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The Effects of Salvia officinalis Extract on Salivary Flow Rate in Rats

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Abstract: Sialorrhea is a common clinical problem in children and adults that can have important social and medical consequences.The aim of this was to evaluate the effects of the sage extract in the salivary flowrate in rats. In this experimental study, 18 rats were divided into three equal groups: Group A: in this group a single dose of 10 mg kg^{-1} of the salvia officinalis extract was injected; Group B: in this group a single dose of 10 mg kg^{-1} of saline was injected and Group C: that rats received a single dose 10 mg kg^{-1} of atropine. Then, the amount of saliva after injection in four 7 min intervals was measured consecutively and results were analyzed with multiple t-test ($p<0.05$ was considered significant).The result showedafter injection of atropine salivation was significantlylower as compared to other groups. The most of the reduction was in time points of 14 and 21 min, respectively. In addition, the salivary flow rate did not decreased after injection of salvia officinalisextract in comparison with normal saline. The findings of this study demonstrated that salvia officinalis extractcannot cause asignificant reduction thesalivary flow rate in rat.

Key words: Atropine, salvia officinalis, saliva, sialorrhea, Iran

INTRODUCTION

Sialorrhea is defined as an inability to control oral secretions which is manifested by drooling of saliva from the oral cavity. Sialorrhea also called as drooling is considered a normal issue in children under 4 years, it is a common problem in children with neuro-immune deficiency (such as mental retardation or cerebral palsy) as well as adults with Parkinson's disease and or stroke (Hockstein *et al*., 2004; Fuster *et al*., 2007). Although, the pathophysiology of this disease is multifactorial but generally its causes are divided into three categories: the excessive saliva production; the inability to keep the saliva in the mouth and trouble swallowing (Bavikatte *et al*., 2012). Different clinical factors and conditions are involved in creating diseases. As it was said, nervous diseases have most associations to their causes but one of its most common causes is tooth infection and tooth decay. Diagnosis of the cause of the creation of these diseases is a vital strategy for selecting appropriate treatment for them (Ravishankar *et al*., 2013).

n general, the treatment ofsialorrhea has been faced almost always with problems and it is relatively difficult. So that treatments available are placed in a variety, including preservative treatments and invasive interventions such as surgery. Today, the most common preservative treatments include changes in dietary habits, oral-motor exercises, botulinum toxin injections and medications (Lakraj *et al*., 2013). For so much time, anticholinergic medications are used as first line treatment in these patients. Through the parasympathetic inhibition these medications reduce harassment of salivary glands (Hockstein *et al*., 2004). Medications that are commonly used in these patients include: benztropine, atropine drops, glycopyrrolate andipratropium bromide (Glick *et al*., 2014). Atropine is a medication with anticholinergic effects that its therapeutic effect on patients with sialorrheahas been provenin some studies (Hyson *et al*., 2002; Rapoport, 2010); in addition to very good effects of these medications, they create numerous side effects for patients (Hockstein *et al*., 2004).

Today, herbal medicines, because of less side effects, have attracted the attention of many researchers. The Salvia officinalis (sage), a darkplant isthe most valuable member of the family Lamiaceae. Different pharmacological effects of this herb such as antimicrobial, wound healing, antioxidant and anti-inflammatory effects, have been proven (Hosseinzadeh *et al*., 2009; Fu *et al*., 2013). Some studies recently have shown anticholinergic effects of this medicinal plant contains effects (Kennedy *et al*., 2006; Scholey *et al*., 2008). Current evidence indicates that likely Salvia officinalis can be used in the treatment ofpatients withsialorrhea. Therefore,

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this study aimed to evaluate the effects of herbal medicine of salvia officinalis (Sage) on salivary flow rate in rats.

MATERIALS AND METHODS

Study design: This experimental animal study was approved according to the guidelines of the ethics committee of Ahvaz Jundishapur University of Medical Sciences. In this study, 18 female wisterrats, weighted 200-300 g was used. The rats were kept in the room specifically for the animals in a temperature of 24°C and the lighting-darkness period: 12 h darkness and 12 h lighting by considering starting of lighting period at 8 in the morning. The rats were fed with the purified water and convenience food for rats. Rats were randomly divided into equal three groups, Group A: in this group a single dose of 10 mg kg^{-1} of the sage extract intra-peritoneally was injected; Group B: in this group a single dose of 10 mg kg^{-1} of saline was injected intra-peritoneally and Group C: that rats received asingle dose 10 mg kg^{-1} ofatropine intra-peritoneally

The preparation of the first salvia officinalis extract: The leaves of the plant were dried in the shadow in a good condition and powdered by the household mixer device. Then, the value of 120 g of dried herb powder with a proportion of 83%, ethyl 96 and 17% of distilled water soaked and mixed and were kept for 72 h. After filtering the solution using the vacuum evaporation method, it was extracted by a rotary device (Heidolph, Germany). The obtained extract was finally concentrated during 24 h under the Fume hood. The obtained extract was mixed with normal saline and was ready for infusion.

