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Research Article

Effect of *Salvia sahendica* Extracts on Neuromuscular Transmission in Chick Biventer Cervicis Muscle

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ABSTRACT

Background: The *Salvia sahendica*, a known folk medicine, possesses antibacterial, antifungal, anticancer properties and the improvement effect on the memory system. Because memory improving effects of the other species of this genus (*salvia officinalis*) is mediated by interacting with muscarinic and nicotinic cholinergic systems. It seems that *S. sahendica* may also follow the same mechanisms.The purpose of the present study is to investigate the anticholinesterase effect of *Salvia sahendica* extracts,in the pharmacologic methods. ***Method:*** The study was done by using the Dose-response curves and Twitch tension techniques in chick biventer cervicis. The ability of extracts to modulate cholinesterase activity were assessed by obtaining concentration–response curves to acetylcholine and carbacol in the absence or presence of extracts. The isolated tissues were exposed to extracts (methanol, dichloromethane and hexane extracts) and responses were recorded, with $p < 0.05$ indicating significance. ***Results:*** Based on our finding, methanol extract of *S. sahendica*, has not significant change in the dose-response curve for acetylcholine, enzyme but elicited reduction effect at carbacol dose-response curve. All other extracts showed the significant reduction in Dose-response curves and Twitch tension. There was a direct relationship between hexane extract´s concentration and reduction of contraction on log dose-response curves for acetylcholine and carbachol. Hexane extract (75µg/ml) blocked muscle twitching and yield in a complete blockage in the presence of tubocurarine, dichloromethane extract decreased twitch height. The effect of tubocurarine was not abolished by extract but also had cumulative effects. ***Conclusion:*** These results suggest that the relaxant effects of *S. sahendica* extracts on neuromuscular junction are elicited by post junctionally and curare–mimetic effects. It seems that, methanol extract has anticholinsterase activity but this effect will be affected by the inhibitory effect of the extract and inhibited.

Introduction

Salvia genus belongs to the *Lamiaceae* family and comprises of about 900 species. Numerous species of the genus *Salvia* have been used in traditional medicine, and extensive studies have been conducted to elucidate their pharmacological effects and their biologically active compounds, as well. Recently, some studies have performed in order to examine the impacts of different *Salvia* species on CNS. According to these studies, sage species affects numerous processes potentially related to brain function, and can be sued for prevention or treatment of Alzheimer's disease.[1]

Salvia sahendica is an endemic species which grows in Azerbaijan and known by the common name “Doshan Almasi". Several compounds have been isolated from *S. sahendica* aerial parts belong to sesterterpene, sesquiterpene, diterpene, triterpene, steroid and flavonoid groups.[2] Rustaiyan et al., have reported 24 components from the essential oils of leaves and stems of the plant, among them α-pinene (29.4%) and β-pinene (34.8%) were the major compounds.[3] *S. sahendica* has been reported to exhibit antifungal and antibacterial[4] cytotoxic[5] anti-tumor[6] *and* anticonvulsant effects.[7] A behavioral and biochemical study has showed that treatment with *S. sahendica* can protect rats against apoptosis and memory impairment induced by the β amiloide injection.[8]

Shaerzadeh et al[9] indicated that *S. sahendica* prevents harmful effects of reactive oxygen species by stabilizing mitochondrial membranes and inhibiting ER

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stress, which is early phenomenon in Alzheimer disease. Previously, memory improving effect of *Salvia officinalis* has documented.[10]

There are several mechanisms to enhance memory, one of which is use of the anti-cholinesterase agents.[11] Cholinergic hypothesis is an important therapeutic strategy in patients afflicting from Alzheimer disease result from a deficit of cholinergic function in brain. One of the most promising methods for treating this disease is to increase the acetylcholine level in the brain using acetyl cholinesterase (AchE) inhibitors.[12] Because memory improving effects of *S. officinalis* is mediated by interaction with muscarinic and nicotinic cholinergic systems,[10] it seems that *S. sahendica* may also follow the same mechanisms.

