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Evaluation of systemic effects of ginger fractions on salivation in rats

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ABSTRACT

Various herbal drugs and treatment modalities claim to stimulate salivary flow. Since the effect of ginger on increasing salivation and cholinergic activity has been shown in previous studies, we decided to evaluate the systemic effects of ginger extracts on salivation. The extracts of ginger fractions (Petroleum ether, Diethyl ether, Dichloromethane, Chloroform, Ethyl acetate, Methanol, Watery) and Total methanolic extract of ginger were prepared. In this experimental investigation ten groups of rats for seven types of ginger fractions, total ginger extract and negative and positive control groups were studied (n=7 for each group). Saliva volumes were measured gravimetrically after intraperitoneal (I.p.)injection of extracts during four continuous seven minute intervals. Systemic injection of ginger extract and each of the seven fractions did not result in an increase in saliva secretion. After injection of six extracts (Petroleum ether, Dichloromethane, Chloroform, Ethylacetate, Methanol, Watery) a significant decrease in the saliva secretion occurred (P<0.05). A decrease in salivation may be explained by dual activity of ginger (cholinergic and calcium antagonist). Use of different doses of ginger extracts (especially aqueous extract) or other preparations of ginger may be helpful for future studies on the effects of ginger on salivation.

Key words: Ginger; Herbal; Rat; Salivation; Xerostomia

INTRODUCTION

The subjective sensation of dry mouth, xerostomia, is a well recognized problem in adults [1,2] however relatively little attention has been paid to this issue in children. Because infants drool and young children always seem to have an excess of watery saliva, there is an unfounded belief in the dental profession that children cannot or do not suffer from salivary hypofunction, i.e. xerostomia or dry mouth. Regrettably, this is not so. Many children with special needs or complicating medical factors can suffer significant impairment of salivary function[3].The factors which contribute to salivary dysfunction in children are, in broad terms, no different from those in adults. The most common causes of salivary gland dysfunction leading to xerostomia include: medication, systemic disease, radiation therapy, malnutrition, local factors, salivary gland aplasia and salivary gland tumors[4-11].

Treatment that is available for the dry mouth patients can be divided into four main categories: Preventive therapy[12], Symptomatic treatment[11,13],local or topical salivary stimulation[14,15] and systemic

stimulation[9,10].

Traditionally, ginger has been used to treat a wide range of diseases including gastrointestinal disorders, such as stomachaches, abdominal spasm, nausea, and vomiting, as well as in arthritis and motion sickness[16-18].

Phytochemical studies have demonstrated that the plant is rich in a large number of substances, including gingerols and shogaols[19-21]. These compounds possess diverse biological activities such as antioxidant, anti-inflammatory, and anticarcinogenic properties [22,23].

An effective method in treatment of xerostomia is systemic stimulation of saliva secretion, thus use of medications such as pilocarpine as a systemic stimulator of saliva secretion has been used as an effective therapy, but its high cost and undesirable side effects limit its usage[24]. Furthermore, there is an increasing trend in use of herbal medicine as an alternative to the synthetized agents[21,25-27].

The effect of ginger on salivation rate has been studied before [28], the objective of the current study was to evaluate the systemic effects of ginger fractions on salivation in rats.

MATERIALS AND METHODS

Animals

Adult male rats (weight: 200-300 g) from NMRI strain were used. The animals were bred and housed at the animal facility of neuroscience research center of Kerman University of Medical Sciences. The animals were kept in a well cross ventilated room with controlled temperature and humidity and a standard 12h light: 12h dark cycle. Standard rodent food and tap water were available. Ethical considerations of ethic committee of Kerman University of medical sciences for animal studies were considered in the present research (code: K/87/87).

Ten groups of rats including seven fractions, total ginger extract and negative and positive control groups were used (n=7 for each group) [28].

Plants

Total methanolic extract 70° of ginger and seven ginger fractions were injected intraperitoenally to the animals in each group. Ginger fractions were as follows: Petroleum ether, Diethyl ether, Dichloromethane, Chloroform, Ethyl acetate, Methanol and Water based.

