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Original Article

Effects Of Curcumın, Sulforaphane And Intralıpıd İn The Management Of Organophosphate Toxİcİty

Organofosfat Zehirlenmelerinde Kurkumin, Sülforafan ve İntralipidin Etkilerinin Değerlendirilmesi

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Abstract

Background: Organophosphates (OP) are a group of chemical agents with frequent exposure due to accidents, suicide attempts or occupational reasons consisting of agricultural and industrial usage. The aim of the present study is to examine the influence of curcumin (CUR), sulforaphane (SFN) and intralipid treatments with known antioxidant effects in OP poisoning.
Material and method: In the experimental study, an OP toxicity model was created by administering a dose of 30 mg / kg p.o malathion (MAL) to 30 rats divided into five equal groups, excluding the control group. Electrocardiography (ECG) and electromyography (EMG) examinations were performed on all rats at the 2nd and 12th hours and CUR, SFN and intralipid were administered in certain doses to rats other than the control group. In the liver and kidney tissue samples taken after the rats were sacrificed at the 24th hour; Superoxide Dismutase (SOD), Active Glutathione (GSH), Malondialdehyde (MDA) levels were assessed, whereas in the serum; Alanine aminotransferase (ALT), Aspartate transaminase (AST), urea, creatinine and pseudocholinesterase (PChE) levels were studied.
Results: PChE levels of the rats were significantly lower in the OP group, and ALT and AST levels were significantly higher. There was no significant difference in the levels of SOD, MDA and GSH. ECG and EMG results were evaluated as normal in all groups.
Conclusion: the present study, it is thought that CUR may have a therapeutic effect on liver tissues in OP toxicity, while SFN and intralipid may be effective in liver tissues by showing antioxidant properties and reducing organophosphate-induced pseudocholinesterease suppression, respectively. Additionally, it is suggested that ECG and EMG alone are not sufficient in evaluating cardiotoxicity and neurotoxicity in the acute period.
Keywords: Organophosphate poisoning, Curcumin, Sulforaphane, Intralipid

ÖZ

Amaç: Organofosfatlar, tarım ve sanayide kullanılan, kaza, intihar amaçlı veya mesleksel nedenlerle insanların da sıkça maruz kaldığı bir grup kimyasal ajandır. Çalışmamızın amacı organofosfat zehirlenmesinde, antioksidan etkinliği bilinen CUR, SFN ve intralipid tedavisinin etkilerini incelemektir.
Gereç ve Yöntem: Deneysel olarak yapılan çalışmamızda beş eşit gruba ayrılmış 30 adet rata, kontrol grubu hariç tutularak 30 mg/kg p.o. dozunda MAL verilerek organofosfat zehirlenmesi modeli oluşturuldu. Tüm ratlara, 2. ve 12. saatte elektrokardi EKG ve EMG tetkikleri yapıldı ve yine bu saatlerde kontrol grubu dışındaki ratlara CUR, SFN ve intralipid maddeleri belirli dozlarda verildi. 24. saatte ratlar sakrifiye edildikten sonra alınan karaciğer ve böbrek dokularında; SOD, GSH, MDA düzeyleri, serumda ALT, AST, üre, kreatinin ve PChE düzeyleri çalışıldı.
Bulgular: Ratların PChE düzeyleri organofosfat grubunda anlamlı şekilde düşük, ALT ve AST düzeyleri ise anlamlı olarak yüksek bulundu SOD, MDA ve GSH üzerinde anlamlı etki oluşmadı. EKG ve EMG tüm gruplarda normal olarak değerlendirildi.
Sonuç:Çalışmamızda, organofosfat zehirlenmesinde, kurkuminin karaciğer dokularında iyileştirici bir etkisinin olabileceği, sülforafanın karaciğer dokularında antioksidan özellik göstererek, intralipidin de organofosfata bağlı psödokolinestereaz baskılanmasını azaltarak etkin olabileceği düşünülmektedir. Bunun yanında akut dönemde kardiyotoksisite ve nörotoksisiteyi değerlendirmekte EKG ve EMG'nin tek başlarına yeterli olmayacağı öngörülmektedir.
Anahtar Kelimeler: Organofosfat zehirlenmesi, Kurkumin, Sülforafan, İntralipid

INTRODUCTION

Organophosphate (OP) compounds are highly fat-soluble compounds formed as a result of the reaction of alcohol and phosphoric acid. They can be absorbed from the skin, conjunctiva, oral mucous membranes, gastrointestinal and respiratory tracts. The severity and duration of intoxication varies depending on the dose taken, the route of intoxication, chemical structure of the OPs and the metabolic rate of the body (1-2).