The purpose of the present study is to investigate the effects of *S. sahendica* on the neuromuscular transmission. In vitro models of cholinergic transmission, including chick biventer cervicis nerve-muscle preparations, are used in this study.

Materials and Methods

Plant material

Aerial parts of *S. sahendica* were collected from barren lands around the Tabriz-Basmenj Road, Iran, during flowering season (2013) and a voucher specimen (Tbz-FPh 736) was deposited in the Herbarium of the Faculty of Pharmacy, Tabriz University of Medical Sciences. The plant materials were dried in the room temperature, after wards were kept in the closed containers in cold room.

Extraction

The dried and powdered plant material (130 g) was extracted with organic solvents including hexane, dichloromethane and methanol using a Soxhlet extractor at 45 °C for 8 hours. The solvent of extracts were removed under vacuum and low temperature.

Chemicals

Acetylcholine, Physostigmine and Tubocurarin were obtained from Sigma. Carbachol, ethyl acetate and Krebs-Henseleit solution salts of the following composition (mM): NaCl (118), KCl (4.7), KH_2PO_4 (1.2), $NaHCO_3$ (25), $MgSO_4 \cdot 7H_2O$ (1.2), $CaCl_2$ (2.5), glucose (11.1), were purchased from Merck (Germany) and hexane, dichloromethane and methanol prepared from Caledon (Canada).

Chick biventer cervicis preparations

The chick biventer cervicis muscle have both focally innervated twitch-producing fibers and multiply innervated contracture-producing fibers. Thus, it can be stimulated by exogenously applied cholinomimetic agonists, as well as by stimulation of its motor nerve. These make able prejunctional effects to be distinguish from postjunctional effects.

Chickens aged 7-14 days were anaesthetized with ether and then Scarified. The surgery was similar to that explained by Ginsborg & warriner.[13] The skin of the animal's neck was cut, and then incised rods cervicis along the midline from the skull to below the base of the neck. The two biventer cervicis muscles were observed on either side of the midline. A string was tied to the upper end of the tendon and the ring was created in that area. The other thread was tied to the distal end of the muscles. Muscle was carefully separated from the body. Threading the upper end muscle was passed through the electrode and was attached hooks into the organ bath .The electrode was lowered until it was in contact with the tendon surrounding the nerve. Threading the lower end of the muscle to the transducer (LETICA, Spain) was attached, transducer valve suitably connected to a pen recorder (AD Instrument). One gram resting tension was applied to the muscle. Electric excitements with Pulse rate 0.1 Hz, Pulse width 500 μs, the minimal voltage that can cause maximum contractions, were applied. The organ bath (20ml capacity) contained Krebs-Henseleit [14] solution which was kept at a stable temperature between 37˚C and 40˚C and which was well blend with a mixture containing 95% oxygen and 5% carbon dioxide. The preparation remained in good state for several hours.

Data Analysis

The dose -response curve to acetylcholine and carbachol were expressed as the mean ± standard error of the mean (±SEM) (n = 5-6). Data analyses were performed using the one-way (repeated) analysis of variance (ANOVA) for multiple comparison and Student t-test, with $p < 0.05$ indicating significance.

Results and Discussion

Dose-response curves for acetylcholine and carbachol

The dose-response curve for Carbachol in the presence of different concentration of extract was obtained. In the log dose-response curve for carbachol in the presence of extract, without shift, reduction in the maximum response was observed (Fig .1, 2 and 3), that indicated non-competitive but reversible inhibition.

Except the curve of the methanol extract (Fig. 4), in the other dose-response curves for acetylcholine, decrease in maximum contractile response was observed (Fig.5 and 6), resulting noncompetitive inhibition effect. These results suggest a direct depressing effect on the muscle fibers. The effect of dichloromethane extracts on log dose-response curves for carbachol is more than the effect on Log dose-response curves for acetylcholine, Due to no increase in response to exogenous acetylcholine and carbachol there are no anticholinsterase activities .In the methanol extract, dose-response curve for acetylcholine has not significant change, acetylcholine was destroyed by anti-cholinesterase enzyme but carbachol wasn't .It seems that this extract has anticholinsterase activity but this effect will be affected by the inhibitory effect of the extract and inhibited.

