group 1 control: rats fed on normal diet and received an injection of saline intraperitoneally; Group 2 crude green tea: rats were orally injected with 1% crude extraction of green tea per kg rats; Group 3 malathion: rats received 135 mg kg^{-1} b.wt. day^{-1} (Malathion was dissolved in saline (0.9% NaCl), mixed manually and administered intraperitoneally (i.p.)); Group 4 malathion + green tea crude: rats received 135 mg kg^{-1} b.wt. day^{-1} malathion along with 1% crude extraction of green tea. The animals were weighed and autopsied for detailed histopathological and haematological studies on the last day after final dosing.

Crude Extraction

The pan-fired green tea leaves were purchased from a local supermarket. With a tea/water ratio of 1/100 (w/v) and the tea leaves were steeped in water at 50°C for 20 min. The mixture was filtered with a 200 mesh sieve, and the filtrate contained in a beaker was cooled to 10°C in an ice water bath, and then centrifuged at 7200 g for 15 min to remove impurities (Wall *et al*., 1996).

Parameters Studied

Histological Studies

Animals were necropsed after 5 weeks. The blood and tissue Specimens from the lung were collected immediately after slaughter, the lung tissues fixed in 10% formalin embedded in paraffin wax, sectioned at 5 μm and stained with hemtoxylin and eosin (Drury and Wallington, 1980).

Haematological Studies

The haemoglobin content was estimated by standard procedure of Crossby *et al*. (1954). Also, The WBC and RBC were counted on neubauer haemocytometer (Lynch *et al*., 1969).

Statistical Analysis

Difference between the control and treated groups were evaluated statically by using Student's t-test. The data are expressed as mean± SEM. Significance was set at $p<0.05$.

RESULTS

Male rats exposed to malathion (135 mg kg^{-1} b.wt. day^{-1}) for a period of 5 weeks showed signs of lung toxicity. There were no significant differences in body weight at the end of the experimental period among the treated groups as compared with control (Table 1).

Administration of Malathion brought about no significant changes in blood hemoglobin when compared to control except increase in WBC count ($p\leq 0.05$) and significant decrease in RBC count ($p\leq 0.05$) (Table 2).

Histopathological findings of lung tissue samples were significantly different in comparison of controls and the experimental rats. Where, in control group, the large bronchi, the surface epithelium rests on a basement membrane, below which there is an elastin-rich layer of connective tissue; together these elements comprise the bronchial mucosa. Beneath the bronchial mucosa lies the submucosa, in which submucosal glands, cartilage, nerves, ganglia, and branches of the bronchial artery may be found. There is no clear histologic boundary between mucosa and submucosa. Lung of rats injected with malathion and treated with crude green tea were showed lung tissue as seen in normal lung of control rats (Fig. 1a, b and f). But in injected rats with malathion group, the main findings were neutrophil infiltration in the walls and spaces of the alveoli, pulmonary edema, pulmonary fibrosis, histiocyte infiltration in the alveoli, restructuring of alveolar walls and microgranulation. Examination of lung section of the injected rats with malathion and treated with green tea extracted illustrated the role of green tea in the protection of lung against the effect of the toxicity of malathion pesticide. Increased alveolar macrophages and interstitial neutrophil infiltration and minimal fibrosis were detected in malathion group. Microscopic examinations revealed epithelial desquamation and vacuolation in large and medium airways as well as terminal bronchioles. Cellular crowding expressed as an irregular epithelial lining and indicative of a very early hyperplasia and a reduction of apical blebs (Fig. 1c, d and e).