The main mechanism of action of OP compounds is that they cause excessive stimulation at the cholinergic junction by inhibiting serum acetylcholinesterase (AChE) and cholinesterase enzymes (3). The measurement of AChE levels is a valuable diagnostic criterion that can be used as a mortality and morbidity marker (4,5). Pesticides have been shown to induce oxidative stress by causing the formation of free radicals and leading to changes in the antioxidant or oxygen-free radical scavenger systems of cells (6).

Curcumin (CUR), which is obtained from the Curcuma longa plant, is a spice of the polyphenol super family, widely used in India. Numerous studies have shown that CUR has antioxidant, anti-inflammatory, and anti-cancerous properties (7).

Sulforaphane (SFN) is an anti-carcinogenic compound, first discovered to be a potent phase-2 detoxification enzyme activator, found in vegetables such as broccoli and Brussels sprouts (8,9).

Lipid emulsion is a compound containing soybean oil, egg phospholipids and glycerin, used parenterally in patients whose oral nutrition is not adequately provided. Lipid emulsion is generally referred to by its brand name; intralipid. It is used in different concentrations including 10%, 20%, 30% and it contains linoleic acid, omega 6, omega 3, and alpha-linolenic acid (10).

The aim of this study is to reveal the possible protective effects of CUR, SFN and intralipid solution against the damage on the heart, liver and kidney tissues caused by malathion (MAL) poisoning, which is one of the derivatives of OP.

MATERIALS AND METHODS

In this study, 30 Wistar Albino rats weighing 250-300 g were used. The rats were fed with ready-made pellet rat food under standard conditions (21-22 ° C, 55-65% humidity, 12 hours light-12 hours dark) and there were no limitations on the consumption of drinking water.

The rats were divided into five groups of six. Group 1 was determined as the control group and there were no administrations, while the other groups received 30 mg / kg OP (p.o.). Group 2 rats received no additional agent. Group 3 rats were given the first dose of CUR (100 mg / kg p.o.) 2 hours after the OP administration and CUR (100 mg / kg p.o.) at the 12th hour. Group 4 rats were administered OP + SFN 2 mg / kg (i.p) in 2 doses at the 2nd and 12th hours. Group 5 rats received intralipid fat emulsion (18.6 mg / kg p.o) at the 2nd and 12th hours. Electrocardiography (ECG) recordings were performed twice; at the 2nd hour (onset) and the 12th hour. After the rats were anesthetized with 40 mg / kg ketamine and 4 mg / kg xylazine, ECG recordings were performed in the prone position. The data were evaluated using the BIOPAC System. The P wave, P-R distance, QT interval, T wave duration and heart beats per minute were calculated. Any arrhythmias and findings outside of the normal durations and amplitudes were interpreted as abnormal ECG findings. Bazzet formula was used to calculate QTc. Results were analyzed via the Mann-Whitney U Test. After the ECG recordings were completed, electromyography (EMG) recordings were started without any additional anesthetics. EMG recordings were performed on all groups 2 times in total; at the 2nd hour (onset) and at the 12th hour. BIOPAC MP 100 Acq. system (Santa Barbara, USA) was used for the EMG recordings by the supramaximal stimulation of the right sciatic nerve with a bipolar subcutaneous needle. The results were evaluated using the BIOPAC Acq. Knowledge software and analyzed by the Mann-Whitney U test. At the end of the 24th hour, venous blood samples were taken into EDTA tubes to evaluate the biochemical parameters (ALT, AST, PChE, urea and creatinine) of the rats. Rats were sacrificed following the administration of 40mg / kg pentobarbital. Kidney and liver tissue samples were taken from the postmortem rats.