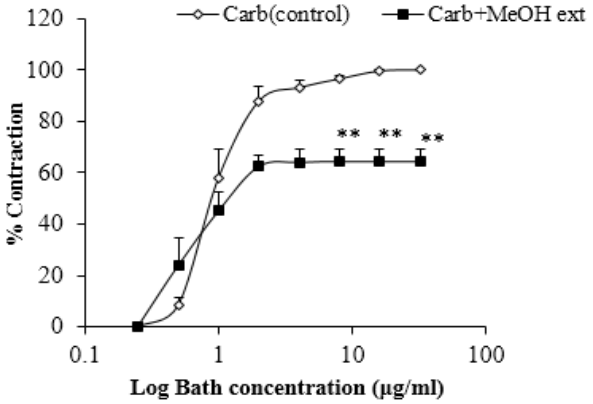

Figure 1. Effect of methanol extract (MeOH ext) (100µg/ml) of *Salvia Shandica* on the cumulative concentration–response curve of carbachol (carb) on chick biventer cervicis muscle. Results are expressed as mean±S.E.M. of percentage of maximum contraction induced by carb. n=6; **P < 0.01.

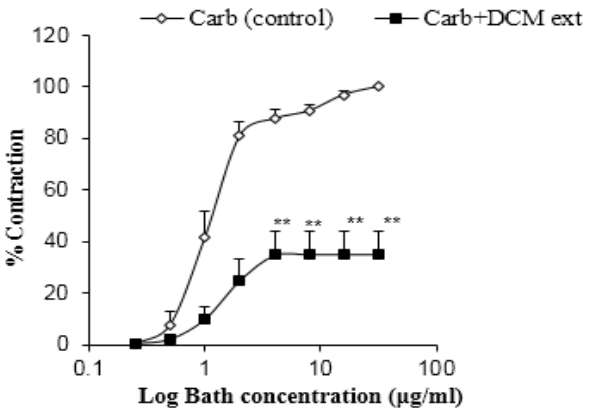

Figure 2. Effect of dichloromethane extract (DCM ext) (50µg/ml) of Salvia Shandica on the cumulative concentration–response curve of carbachol (carb) on chick biventer cervicis muscle. Results are expressed as mean±S.E.M. of percentage of maximum contraction induced by carb. n=6; **P < 0.01.

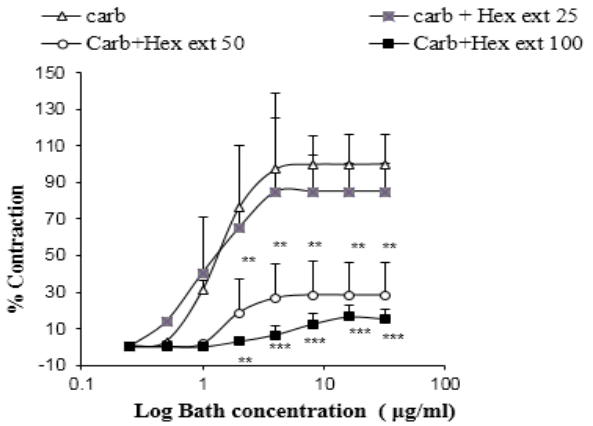

Figure 3. Effects of hexane extract (Hex ext) (25, 50, 100µg/ml) of Salvia Shandica on the cumulative concentration–response curve of carbachol (carb) on chick biventer cervicis muscle. Results are expressed as mean±S.E.M. of percentage of maximum contraction induced by carb. n=6; **P < 0.01, ***: p<0.001.

