European Review for Medical and Pharmacological Sciences 2012; 16(Suppl 3): 144-148

Effects of sulforophane and curcumin on oxidative stress created by acute malathion toxicity in rats

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Abstract. – BACKGROUND AND OBJECTIVES, Organophosphate insecticides (OPIs) are widely used in agriculture and horticulture for controlling insects in crops, ornamentals, lawns, fruits, and vegetables. But, there have not yet any study about effects of sulforophane (SFN) and curcumin (CUR) on the oxidative stress created by acute toxic effects of malathion (MAL) as an OPI often causing human and animal poisoning.

AIM, The aim of this study was to investigate the effects of SFN and CUR on the oxidative stress created in the lung, liver, and kidney tissues of rats by acute MAL toxicity.

MATERIALS AND METHODS, Thirty-six mature Sprague Dawley rats weighing 200-250 g were used. The rats were randomly divided into six groups: unmedicated control, SFN, CUR, MAL control, MAL + SFN, and MAL + CUR. Tissue samples were analyzed for glutathione (GSH), malondialdehyde (MDA), and nitric oxide (NO) levels in the lung, liver, and kidney tissues. Biochemical parameters were measured colorimetrically by using a spectrophotometer.

RESULTS, No statistically significantly difference was found when comparing the unmedicated control, SFN, and CUR groups. MAL significantly increased MDA levels in the liver and kidney tissues, but SFN and CUR these levels. MAL did significantly reduce the GSH levels, but SFN and CUR increased these levels by blocking the MAL effect in the liver tissues. Also, MAL significantly increased the NO levels, depending on the severity of the tissue damage, and SFN and CUR attenuated to NO levels and remained under the effect of MAL.

CONCLUSIONS, SFN and CUR, which showed similar effects, could be used to protect against the oxidative stress caused by acute malathion intoxication.

Key Words:

Curcumin, Malathion, Organophosphate, Oxidative Stress, Sulforophane.

Introduction

Organophosphorus insecticides (OPIs) are primarily recognized for their neurotoxic effects in mammals through the inhibition of acetylcholinesterase (AChE), which leads to the accumulation of acetylcholine[1]. Recent evidence suggests that OPIs may exert direct actions on the muscarinic and nicotinic receptors when their concentrations in the circulation exceed the micromolar level[1,2].

Malathion (MAL), or O-dimethyl-S1-2-di(ethoxycarbonyl)-ethylphosphorodithioate, was selected as the toxic material in this study. This OPI is widely used in agriculture and houses, as well as in public health programs for the control of the vectors of diseases. However, MAL is a major cause of poisonings and deaths, mainly in developing countries[3,4]. Recent studies have related the acute and chronic exposures to malathion and their effects on the induction of oxidative stress in the blood and the various tissues of rats[5,6].

Although many studies have investigated how MAL causes oxidative stress, these researches provide insufficient information on the treatments for the oxidative stress caused by MAL or on the medications with potentially protective effects. Therefore, this work aimed to examine the potentially protective effects of sulforophane (SFN) and curcumin (CUR) against the oxidative damage created simultaneously in the lung, liver, and kidney tissues by malathion. CUR has been shown to have antioxidant activity at a level comparable to Vitamins C and E. MAL is known to facilitate the discarding of several reactive oxygen radicals, particularly superoxide anion radicals with nitrogen dioxide radicals and hy-

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droxyl radicals[7-9]. SFN, an isothiocyanate, anticarcinogen, found in cruciferous vegetables such as broccoli and brussel sprouts, was first identified as a potent inducer of phase 2 detoxification enzymes[10,11]. One exciting new avenue of SFN research focuses on the inhibition of histone deacetylase (HDAC) activity[12,13]. SFN was first reported to inhibit HDAC activity in human colon cancer cells[14] and then in various human prostate lines[15]. SFN retarded the growth of prostate cancer xenografts[16] and suppressed spontaneous intestinal polyps in mice[17].

This research had three basic aims:

1. To research the oxidative stress created by malathion in rats' lung, liver, and kidney tissues;
2. To compare the effects of SFN and CUR used alone on the oxidative mechanism;
3. To define whether SFN and CUR treatment was beneficial as a new protective medication for acute MAL toxicity.

Materials and Methods

Animals, Care, and Nutrition

Thirty-six mature female Sprague Dawley rats weighing 200-250 g were randomly divided into six groups of six rats each. The animals were kept under laboratory conditions with a 12-hour light/dark cycle and a room temperature of 21±3ºC. The study was approved by the Selcuk University Experimental Medical Research Center's Experimental Animals Ethics Committee.

Animals and Treatment

36 rats were randomly divided into six groups: unmedicated control, SFN control (Sigma S4441 – 0.5 mg/kg via oral [po]), CUR control (*Curcuma longa* Turmeric/Sigma C1386, 1000 mg/kg via po), MAL (Fluka-36143) control (200 mg/kg via po), MAL + SFN, and MAL + CUR. Twenty-four hours after the administration of the medication, the animals were anesthetized with ketamine (50 mg/kg via intra peritoneum [ip]) + xylazine (5 mg/kg dose ip) and then sacrificed. Measurements were taken of malondialdehyde (MDA), glutathione (GSH), and nitric oxide (NO) in the lung, liver, and kidney tissues.