Statistical Analysis

Obtained findings (ALT, AST, urea, creatinine, PChE, SOD, GSH (Active Glutathione) and MDA) statistical calculations were made using the SPSS 10.0 package program. The data obtained in the study were expressed as “mean ± standard deviation” (X ±SD). By applying analysis of variance (ANOVA) and Tukey's HSD (honestly significant difference) test in groups statistical relationship was determined. $P< 0.05$ was accepted for statistical significance. ECG and EMG findings were compared using the Mann-Whitney U test.

RESULTS

The highest AST value was found in the OP group, while the lowest value was found in the control group. The increase in the OP group compared to the control group was statistically significant. Although the AST levels were observed to be lower in the other

groups in comparison to the OP group, this difference was not statistically significant. The ALT increase in the OP group was found to be statistically significant compared to the control group, while the lowest ALT value in the OP + SFN group was not statistically significant compared to the other groups as presented in Table1.

Table 1. AST, ALT, UREA, Creatinine, PChE Measurements in Serum

GROUPS	AST U/L	ALT U/L	Urea mg/dl	Creatinine mg/dl	PChE U/L
Group 1 (Control)	80.5±3.2 p:0,630	68.7±1.9 p:0,057	15.0±1.2 p:0,088	0.3±0.0 p:1,924	838.4±0.0 p:0,916
Group 2 (OP)	799.4±2.0* p<0,05	257.9±0.9* p<0,05	17.3±3.3 p:1,934	0.4±0.0 p:3,280	136.0±0.0* p<0,05
Group 3 (OP+CUR)	387.0±2.5 **, ¥, Ω p<0,05	183.6±0.0 p:0,560	18.0±1.0 p:0,297	0.3±0.0 p:0,523	390.2±0.0 p:0,190
Group 4 (OP+SFN)	533.9±1.6 p:0,093	110.5±0.2 p:0,542	22.8±2.1 p:0,562	0.4±0.0 p:0,733	583.3±0.0 p:0,779
Group 5 (OP+LIPID)	562.0±2.2 p:0,183	201.1±0.2 p:0,954	25.3±3.4 p:1,180	0.4±0.0 p:0,994	793.5±0.0 **,#,¥ p<0,05

p <0.05 was considered significant.PChE: Pseudocholinesterase OP: Organophosphate, SFN: Sulforaphane, LIPID: Lipid emulsion, CUR: Curcumin. * p <0.05 different from control group; #p <0.05 different from OP + CUR group
** p <0.05 different from OP group; ¥ p <0.05 different from OP + SFN group. Ωp <0.05 different from OP + LIPID group

The level of urea, which is an indicator of kidney function, was measured the lowest in the control group. The highest urea value was in the OP + LIPID group, however this difference was not statistically significant compared to the control group (Table 1). There was no statistically significant difference between the groups in terms of urea and creatinine values.

In the present study, the highest and lowest levels of PChE were observed in the control group and OP group, respectively and this difference was statistically significant. The highest PChE value after the control group was recorded in the OP + LIPID group. The fact that this value was significantly higher compared to the OP group was in favor of LIPID preventing PChE suppression. The values in the OP + CUR and OP + SFN groups were found to be lower than the OP + LIPID group, while there was no significant difference compared with the OP group (Table 1).

There was no statistically significant difference between the groups in terms of SOD and MDA levels in the liver tissue. The highest GSH level was measured in the control group, while the lowest value was measured in the OP group and this difference was statistically significant compared to the control group (p <0.05) as presented in Table 2.