Table 1: Treatment schedule of rats body weight changes through the experiment

	Body weight (g)	
Parameters	Initial	Final
Group 1 (Control)	175.89±7.88	191.67±5.62
Group 2 (Green tea)	174.99±5.54	192.08±5.89
Group 3 (Malathion)	169.98±6.19	190.65±6.16
Group 4 (Malathion and crude green tea)	173.74±5.45	193.01±5.17

Table 2: Blood analysis of malathion treated rats

	Blood		
Parameters	Hemoglobin (%)	RBC (10^6 mm^{-3})	WBC (10^3 mm^{-3})
Group 1 (Control)	14.540±0.432	6.799±0.095	5.921±0.992
Group 2 (Green tea)	14.811±0.322	6.983±0.134	6.012±1.012
Group 3 (Malathion)	13.702±0.199^{NS}	5.490±0.160*	7.205±0.123*
Group 4 (Malathion and crude green tea)	14.034±0.545	6.576±0.157	6.732±0.972

NS: Non-significant, Values±SEM , *p- value<0.05 Significant

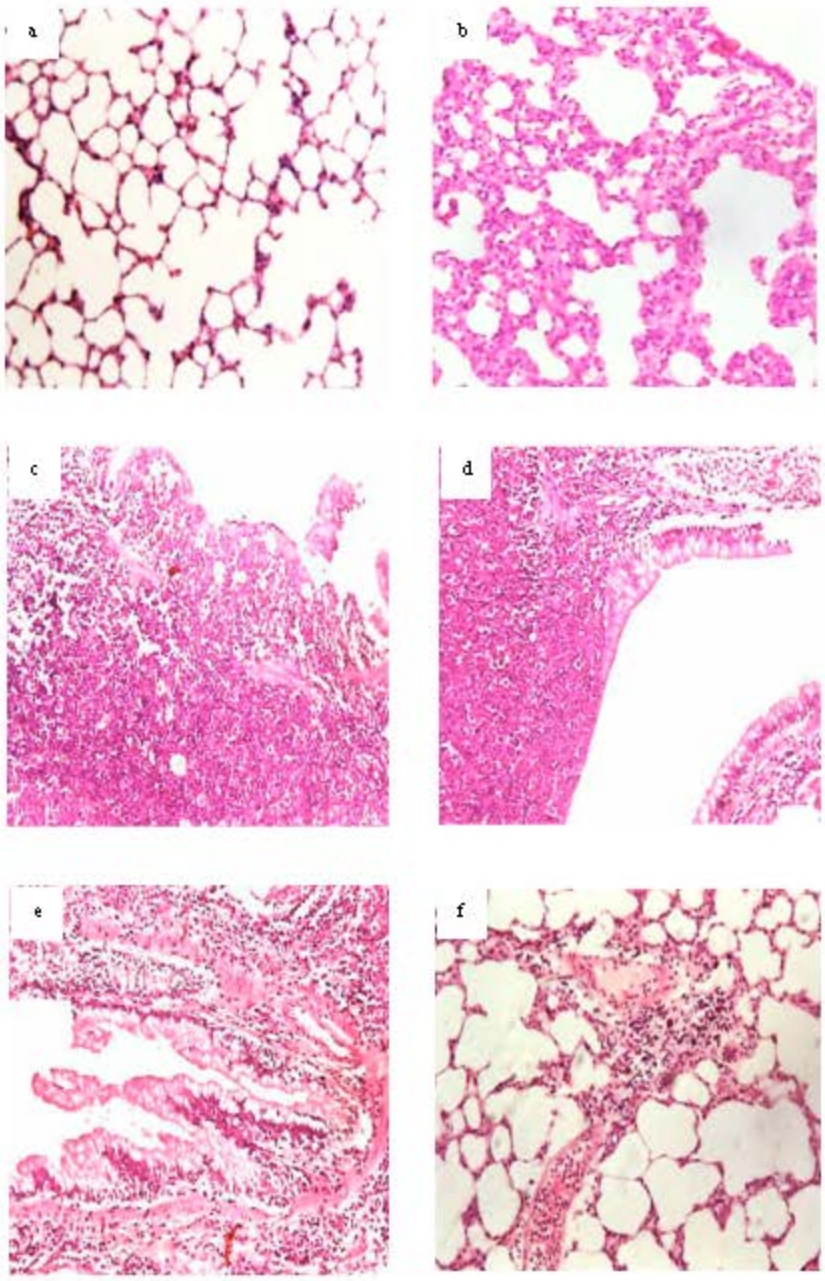

Fig. 1: There is simplification of airspaces structure (a, b) Alveolar cell hyperplasia. A reactive proliferation (alveolar cell hyperplasia) is common after injury. This case illustrates how this appears as a single row of cells protruding from the alveolar surface. Alveolar macrophages and interstitial neutrophil infiltration were present. The interstitium shows edema, inflammatory cells, and some fibrosis consistent with recent lung injury (c, d, e). Lung tissue in and (f) is seen as in normal lung of control rats. Hematoxylin and Eosin stain X 250

DISCUSSION

Herbal medicine uses any plant part such as the root, bark, stem, seed, flowers, or leaves as a means for treatment. Many of the modern drugs now used are based on native herbal wisdom. For example, medications like anthralin, aspirin, and alkaloids were originally herbal medications (John and Sumaira, 1998).

In any case and irrespective of the organophosphorus pesticides (Ops) type and its toxicity, the oxidative stress seems to be attenuated by non-enzymatic nutritional antioxidants such as vitamin E and C (Ahmed *et al*., 2000). In this regard, OP insecticides containing the P = S bond (called "thion ") are converted to P = O (called "oxon ") by a microsomal system of enzymes called mixed-function oxidases (MFO), among which the enzyme cytochrome P450 (CYP450) plays a major role. The oxons are highly toxic compounds that account for the profound cytotoxic effects of OP.

OPs cause severe environmental contamination and potential health hazards including acute and sub-chronic cases of human accidental poisoning (Curl *et al*., 2002; Abdollahi *et al*., 2004).