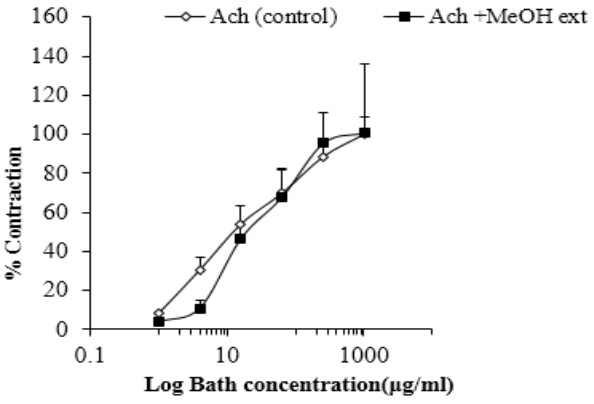

Figure 4. Effect of methanol extract (MeOH ext) (100µg/ml) of Salvia Shandica on the cumulative concentration–response curve of acetylcholine (Ach) on chick biventer cervicis muscle. Results are expressed as mean±S.E.M. of percentage of maximum contraction induced by Ach. n=6.

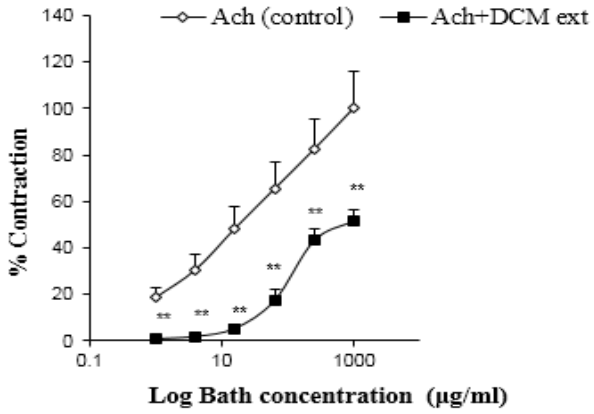

Figure 5. Effect of dichloromethane extract (DCM ext) (50µg/ml) of Salvia Shandica on the cumulative concentration–response curve of acetylcholine (Ach) on chick biventer cervicis muscle. Results are expressed as mean±S.E.M. of percentage of maximum contraction induced by Ach. n=6; **: p<0.01.

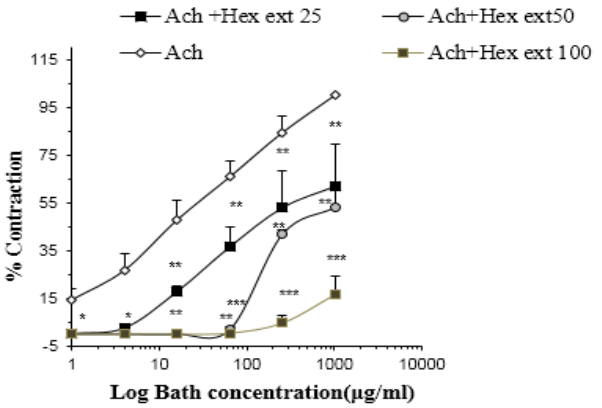

Figure 6. Effect of hexane extract (Hex ext) (25, 50, 100µg/ml) of Salvia Shandica on the cumulative concentration–response curve of acetylcholine (Ach) on chick biventer cervicis muscle. Results are expressed as mean±S.E.M. of percentage of maximum contraction induced by Ach. n=6; *: p<0.05, **: p<0.01, ***: p<0.001.

As could be seen in figure 7, there was a direct relationship between hexane extract´s concentration and reduction of contraction on log dose-response curves for acetylcholine.

