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Effect of extract of *Varthemia persica* DC on whole blood acetylcholinesterase activity in rats

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ABSTRACT

Varthemia persica DC. is an aromatic plant, from the Asteraceae family, wildy growing in the central provinces of Iran. Sesquiterpene lactones that are found in *Varthemia* species have protective effect against organophosphates toxicity. This study aimed to assess the Effect of *Varthemia persica* extract on acetylcholinesterase (AChE) activity. In this study, 0.4 mg normal saline was injected intra peritoneally in 12 control rats. 0.4 ml ethyl paraoxon was injected intraperitoneally in the reference group. In positive control group, after ethyl paraoxon injection, 0.4 ml pralidoxime was injected. In the first group (test 1) ethanolic *Varthemia persica* suspension was injected. In the second group (test 2), during the 6 days before toxin injection, daily injection of *Varthemia persica* was made. In test 3 group, suspension injection was repeated in 5 following days. In test 4 group, dichloroethanic suspension was injected. And in the last group (test 5) a high dose of suspension (1g/ml) was injected. The blood sample was collected 30 minutes after final injection. AChE activity was measure. The results showed that AChE activity was significantly improved only in the second and third groups and in other test groups, *Varthemia persica* suspension don't improved AChE activity. Based on the present finding it seems that the *Varthemia persica* extract is only effective if it used frequently and a single dose even in high dose is not effective.

Keywords: *Varthemia persica*, Acetylcholinesterase, Organophosphates, Ethyl paraoxon

INTRODUCTION

Throughout previous decades, organophosphates have been mostly used as insecticides and pesticides. Exposure to these products or their residues could cause acetylcholinesterase (AChE) inhibition and neurodevelopmental consequences including smaller head circumference [1] and more abnormal primitive reflexes [2]. AChE is an enzyme responsible for termination of impulse transmission via degrading of the excitatory neurotransmitter, acetylcholine (ACh) [3]. The toxicity of organophosphorus (OP) is capable of inhibiting AChE irreversibly, forming a bond with a serine remainder at the active site [4]. There are therapeutic regimes for organophosphate toxicity including muscarinic receptor antagonists and ACHE reactivators [5].

Medicinal plants are a key resource for discovering new drugs [6-9]. They have been used in traditional medicine for prevention of human disorders [10-17]. Also the effects of them have been investigated in a lot of studies [18-24]. They have been shown the antioxidant activity, anti-inflammatory, anti-hyperlipidemic effects, etc. In this regards, *Varthemia persica* DC. is an aromatic plant, from the Asteraceae family, wildy growing in the central provinces of Iran [25]. Phenolic compounds and coumarin are the most efficient component of this plant [26]. The essential oil of this plant has several terpenoid including monoterpenes (3.28%), sesquiterpenes (44.91%), oxygenated sesquiterpenes (36.49%) and oxygenated monoterpenes (1.82%) [27]. Sesquiterpene lactones that are found in

Varthemia species have protective effect against organophosphates toxicity [28]. A previous study on aerial parts of *Varthemia persica* identified numbers of volatile oil components including β -Eudesmol [27]. Moreover, the protective effect of β -Eudesmol on anti-cholinesterase organophosphorus evaluated and it has been shown that β -Eudesmol boost isopropyl fluoro phosphate [29]. However to the best of our knowledge there is no study evaluating the effect of *Varthemia persica* extract on cholinesterase, so this study aimed to assess the effect of *Varthemia persica* extract on acetylcholinesterase activity.

MATERIALS AND METHODS

Wild *Varthemia persica* DC was collected from the high mountains of Natanz, Iran during September and dried in shadow and Lab temperature before extraction. 100 grams of dried plant were re-dissolved with one liter of methanol and dichloromethane for 48 hours. The solution was filtered with filter paper and then concentrated with rotary in 40 degree centigrade. To produce 250mg/dl stable suspension for injection, 4 grams of this extract, 0.5 milliliter tween and 19.5 milliliter normal saline were used.

Adult female rats (200-225 g) were kept at $32\pm 2^\circ\text{C}$ with the 12 hours daily light-dark cycle. 12 samples were entered in the control group and 0.4 mg normal saline was injected intraperitoneally. 0.4 ml ethyl paraoxon was injected intraperitoneally in the reference group. In positive control group, one minute after ethyl paraoxon injection, 0.4 ml pralidoxime 0.125 molar in normal saline was intraperitoneally injected. In the first group (test 1) 0.4 ml ethanolic *Varthemia persica* suspension (250mg/dl) was injected (one minutes after paraoxon injection). In the second group (test 2), during the 6 days before toxin injection, daily injection of *Varthemia persica* suspension was made. In test 3 group, one minute after the toxin injection the *Varthemia persica* suspension was injected and the suspension injection was repeated in 5 following days. In test 4 group, dichloroethanic suspension was injected (one minute after paraoxon injection). And in the last group (test 5) a high dose of suspension (1g/ml) was injected. The blood sample was collected 30 minutes after final injection.