Technique Intra-peritoneal injection: At first mixture of 75 mg kg^{-1} ketamine (Alfasan, Holland) and 5 mg kg^{-1} xylazine (Alfasan, Holland) was injected intra peritoneal to create generalized anesthesia. Rats were held with their abdominal exposed in the left hand and then, the needle was injected deeply into the abdominal cavity in the lower right quadrant. The needle angle was 15-20° and the depth of the injection was approximately 5 mm.

Measuring the amount of saliva: In order to maintain the anesthetized rats' body temperature at 37°C, they were placed on a thermal pad. Before collecting the saliva, the oral cavity was dried by cotton pellets with the specified weight. So that the cotton pellets were placed under the tongue at the rat's mouth between the teeth and cheeks for 10 min. After removing the cotton pellets by by a scale (Sartorius, Germany), their weights were measured. The weight difference between the cotton pellets before and after mouth-drying was considered as the base amount of the secretion saliva of these rats. The flow rate of saliva was determined gravimetrically, assuming that the specific of saliva is 1 (1 g equals 1 mL of saliva).

After the measurement of the baseline secreted saliva, sage extract, normal saline and atropine, as described were injected intra-peritoneally (10 mg kg^{-1}). Rats were randomly divided into equal three groups, Group A: in this group a single dose of 10 mg kg^{-1} of the salvia officinalis extract was injected; Group B: in this group a single dose of 10 mg kg^{-1} of saline was injected and Group C: that rats received a single dose 10 mg kg^{-1} ofatropine. The rate of saliva secretion was measured in four time periods of 7 min (7, 14, 21 and 28). Finally, the rats were sacrificed with intra muscular injection trought an overdose of pentobarbital (Ulfasan, Amesterdam, Netherlands).

Data were calculated and analyzed by descriptive statistics; then in order to compare the average between the different groups in different time points, the multiple t-test was used. The $p<0.05$ was considered to be significant.

RESULTS

The results of the present study showed that salivary flow rate decreased significantly after injection of atropine. Comparison between atropine (Group C) and saline (Group B) indicated that the maximum effects of the atropine was seen in the time points 14 and 21 min. Also, on these points a significant difference can be seen between the effects of atropine and normal saline ($p = 0.0009$). While in the rest of the time points, the difference between the effect of atropine and normal saline was not statistically significant (Fig. 1, Table 1).

Comparison between atropine (Group C) and salvia officinalis extract (Group A) showed that there was a more difference between the effect of atropine compared with salvia officinalis extract at the time points of 14 and 21 min more and the difference was statistically significant ($p = 0.003$). But at other time points, no significant difference was observed .

A review intragroup results of salvia officinalis (A) showed that the salivary flow rate in minutes 14 and 21 have been reduced slightly compared to the basic saliva, but this reduction was not statistically significant.

Comparison between two groups of Group A and Group B did not show significant differences at different time points between the two groups.

Table 1: Saliva secretion (Mean±SD) before and after injection of salvia officinalis extract, atropine and saline in four continuous 7 min intervals

	Zero		Seven		Fortheen		Twenty one		Twenty eight		
Drug	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	p-values
Saline	0.0175	0.003082207	0.0175	0.003082207	0.0173	0.003076795	0.0173	0.003076795	0.0173	0.00301662	0.86
Salvia officinal is extract	0.0196	0.003444803	0.0185	0.004816638	0.0180	0.004816638	0.0180	0.005244044	0.0180	0.005573748	0.24
Atropine	0.0200	0.004589845	0.01133	0.05281162	0.0093	0.00294392	0.0088	0.003060501	0.0105	0.02403331	<0.001