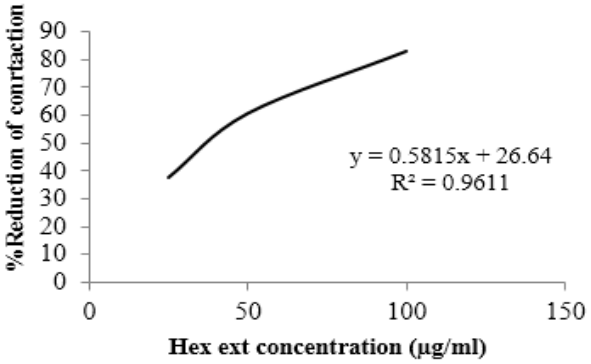

Figure 7. The effect of hexane extract´s dose on reduction of contraction on log dose-response curves for acetylcholine on the CBC muscle preparation.

The effect of extract on twitch and exogenous acetylcholine responses

Table 1 show that the twitch responses of chick biventer cervicis evoked by nerve stimulation were reduced in the presence of *S. sahendica*, 75µg/ml of hexane extract blocked muscle twitching and yield in a complete blockage. Blocking of twitch responses with considering the effects the exogenous acetylcholine and carbachol could occur due to a postsynaptic effect through blockage of acetylcholine receptors or intracellular muscles contraction pathways.[15]

Table 1. Effect of *salvia sahendica* on twitch response.

Extract	Dose (µg/ml)	Reduction of twitch height%
Methanol	100	14
	200	56
Dichloromethane	25	11
	50	27
Hexane	25	20
	50	80
	75	100

Figure 8 shows a decrease on exogenous acetylcholine response after exposure to different concentrations of extract. At dichloromethane extract (FIG 8.B), there was a series of periodic increase in resting tension, coinciding with the reduction of twitch height. The effect of methanol and dichloromethane extracts were reversed by washing intermittently for about 45 min but the effect of hexane extract was irreversible.