Table 2. SOD, GSH, MDA levels in liver tissue

Groups	SOD(L) (nmol/g tissue)	GSH (L) (nmol/g tissue)	MDA (L) (nmol/g tissue)
Group 1 (Control)	0.03±0.01 p:0,145	11.6±3.8 p:1,136	0.94±0.13 p:0,182
Group 2 (OP)	0.05±0.01 p:0,744	4.7±2.15* p <0,05	1.51±0.29 p:1,234
Group 3 (OP+CUR)	0.03±0.01 p:0,093	4.9±1.05 p:0,262	1.33±0.05 p:0,785
Group 4 (OP+SFN)	0.04±0.01 p:0,542	8.6±2.93**, ¥, Ω p <0,05	1.56±0.22 p:0,092
Group 5 (OP+LIPID)	0.05±0.01 p:0,560	4.8±2.41 p:0,365	1.00±0.09 p:1,164

SOD: Superoxide Dismutase, GSH: Active Glutathione, MDA: Malondialdehyde p <0,05 was considered significant. OP: Organophosphate, SFN: Sulforaphane, CUR: Curcumin, L: Liver, LIPID: Lipid emulsion* p <0,05 different from control group; #p <0.05 different from OP + CUR group ** p <0.05 different from OP group; ¥ p <0.05 different from OP + SFN group

Ωp <0.05 different from OP + LIPID group

The highest GSH level after the control group was recorded in the OP + SFN group. The difference between the GSH levels of the OP + SFN group and the OP, OP + CUR and OP + LIPID groups was statistically significant (Table 2).

No statistically significant difference was found between the control group and the other groups in terms of SOD, GSH and MDA levels in kidney tissue as seen in Table 3.

Table 3. SOD, GSH, MDA levels in kidney tissue

Groups	**SOD (Kidney) (nmol/g tissue)**	**GSH (Kidney) (nmol/g tissue)**	**MDA (Kidney) (nmol/g tissue)**
Group 1 (Control)	0.06±0.16 p:0,063	0.73±0.3 p:1,93	2.4±0.6 p:1,54
Group 2 (OP)	0.03±0.01 p:1,18	2.03±0.1 p:0,89	1.7±0.3 p:0,72
Group 3 (OP+CUR)	0.07±0.01 p:1.01	1.81±0.6 p:0,16	2.1±0.2 p:0,43
Group 4 (OP+SFN)	0.07±0.01 p:1.59	0.86±0.2 p:1,15	3.7±1.2 p:2,39
Group 5 (OP+LIPID)	0.07±0.01 p:0,23	1.66±0.6 p:0,56	1.7±0.2 p:1,52

p <0.05 was considered significant. OP: Organophosphate, SFN: Sulforaphane, LIPID: Lipid emulsion, CUR: Curcumin

When the ECG findings of the toxicity model were observed, a prolonged QTc interval was detected in the recordings of a rat in the OP group compared to other rats, however there was no statistically significant difference (p> 0.05). Lastly, there were no abnormal EMG findings in any of the rats.

DISCUSSION

In the present study, which investigated the effects of curcumin, sulforaphane and intralipid in the management of organophosphate toxicity; it was determined that in the OP group compared to the control group AST and ALT values measured in serum to evaluate liver tissues were found to be significantly increased. This indicates the damage to the liver tissues in OP poisoning.

There are many studies in the literature indicating a decrease in liver enzymes after CUR administration in OP poisoning and its restorative effect on liver tissue (16-19). In the present study, AST and ALT levels were found to be decreased in the group treated with CUR, in accordance with the literature. However, only the AST levels presented a statistically difference in comparison to the OP group; this may be because AST is also a marker of cardiac and skeletal muscle damage besides the liver.

SFN is a compound mostly found in vegetables such as Brussels sprouts and broccoli, which was first determined to activate phase 2 detoxification enzymes and has been shown to have anti-carcinogenic effects (20,21). On the other hand, the results of the present study indicated no beneficial effects of SFN on liver and kidney tissues.

Although OPs are highly lipophilic compounds, according to the biochemical data, it was observed that intralipid had no protective effect on liver and kidney tissues. However, the researchers believe that no renal damage occurred in the rats of the MAL toxicity model. This may be due to insufficient dosing or time required for damage to occur.

PChE is an enzyme that shows OP exposure and can be easily measured in blood. The PChE level was found to be suppressed in all groups except the control group. Although this result suggests that the intoxication model was formed properly, due to the fact that the experiment was terminated in the acute period, it could not be determined whether these values were significant in terms of clinical course.

