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Original article

Taurine alleviates malathion induced lipid peroxidation, oxidative stress, and proinflammatory cytokine gene expressions in rats

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ABSTRACT

The present study was considered to evaluate the protective effect of taurine on malathion-induced toxicity in rats. Totally, 48 male rats were divided into 6 equal groups: 0.5 ml physiological salt solution was given orally to control rats. 0.5 ml corn oil was given orally to rats in corn oil group. Malathion at dose of 27 mg/kg (1/50 of LD_{50}) was dissolved in 0.5 ml corn oil and given to orally rats in malathion group. The other groups; malathion (27 mg/kg) and taurine (dissolved in 0.5 ml physiological salt solution) at dose of 50, 100, and 200 mg/kg were given orally to rats for 30 days, respectively. Malathion treatment decreased acetylcholinesterase levels in serum (30%) and liver (25%) compared to the control group. Malathion resulted in a significant increase in malondialdehyde levels whereas decreased glutathione levels, superoxide dismutase, and catalase activities in rats. Also, IF-γ, IL1-β, TNF-α, and NFκB mRNA expression levels were found to be increased 5, 1.7, 2.3, and 2.5 fold in malathion treated rats compared to control, respectively. However, treatment of taurine, in a dose-dependent manner, resulted in a reversal of malathion-induced lipid peroxidation, antioxidant enzyme activities, and mRNA expression levels of proinflammatory cytokines. Moreover, taurine demonstrated preventive action against malathion-induced histopathological changes in rat tissues. In conclusion, taurine exhibited a protective effect in rats against malathion-induced lipid peroxidation, besides it ameliorated antioxidant status, decreased mRNA expression levels of proinflammatory cytokine and repaired rat tissues.

1. Introduction

Organophosphate insecticides inhibit esterase activities, mainly acetylcholinesterase (AChE), resulting in drastic effects on central or peripheral nervous system. However, both short and long-term toxicologic studies often reveal non-anticholinesterase effects, such as delayed hepatic injury, polyneuropathy, genotoxicity, immunotoxic effects, endocrine effects, and developmental neurochemical and neurobehavioral impairments [1,2]. Malathion is an organophosphate insecticide that is usually used in agriculture and health pest control (mosquito eradication) in public areas [3] and it is known as a safe insecticide for mammalians [4]. In some cases, a residue of organophosphate insecticide can occasionally be found in soil, vegetables, water, and grains [5]. Malathion is converted to its metabolite malaoxon by cytochrome enzyme system in the liver after oral exposure. In addition, its toxicity was determined by the inhibition of AChE activity in serum and tissues. Malathion intoxication has led to oxidative stress due to the production of free oxygen radicals and decreases the antioxidant defense system in the organism [6,7].

Taurine is an essential amino acid for an organism but not utilized in protein synthesis, nevertheless it is found free or in simple peptides. Taurine has been used in the mammalian development and many in vitro studies implicated that lack of taurine is linked to some pathological lesions especially including retinal degeneration, cardiomyopathy, and growth retardation [8]. It plays a crucial role in a metabolic activity such as detoxification, membrane stability, bile acid conjugation, regulation of cellular calcium levels, and osmoregulation. Also, it has been used in many treatments of disease such as cystic fibrosis, hypercholesterolemia, epilepsy, Alzheimer, cardiovascular and hepatic disorders [9–11]. Moreover, some researchers suggest that taurine has a protective effect on oxidative stress caused by chemicals such as cyclosporine A [12], arsenic [13], and ethanol [14] in rats.

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The present study was aimed to the investigate the toxicity of malathion, organophosphorus pesticide, in terms of lipid peroxidation (LPO), oxidative stress, mRNA expression levels of proinflammatory cytokines in liver, and histopathological alterations in brain, kidney, liver, and testis in rats. Also, protective role of taurine against malathion intoxication was evaluated.

2. Materials and methods

2.1. Chemicals

Taurine and malathion were purchased from Sigma (Sigma-Aldrich, MO, USA) and Koruma Tarim A.S. (Kocaeli, Turkey), respectively. Also, other chemicals were of analytical purity and obtained from the manufacturer in Turkey.

2.2. Experimental design

Male Wistar albino rats, 2 months of age (250–350 g), were obtained from Afyon Kocatepe University Experimental and Research Center in Turkey. Animals were kept at 25 °C room temperature, % 50–55 humidity, and 12 h light/dark period. They also fed standard rodent diet and were given clear drinking water. Before the experiment, they were accommodated in animal cages for 7 days. This study was also approved by the local Animal Ethic Committee (49533702-62).

Totally, 48 rats were divided into 6 groups each with 8 rats in the experiment. 0.5 ml physiological salt solution was given orally to control rats. 0.5 ml corn oil was given orally to rats in corn oil group. Malathion at dose of 27 mg/kg [15] (1/50 of LD_{50}; dissolved in 0.5 ml corn oil) was given to orally rats in malathion group. The other groups; malathion (27 mg/kg) and taurine (dissolved in 0.5 ml physiological salt solution) at dose of 50, 100, and 200 mg/kg [16,17] were given orally to rats for 30 days, respectively. End of the experiment, tissue and blood samples were collected from animals under ketamine/xylazine anaesthesia. Blood samples from each group were collected by cardiac puncture into heparinised (for plasma) and non-heparinised (for serum) tubes. Antioxidant enzyme activities were determined in the erythrocytes, for this purpose blood samples were centrifuged at 600*g* for 15 min and plasma kept in a tube than erythrocytes were prepared according to the method of Winterbourn et al. [18]. Liver, kidney, brain, and testis tissue samples were divided into two sections for biochemical and histopathological analysis. Also, a part of the liver was taken for molecular analysis.