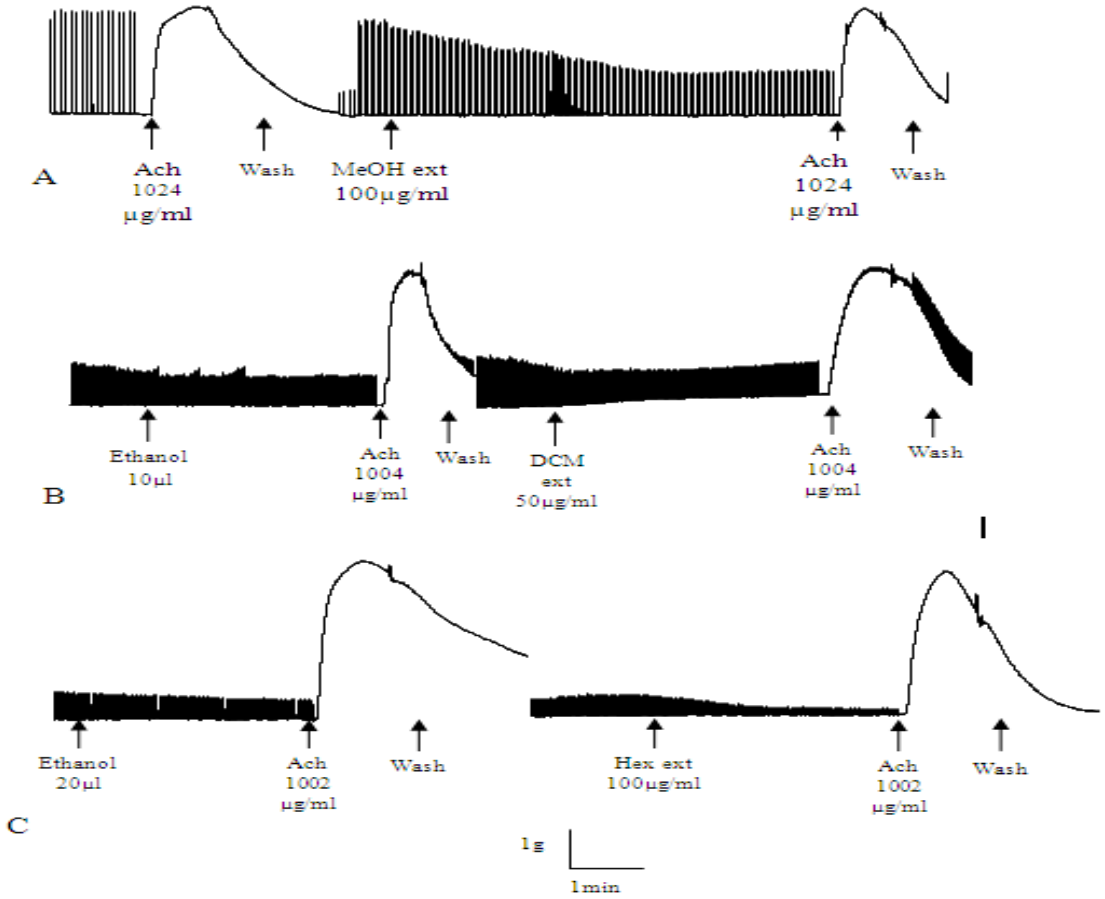

Figure 8. The response to exogenous acetylcholine in the presence of methanol (A), dichloromethane (B) and hexane (C) extract on CBC muscle.

Effect of extract in present of Tubocurarin

Tubocurarin is a non-depolarizing drug.[16] It is a reversible competitive antagonist at nicotinic acetylcholine receptors[17] and reduces the twitch height. In the presence of tubocurarine, *S. sahendica* 25 µg/ml decreased twitch height. The effect of tubocurarine was not abolished by extract but also had cumulative effects; indicated that the extract is not depolarizing blocking agent.

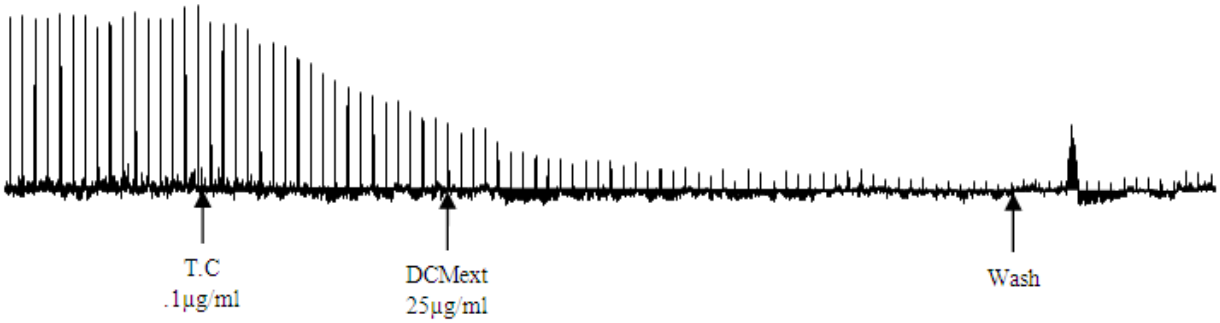

Figure 9. Effects of *S. sahendica* (25 µg/ml) on chick biventer cervices muscle preparation in present of tubocurarin (1 µg/ml).

Conclusion

Results from the present study show that *S. sahendica* has some effects on neuromuscular transmission. There were no data to show the existence of anti-cholinesterase effect. In the log dose-response curve for carbachol and acetylcholine in the presence of extract, without shift, reduction in the maximum response were observed, it seems that the effects of the *S. sahendica* are mostly mediated through postsynaptic effects on neuro muscular junction and there are non-competitive inhibition. This effect may be due to the blockage of neuro-muscular transmission, arising from the effects of the *S. sahendica* on nicotinic receptors of the postsynaptic membrane.[18] Blocking of twitch responses could occur due to a postsynaptic effect through blockage of acetylcholine receptors or intracellular pathways of the muscle contraction.[15] According to the inhibitory effects of *S. sahendica* in response to acetylcholine and carbachol, likely the extract has curare–mimetic effects.[19] Tubocurarine and extract had cumulative effects; indicated that the extract cannot be depolarizing blocking agent.

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Conflict of interests

The author claims that there is no conflict of interest.

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