Biochemical Parameters

Tissue samples from the liver, lungs, and kidneys were collected for analysis of GSH, MDA, and NO concentrations. The tissues were immediately removed and washed with 0.15M KCl (at 4°C). The tissues were then homogenized at ice-cold temperatures (A: 50 mM, H2PO4 ve B: 50 mM $Na_2HPO_4.2H_2O$/A:B (v/v) = 1:1.5) by a homogenizator at 290 g for three minutes. The homogenates were centrifuged at 2400 g at 4°C for 15 minutes. The supernatants were stored at –25°C until they were analyzed. NO was measured colorimetrically using a spectrophotometer (PowerWave XS, BioTek Instruments, Winooski, VT, USA) by the method of Miranda et al[18]. For GSH and MDA concentrations, the UV-1201 (Shimadzu, Kyoto, Japan), the Beutler et al[19], and the Yoshoiko et al[20] methods were used.

Statistical Analysis

The data for the biochemical parameters were analyzed by ANOVA, followed by the post hoc Tukey test. All data was presented as mean ± SE using SPSS Windows 10.0 (Chicago, IL, USA). A value of $p < 0.05$ was considered statistically significant.

Results

The results obtained and the statistical analyses of the results are shown in the Tables I, II. No statistically significant difference was determined between the groups in a comparison of the effects on MDA, GSH, and NO parameters in the liver, kidney, and lung tissues in the control, SFN, and CUR groups. SFN and CUR used alone did not create a negative effect on MDA, GSH, and NO levels in the lung, liver, and kidney tissues. Both medications showed similar effects on these enzymes, so their use alone with respect to an oxidant-antioxidant defense mechanism did not generate any problem (Table I).

In a comparison of the malathion, MAL + SFN, and MAL+ CUR groups, the MDA levels in the liver and kidney tissues were significantly high in the groups that had been given MAL, but by blocking the toxic effect of malathion, SFN and CUR significantly reduced the MDA levels. The GSH levels in liver tissues were significantly reduced by MAL, but SFN and CUR increased the GSH levels by blocking the toxic effects of malathion. When the NO levels were examined, the levels of the groups given malathion were significantly high, and SFN and CUR were determined as having remained under the influence of MAL. The reason for this originated from the excessive increase in NO expression, depending on the severity of tissue damage created by malathion (Table II).

Table I. The effects on MDA (nmol/g protein), GSH (μg/g protein) and NO (nmol/g protein) in liver kidney and lung tissue in the control, SFN and CUR groups.

Parameters		Control (n = 6)	SFN (n = 6)	CUR (n = 6)	*P*
Liver	MDA	16.41 ± 1.32	14.99 ± 2.5	11.32 ± 0.68	Ns
	GSH	15.53 ± 1.05	15.36 ± 2.14	16.29 ± 1.11	Ns
	NO	275.55 ± 41.40	257.54 ± 27.95	212.86 ± 23.48	Ns
Kidney	MDA	8.78 ± 0.88	8.82 ± 0.38	8.92 ± 2.29	Ns
	GSH	8.35 ± 0.36	8.31 ± 0.67	8.29 ± 0.48	Ns
	NO	271.96 ± 24.0	279.62 ± 30.68	276.96 ± 117.43	Ns
Lung	MDA	25.56 ± 2.80	25.67 ± 3.36	25.35 ± 2.38	Ns
	GSH	15.44 ± 1.85	17.31 ± 2.20	21.07 ± 1.34	Ns
	NO	862.23±138.8	851.96 ± 216.53	861.86 ± 120.22	Ns

Ns: not statistically significant.

Discussion

Pesticides have been shown to induce oxidative stress that leads to the generation of free radicals (FR) and changes in the antioxidant or oxygen-free radical scavenging systems in cells. Bagchi et al[21] showed that different classes of pesticides induce the production of reactive oxygen species (ROS) and oxidative tissue damage. Several reports have indicated that the activities of enzymes associated with antioxidant defense mechanisms are altered by insecticides, *in vivo* and *in* vitro[22-25]. However, there are not yet sufficient studies on the oxidative stress caused by malathion in kidneys, liver and luıngs tissues at the same time and on the medications with potential protective effects against the toxic effect of MAL. Especially, there have not yet studies about the protective effects of SFN and CUR against the acute toxic effects of MAL. SFN is an important isothiocyanate found in cruciferous vegetables, such as broccoli and brussel sprouts used frequentlly by people. For this aim we have investigated the effects of SFN and CUR both used alone and with MAL on MDA, GSH and NO levels in lung, liver and kidney tissues at the same time.

Lipid peroxidation (LPO) has been suggested as one of the mechanisms of pesticide-induced toxicity. MDA is a major oxidation product of LPO polyunsaturated fatty acids and increased MDA content is an important indicator of LPO. As has been shown, pesticides increase MDA levels in tissues[22,26-28].