MAL is known to activate various reactive oxygen radicals; superoxide anion, nitrogen dioxide and hydroxyl radicals in particular (22,23). In the present study, there was an increase in the SOD levels of the OP group compared to the control group, however

this difference was not statistically significant. There was no statistically significant difference between the treated groups and the OP group. Due to the lack of research on the relationship between the available drugs and SOD mechanism, a definite conclusion regarding this issue cannot be drawn.

In a study in which experimental MAL toxicity was created, a decrease in GSH in the tissues was found with the increase in serum lipid peroxide (LPO) and glutathione S transferase in the blood (24). In another study, on the contrary, it was reported that GSH levels increased in rats with Fenthion poisoning as an adaptive response to oxidative stress (25). In a study conducted by Alp et al. it was reported that the GSH level in liver tissue decreased significantly in rats with MAL toxicity, whereas it increased in the group given SFN and CUR due to MAL blocking (26). In the current study, similar to the study of Alp et al., the GSH level in the liver tissue of the group receiving MAL decreased significantly compared to the control group, and this difference was statistically significant. There is no consensus on GSH levels in tissues due to OP intoxication in the literature. Additionally, a statistically significant increase was detected in the SFN group compared to the OF group. This suggests that SFN is more effective than CUR and LIPID in preventing oxidative stress in liver tissue caused by MAL toxicity.

It is believed that the major factor contributing to the decrease of cell functions in oxidative stress is the increase of lipid peroxides and the most important and most used marker of lipid peroxidation is the MDA level. There was no significant difference between the groups concerning the MDA levels in liver and kidney tissue. This suggests that in the present study, an injury did not develop in the liver through lipid peroxidation.

In their study, in which EMG recordings were analyzed to evaluate the neuromuscular effects of acute OP intoxication, R.S. Wadia et al. reported that EMG findings were mostly normal in acute poisoning (27). The data obtained in the current study were divided into two as normal and abnormal findings before evaluation. No abnormal EMG findings were found in any of the rats.

In the present study, when the 2nd hour and 12th hour ECG findings of the subjects were evaluated, no ECG disorder was found in any of the subjects, and the EMG values of all subjects were also found to be normal.

CONCLUSION

In summary, as the findings of the present study indicate; curcumin and sulforaphane are effective in preventing liver damage, while intralipid contributes by reducing PChE suppression.

LIMITATION

The number of rats was limited in line with the decision of the ethics committee. Contrary to what was predicted in the hypothesis, no significant difference was found in ECG and EMG. However, there is a need for further research supported by histopathological imaging to reach a definitive conclusion.

Ethical Approval: This experimental study was carried out in Gaziosmanpaşa University Animal Experiments laboratory and Gaziosmanpaşa University Biophysics Department laboratory with project number 2014 HADYEK-38 (permission no: 51879863-05), after the approval of Gaziosmanpaşa University Animal Experiments Local Ethics Committee dated 03.06.2014.

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Literature Review: A.Y.S.
Design: A.Y.S.
Data acquisition: A.Y.S, S.G., N.B.
Analysis and interpretation: S.G., A.Y.S.
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REFERENCES