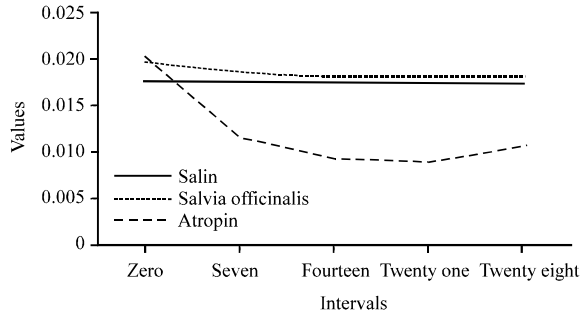

Fig. 1: Saliva secretion before and after injection of three matter in four continuous 7 min intervals

DISCUSION

Sialorrhea is known as the inability to control oral secretions that can have social and important physical implications. So far the different treatments have been suggested for the management of these patients that each has benefits and disadvantage (Hockstein *et al*., 2004). Today, anticholinergic medications areused in abundance in these patients that has been one of the anticholinergic medications is atropine that creates itself effect by blocking Muscarinic receptors in the salivary gland and reducing production of saliva (Glick *et al*., 2014). In this study, the effects of normal saline, atropine and salvia officinalisextract in the saliva secretion rate of in were measured and compared.

The results of the present study showed that the atropine, caused significant reduction in the salivary flow rate at the time points of 14 and 21. So far the different results have been reported in this case that some of these studies were consistent with these results (Glick *et al*., 2014; Hyson *et al*., 2002). Hyson *et al*. (2002) have shown that atropine caused a significant decrease in the production of saliva. Diamant and Feinmesser (1959) compared the amount of the activity of salivary glands during general anesthesia under the effect of the drugs such as atropine, L-hyoscyamine (bellafoline), Scopolamine butylbromide (Buscopan) and Oxyphenonium (Antrenyl), in this study, it became clear that atropine is best antisialagogue to reduce saliva during general anesthesia. Rappaport (2010) also showed that sublingual drops of atropine are a successful treatment for children experiencing sialorrhea (Hyson *et al*., 2002). Also, Mustafa (2013) evaluated the effect of atropine in the treatment of sialorrhea caused by clozapine and showed that the sublingual administration of atropine caused a significant decrease in the saliva rate. While Simone *et al*. (2006) showed that atropine compared with placebo, did not have an effect on the treatment ofpatients with sialorrhea.

Unlike the numerous usefulnesses of atropine in the treatment of sialorrhea, side effects such as urinary tract and vision problems cause limitations of its use (Shirley *et al*., 2013). In addition, prescription of this medication on the underlying diseases such as glaucoma, diseases of the stomach and myasthenia gravis is restricted (Hockstein *et al*., 2004).

So in the search for finding an alternative medicinal with similar effects, we examined the impact of an extract of salvia officinalis in reducing the amount of saliva. The results of this evaluation showed that in comparison with normal saline, extracts of salvia officinalis did not have a significant impact in reducing the amount of saliva. So far, some studies have proven anti-cholinergic effects of the extract of Sage (Kennedy *et al*., 2006; Scholey *et al*., 2008; Savelev *et al*., 2003). First, Savelev *et al*. (2004) in the *in vitro* demonstrated that sage oil causes inhibition of the enzyme acetylcholinesterase. Also, Sergey *et al*. showed that sage in addition to the inhibition of acetylcholinesterase, may also cause inhibition of Butyrylcholinesterase. Kennedy *et al*. (2006) showed that saliva officinalis has anti-cholinergic effects not only in the *in vitro* but also in a dose-dependent state cause changes in the mood of individuals and alerts them by increasing the dose. These results showed that Sage had anti-cholinergic effects in the *in vivo* as well. Following this study, the Scholey *et al*. (2008) indicated that a certain dose of Sage by using of the anti-cholinesterase effect improve memory and attention in man. However, according to our knowledge, no study so far, has shown the effects of Sage on reducing the saliva rate. In general, the results of this study showed that Sage extract in comparison with normal saline did not have a significant effect in reducing the amount of saliva. Also, our findings show that atropine as conventional therapy in these patients had a significant effect in reducing the rate of secretion of saliva more than Sage and also the maximum effects of the drug is in the time points of 14 and 21 min.

Evaluation of effects of salviaofficinalison the amount of secretion of saliva that was done for the first time was one of the strengths of the study while the low sample size and lack of pharmacological manipulation in the salviaofficinalisextract was a limitation of this study. Therefore, it is recommended that the future studies as the higher the sample size to confirm the results of as well as make pharmacological changes on the saliva officinalis extract.

CONCLUSION

The findings of this study demonstrated that salvia officinalis extract cannot cause a significant reduction the rate salivation in rat.

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