2.3. AChE, MDA, GSH, and antioxidant enzymes analyses

Serum and liver AChE activity as a parameter of organophosphate intoxication was assayed with rat AChE ELISA kit (SunRed, Shanghai, China). Tissue and blood malondialdehyde (MDA) levels were measured according to Ohkawa et al. [19] and Draper and Hardley [20], respectively. Reduced glutathione (GSH) levels were measured according to Beutler et al. [21]. Erythrocyte and tissue superoxide dismutase (SOD) activities were determined according to Sun et al. [22]. Catalase (CAT) activities were also determined according to Luck [23] in erythrocyte and Aebi [24] in tissues. Protein and haemoglobin levels were measured according to Drabkin and Austin [25] and Lowry et al. [26], respectively. UV–vis spectrophotometer (Shimadzu 1601) was used for these analyses.

2.4. Determination of mRNA expression levels of proinflammatory cytokines

Total RNA was extracted from fresh-frozen liver tissues using Tripure Reagent (Roche, Germany) according to manufacturer's protocols. Isolated RNA was quantified using a Nanodrop ND-1000 (Thermo, USA). The range of ratio was determined at $OD_{260/280}$ between 1.8 and 2.0. Total RNA (1 μg) was reverse transcribed in a 20 μl reaction mixture using RT^2 HT First Strand kit (Qiagen, Germany) according to the manufacturer's instructions. Specific primers were obtained from Ella Biotech GmbH (Martinsried, Germany) and designed for the amplification of NFκB (forward: 5′-GGGACTATGACTTGAA TGCGGTCC −3′, reverse: CAGGTCCCGTGAAATACACCTCA-A), TNF-α (forward: 5′-CTTCTGTCTACTGAACTTCGG-3′, reverse: GTGCTTGATCT GT-TGTTTCC), IFN-γ (forward: 5′-CACGCCGCGTCTTGGT-3′, reverse: TCTAGGCTTTCAAT-GAGTGTGCC), IL-1β (forward: 5′-CACCTCTCAA GCAGAGCACAG-3′, reverse: GGGTTCCATGGTGAAGTCAAC) and GAPDH (GAPDH forward: 5′-ACCACAGTCCATGCCATCAC-3′, reverse: TCCACCACCCTGTTGCTGTA). qRT-PCR was performed on a Rotor-Gene Q using RT^2 qPCR SYBR Green ROX mastermix (Qiagen, Gemany). The gene expression levels in each sample analysed in triplicate and normalized to the expression level of GAPDH. The results are expressed as relative gene expression using the $2^{-\Delta\Delta Ct}$ method.

2.5. Histopathological analyses

Liver, kidney, brain, and testis tissues were taken in 10% formalin solution for histopathological analysis and fixed in 10% formalin solution for 48 h. The tissue was dehydrated by passing through graded alcohol (from 70% to 100%). Tissues were cleaned in xylene and embedded in paraffin. Then, tissues were separated by 5–6 μm sections and stained with hemotoxylin-eosin (H & E). As a result, each section was examined under a light microscope (Microscopic Digital Image Analysis System with Olympus BX51 and DP20, Tokyo, Japan).

2.6. Statistical analyses

Data obtained from experimental animals were expressed as means and standard deviation of means (± SD) and analysed using one-way analysis of variance (ANOVA), followed by Duncan test on the SPSS (20.0) software computer program. A difference in the mean values of $p < 0.05$ was considered to be significant.

3. Results

3.1. Effect on AChE, MDA, GSH, and antioxidant status

Organophosphorus substances inhibit AChE activity, resulting in a decreased enzyme activity. In this study, it was determined that malathion treatment decreased AChE levels at 30% in serum and 25% in liver compared to the control group (Table 1) ($p < 0.05$). In contrast, taurine treatment in a dose-dependent manner improved AChE levels at 16–33% in serum and 15–25% in liver ($p < 0.05$). The level of MDA is widely used as a marker of free radical-mediated LPO. A significant increase in the levels of whole blood, liver, testis, brain and kidney MDA levels was observed in malathion group ($p < 0.05$). However, depending on the dose of taurine, MDA levels in whole blood and tissues were significantly

Table 1
The effect of malathion (27 mg/kg) and 50, 100, and 200 mg/kg taurine (T) plus malathion (27 mg/kg) on acetylcholinesterase levels in serum of rats.

Groups	Serum (pg/ml)	Liver (pg/ml)
Control	60.24 ± 5.38^{a}	43.01 ± 4.39^{a}
Oil	59.22 ± 4.07^{a,x}	41.85 ± 4.02^{a,x}
Malathion	42.71 ± 2.75^{c,z}	32.55 ± 4.12^{b,z}
T_{50} + Malathion	49.89 ± 5.45^{b,y}	37.21 ± 6.60^{ab,yz}
T_{100} + Malathion	55.97 ± 4.93^{a,x}	39.96 ± 7.65^{a,x}
T_{200} + Malathion	56.49 ± 7.46^{a,x}	40.05 ± 5.47^{a,x}

Mean ± standard deviation; n = 8; ^{a,b,c} In the same column values with different letters show statistically significant differences compared to control ($p < 0.05$); ^{x,y,z} In the same column values with different letters show statistically significant differences compared to oil ($p < 0.05$).