1. Robey WC, Meggs WJ. Insectisides, Herbicides, Rodenticides. In: Tintinalli JE, KelenGD, Stapczynski JS Emergency Medicine A Comprehensive Study Guide. 6th ed. NewYork NY, McGraw-Hill 2004; 1134-1143.
2. Karalliedde L, Edwards P, Marrs TC, Food Chem. Variables influencing the toxic response to organophosphates in humans. Toxicology 2003; 41: 1-13.
3. Eddleston M, Eyer P, Worek F, et al. Differences between organophosphorus insecticides in human self-poisoning: a prospective study. Lancet 2005; 366:1452- 9.
4. Yamashita M, Tanaka J. Human mortality in organophosphate poisonings. Vet Human Toxicol 1997; 39: 84-85.
5. Brahmi N, Mokline A, Kouraichi N, et al. Prognostic value of human erythrocyte acetyl cholinesterase in acute organophosphate poisoning. Am J Emerg Med. 2006; 24(7): 822–7.
6. Kwong TC. Biochemistry and Clinical Toxicology: Organophosphate pesticides. Ther Drug Monit 2002; 24: 144-9.
7. Vallianou NG, Evangelopoulos A, Schizas N, et al. Potential Anticancer Properties and Mechanisms of Action of curcumin. Anticancer Res. 2015 Feb;35(2):645-51.
8. Juge N, Mıthen RF, Traka M. Molecular basis for chemoprevention by sülforafan: a comprehensive review. Cell Mol Life Sci 2007; 64: 1105-27.
9. Myzak M, Dashwood RH. Chemoprotection by sülforafan:keep one eye beyond Keap1. Cancer Lett 2006; 233: 208-18.
10. Foxall G, McCahon R, Lamb J, et al. Levobupivacaine-induced seizures and cardiovascular collapse treated with Intralipid. Anaesthesia 2007; 62: 516-8.
11. Paknikar K M, Nagpal V, Pethkar A V et al. Degradation of lindane from aqueous solutions using iron sulfide nanoparticles stabilized by biopolymers 2005; 6: 370-4
12. Khan S, Khan NN. The mobility of some organophosphorus pesticides in soils as affected by some. Soil Sci; 142: 214-220.
13. Guven M, Ünlühızarcı K, Göktaş Z, Kurtoğlu S. Intravenous organophosphate injection: An unusual way of intoxication. Hum Exp Toxicol 1997; 16: 279-80.
14. Namba T, Greenfield M and Grob D. Malathion poi- soning. A fatal case with cardiac manifestations. Arch Environ Health 1970; 21: 533–40.
15. Farago A. Fatal, suicidal malathion poisonings. Jusic A and Milic S. Neuromuscular synapse testing in two cases of suicidal organophosphorous pesticide poi- soning. Arch Toxicol 1967; 23: 11–6.
16. Preeti Y, Sunil EJ, Kumar V, et al. Protective efficacy of 2-PAMCl, atropine and curcumin against dichlorvos induced toxicity in rats, 2012 Mar;5(1):1-8.
17. Dwivedi & Flora, Concomitant exposure to arsenic and organophosphates on tissue oxidative stress in rats Food Chem Toxicol.2011 May;49(5):1152-9
18. Dwivedi, N., Bhutia, Y. D., Kumar, V., Yadav, P., Kushwaha, P., Swarnkar, H., & Flora, S. (2010). Effects of combined exposure to dichlorvos and monocrotophos on blood and brain biochemical variables in rats. Human & Experimental Toxicology, 29(2), 121–9.
19. Perananthamet S, Shaha K, Sahai A, et al. A hospital based epidemiological study of deaths due to organophosphorus compound poisoning. IAJPR 2014; 4: 3773-9.
20. Juge N, Mithen RF, Traka M. Molecular basis for chemoprevention by sülforafan: a comprehensivereview. Cell Mol Life Sci 2007; 64: 1105-27.
21. Myzak M, Dashwood RH. Chemoprotection by sülforafan:keep one eye beyond Keap1. Cancer Lett 2006; 233: 208-18.
22. Thıyagarajan M, Sharma SS. Neuroprotective effect of Curcumin in middle cerebral artery occlusion induced focal cerebral ischemia in rats. Life Sci 2004; 74: 969-85.
23. Sreejayan and Rao MN. Nitric oxide scavenging by Curcumin. J Pharmacy Pharmacol 1997; 49: 105- 7.
24. Ahmed RS, Seth VS, Pasha T, Banerjee BD. Influence of dietary ginger (Zingiber officinales Rosc) on oxidative stres ınduced by malathion in rats. Food Chem Toxicol 2000; 38: 443-50
25. Karalliedde L. Organophosphorus Poisoning and Anaesthesia. Anaesthesia. 1999; 54: 1073-88.
26.Tuzcu K, Alp H, Özgur T, et al. Oral intralipid emulsion use: a novel therapeutic approach to pancreatic b-cell injury caused by malathion toxicity in rats Drug Chem Toxicol 2014; 37: 261–7
27. Neurological manifestations of organophosphorous insecticide poisoning. J Neurol Neurosurg Psychiatry. 1974 Jul; 37(7): 841–7.