In the present study, there is no significant changes in blood hemoglobin when compared the treated groups to control except increase in WBC count ($p<0.05$) and significant decrease in RBC count ($p<0.05$). Siddiqui *et al*. (1987) reported that the total erythrocyte count reduction is due to physiological dysfunctioning of haemopoietic system as the results of poisoning with the pesticide. Also, increased leucocyte count was also reported (Padamaja *et al*., 2000).

In the histological investigations, the normal architecture of bronchi was present in group 1 and 2 and bronchi of rats injected with malathion and treated with green tea extract were showed similar tissue as previously seen in normal bronchi of control rats. Tos-Luty *et al*. (2003) reported that histopathologic studies of the lungs in animals which were applied a higher dose of the preparation widening of interalveolar septa was observed, with the presence of pulmonary phagocytes. In submicroscopic studies, after administration of both doses (oral and dermal doses of malathion), the endothelium of pulmonary alveoli was swollen.

The green tea contains agents that have chemopreventive activities. These include caffeine, flavandiols, flavanoids, phenolic acids as well as the alkaloids theobromine and theophylline, also, the polyphenols have the greatest effect with respect to chemoprevention (Lu *et al*., 2002). So, the repair and recover of lung tissue injuries occur in group 3 (rats were injected with malathion and treated with extract green tea) due to the antioxidant, antitoxin and chemopretective properties of different components of green tea. Also, Cao *et al*. (1996) reported that the antioxidant capacity of tea and tea polyphenols has been assessed by several methods. Using the Oxygen Radical Absorbance Capacity (ORAC) assay, it has been found both green and black tea have much higher antioxidant activity against peroxyl radicals than vegetables such as garlic, spinach and Brussels sprouts.

The present study may be the first investigation between the malathion toxicity in lung and the effects of green tea, so, it recommend that using crude green tea or active biological components in prevention the toxicity of pesticides such as malathion. Also, our findings open the door to future studies examining the pharmacological potential of green tea polyphenols in health and antitoxines.

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