Blood samples were collected at -20°C until being assayed. AChE activity was measured with a spectrophotometer at 436 nm and total hemoglobin concentration was measured with spectrophotometer at 546 nm. AChE activity and specific Activity of AChE was calculated by equations [30].

Statistical analyses were achieved using SPSS 15 software. Analysis of variance (ANOVA) was used to compare means of enzyme activity between tests and references groups.

RESULTS AND DISCUSSION

In this study the effect of different treatment with of *Varthemia persica* extract on acetylcholinesterase activity was assessed. The result showed a significant improvement after injection of 0.4 ml ethanolic *Varthemia persica* suspension (250 mg/dl) repeatedly before or after toxin injection in comparison with reference group.

Table 1 shows acetyl cholinesterase activity in different groups in comparison with reference group (ethyl paraoxon). After injection of toxin AChE activity significantly decreased (more than $5 \mu\text{mol/l.min}$). Pralidoxime significantly improved AChE activity (from 1.19 to $4.34 \mu\text{mol/l.min}$). Compared with those receives the only toxin, in control group, positive control group and group 2 that received daily injection of *Varthemia persica* during the 6 days before toxin injection and group 3 that received *Varthemia persica* suspension repeatedly in 5 following days, AChE activity was significantly improved. The same results was observed for specific activity of AChE (table 2).

Table 1: AChE Activity in different groups

Groups	n	AChE Activity ($\mu\text{mol/l.min}$)
Control	12	$6.46\pm 2.31^*$
Reference	12	1.19 ± 0.73
Positive control	6	$4.34\pm 1.01^*$
Test 1	12	1.17 ± 1.16
Test 2	6	$2.05\pm 0.90^*$
Test 3	6	$1.77\pm 0.76^*$
Test 4	6	1.05 ± 0.41
Test 5	6	0.81 ± 0.85

Data (AChE Activity) has been shown as mean \pm SD; * Significant difference with reference group ($P < 0.05$).

Table 2: Specific Activity of AChE in different groups

Groups	n	Specific activity of AChE ($\mu\text{mol/L}\cdot\text{min}$)
Control	12	0.0074 \pm 0.0026*
Reference	12	0.0014 \pm 0.0009
Positive control	6	0.0045 \pm 0.0007*
Test 1	12	0.0013 \pm 0.0013
Test 2	6	0.0022 \pm 0.0009*
Test 3	6	0.0019 \pm 0.0007*
Test 4	6	0.0011 \pm 0.0004
Test 5	6	0.0009 \pm 0.0009

Data (Specific activity of AChE) has been shown as mean \pm SD; * Significant difference with reference group ($P < 0.05$).

Organophosphorus pesticides are available worldwide in agriculture and household gardens to use as insecticides and cause public-health problem across much of rural Asia [31]. OP as an irreversible cholinesterase inhibitors inhibit acetylcholinesterase activities and cause accumulation of ACh at synapses, and trouble of neurotransmission in nervous systems [32]. The most common reported symptoms are vomiting, abdominal pain, hypersalivation, respiratory distress, and low level of consciousness and muscle twitch [33]. Despite the severity and require intensive care management, diagnosis and suitable treatment are often lifesaving [34]. In treating sever or moderate OP toxicity oximes are supposed to be effective, and mostly used [32]. Sodium bicarbonate is sometimes used in place of oximes for treatment of OP poisoning in Iran [35]. Beta-eudesmol, existing in Chinese herb antagonizes toxicity of organophosphate, reduce the incidence of convulsions and reverse the neuromuscular failure [36]. Beta-eudesmol (31.7%) was detected from Water extracted essential oil from *Varthemia persica* aerial parts [37]. Eudesmol, a sesquiterpenol present in *Varthemia persica* has a protective effect against organophosphates toxicity [38].

In the current study we used different treatment with ethanolic *Varthemia persica* suspension on the AChE activity as a marker for OP poisoning and found that a single dose of this suspension is not effective on AChE activity, however repeated dose before or after the ethyl paraoxon injection significantly improved its activity. The mechanism by which just repeated doses are effective is not clear. However, it has been shown that the plants with phenolic compounds have antioxidant activities [39-57], and antioxidants, in some cases, have different or paradoxical responses [58-65], which worth examining their mechanism actions.

CONCLUSION

Based on the present finding it seems that the *Varthemia persica* extract is only effective if it is used frequently and a single dose even in high dose is not effective.

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