The acute exposure of rats to dimethoate was found to alter the activity of several antioxidant enzymes in their livers and brains[29]. Following acute administration of chlorpyrifos in rats, the levels of thiobarbituric acid reactive substances (TBARS) increased in the livers[25], brains[30], and kidneys[31]. High doses of diisopropylfluorophosphate were found to trigger excessive NO production in the brain[32]. Gültekin et al[33] reported that after the application of the

Table II. The effects on MDA, GSH and NO levels in liver kidney and lung tissue in the MAL + SFN, MAL + CUR and MAL groups.

Parameters		Control (n = 6)	MAL (n = 6)	MAL + SFN (n = 6)	MAL + CUR (n = 6)	*P*
Liver	MDA	16.41 ± 1.32^{b}	22.82 ± 1.53^{a}	14.50 ± 2.1^{b}	13.55 ± .93^{b}	< 0.01
	GSH	15.53 ± 1.05^{b}	10.05 ± 0.91^{c}	15.22 ± 0.70^{b}	18.33 ± 0.35^{a}	< 0.01
	NO	235.55 ± 41.40^{b}	240.77 ± 95.4^{b}	241.20 ± 57.9^{b}	242.57 ± 23^{b}	< 0.01
Kidney	MDA	10.78 ± 0.88 b	19.64 ± 1.11^{a}	16.45 ± 1.40^{a}	15.27 ± 3.4^{a}	< 0.01
	GSH	9.95 ± 0.36	9.70 ± 1.12	13.62 ± 1.60	11.08 ± 2.5	Ns
	NO	271.96 ± 24.0^{b}	574.38 ± 37.5^{a}	551.26 ± 67.4	503.6 ± 46.7^{a}	< 0.01
Lung	MDA	25.56 ± 2.80	25.82 ± 2.4	21.95 ± 1.9	19.51 ± 1.2	Ns
	GSH	15.44 ± 1.85	15.39 ± 0.9	15.46 ± 1.4	15.53 ± 1.18	Ns
	NO	862.23 ± 138.8	956.34 ± 188	923.72 ± 153	931.74 ± 51	Ns

Ns: not statistically significant. ^{a,b,c}Mean values with same superscripts (a, b, c) showed similar results.

OPI, chlorpyrifos, the MDA level increased in erythrocytes and this could be the cause of ROS and FR. In this study, the acute application of MAL induced oxidative stress and increased MDA levels in kidney and liver tissues. Also, SFN and CUR, by blocking the toxic effect of malathion, significantly decreased the MDA levels.

Nitric oxide (NO) has been implicated in the mechanisms of cell injury and long-term physiological changes in cellular excitability. Yildirim et al[34] found that the NO level in kidney tissue was increased by cisplatin management and erdosteine prevented this increment at a statistically significant level. Increasing evidence has suggested that NO has an important role in modulating oxidant stress and tissue damage. Peresleni et al[36] showed that oxidant stress to the epithelial cells caused an increase in immunodetectable inducible NO synthase (iNOS), which resulted in an elevation in NO release and nitrite production and decreased cell viability[35]. In this study, likewise to Yildirim et al[34] and Peresleni et al[35], the NO levels of the groups that had been given MAL were significantly increased and SFN and CUR were determined as having remained under the influence of malathion. Therefore, the reason for this finding originated from the excessive increase in NO expression depending on the severity of the tissue damage created by malathion.

Reduced glutathione (GSH), which is the other marker agent of this research, has a major role in cellular defense and in the detoxification of reactive intermediates and oxygen radicals. Increased activity of GSH is known to serve as protective response to eliminate xenobiotics[36]. Elevated GSH activity may reflect the possibility of better protection against pesticide toxicity, and it is used as a biomarker for environmental biomonitoring. When GSH activity is inhibited, LPO products accumulate. GSH plays a primary important role in cellular detoxification of toxic aldehydes[37,38]. In a study by Ahmed et al[39] on male rats exposed to the OPI, MAL, while an increase was observed in serum LPO and full-blood glutathione-S-transferase and glutathione reductase activities, a decrease in serum GSH content was referred[39]. In our study, likewise to Ahmed et al[39], GSH levels of the groups that had been given MAL had statistically significantly decreased, but in the groups to which SFN and CUR had been administered, the process of blocking the MAL effect had significantly increased the GSH level in the liver tissues.

In this comparison of all the present data to define the sensitivity of the organs and the effects of medications against malathion, it was determined that the liver was the most sensitive organ against the toxic effects of MAL as the first point of exposure to the drugs. Moreover, the kidneys were more sensitive than the lungs to malathion. Finally, when a comparison was made of the effects of SFN and CUR on GSH, MDA, and NO parameters, it was observed that medications used alone and at the same time against the acute MAL intoxication showed similar effects on oxidative parameters and had a protective effect.

Conclusions

The use of SFN and CUR alone did not have any negative effect or cause oxidative stress in the lung, liver, and kidney tissues, and they could be used for protective purposes against oxidative stress and tissue damage caused by malathion.

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