Ginger selected for the present study (20 g) was washed with distilled water to remove dirt and soil, and shade dried. The dried material was used for a maceration process. The material was extracted twice with methanol (70%). The extract was filtered, pooled, and concentrated at high temperature (+50ºC) on a rotary evaporator (Heidolph, Germany). The extract was suspended in CMC(carboxymethylcellulose)1%(vehicle agent) and tween80 0.25% (suspending agent) and stored in refrigerator within dark containers[28].

In the next stage, the plant powder was obtained with 1-7 solvents[29]ascending order of polarity: petroleum ether 40 -60 solvent to isolate the completely very non – polar ingredients of the plant like resins, di ethyl ether to isolate the non-polar ingredients of the plant like Terpins and some phenol ingredients, dichloromethan to isolate the partially non-polar ingredients of the plant like Diterpenes, chloroform to isolate the ingredients with variable polarity like Alkaloides and ethyl acetate to isolate the ingredients with lower polarity like some Flavonoides).

In each stage 500 g of plant powder was macerated with one litre solvent for 48 hours. This process took place in a cool place with temperature less than 25° C and every 6 hours the plant powder- solvent mixture was shaken for ten minutes. The solvents, added altogether, were concentrated in the vacuum rotary of evaporating apparatus at 50°C and the mentioned solvent evaporated in an oven of 40 C for 48 hours. The remaining undissolved parts of the plant of each extraction were spread and dried and then extraction went on with the next stage solvent, in the same way as mentioned before. Finally, out of one plant powder, seven different extracts in ascending order of polarity, which extract the components of the plant, was obtained. The extracts and pilocarpine were prepared for injection using CMC 1% and Tween80 0.25%.

Saliva collection

Rats were anesthetized using a single intraperitoneal injection of 75 mg kg^{-1} ketamine (Alafsan, Holland) and 5 mg kg^{-1}rampon (Alafsan, Holland). To determine the stage of general anesthesia of rats, 3 criteria including positive hind paw reflex, negative eye blink reflex and breathing rate (between 10-15 breath/min)were checked[28].

The unconscious rats were kept on a thermal pad to maintain their body temperature at the level of 37ºC. Before saliva collection, the oral cavity was wiped and dried with a cotton pellet and then four pre-weighed cotton pellets were inserted in the mouth of each animal: two cotton pellets underneath the tongue and one between the cheek and the teeth on either side. After seven minutes, the cotton pellets were removed and weighed again on a precise digital Sartorius balance (LD 450, Germany) (0.001gr precision). The difference of the weight of the cotton pellets between two determinations was considered as the baseline weight of the saliva secreted. The flow rate of saliva was determined gravimetrically, assuming that the specific gravity of saliva is one (i.e. one gram equals one mililitter of saliva)[24,28].

Following measurement of the baseline secreted saliva, the extracts were injected (100 mg kg^{-1} body weight) intraperitoneally. The rate of saliva secretion was determined at four continuous seven-minute intervals. The investigator was blinded to all of the injected solutions in this study. A negative control(10mlkg^{-1}) 0.25%Tween80 mixed with suspending agent as 1% CMC) and a positive control (4 µ mol kg^{-1}pilocarpine dissolved in 0.25% Tween80 mixed with suspending agent as 1% CMC)[28] were used for comparison of the ginger extracts efficacy.

Statistical Analysis

Data were analyzed using SPSS V.16 (IBM, USA). ANOVA test was used to compare the volume of secreted saliva between different groups. For repeated measurement of saliva secretion, repeated measures ANOVA test was recruited. Considering the different efficacy of drugs and necessity for adjustment, the multiple regression modelwas used to compare the drug effects and estimating the effective dose. $P<0.05$ was considered statistically significant.

RESULTS

In this study, the effect of total methanolic extract of ginger and each of the seven extracted fractions on saliva secretion of the animals was studied. The average saliva secretion rate in four continuous seven-minute intervals after the injection of total methanolic extract of ginger and Diethyl ether extract was not significantly different from baseline saliva secretion.

There was a significant decrease in saliva secretion in comparison with the baseline saliva secretion after the injection of the 6 extracts(Table 1 and Figure1).

Table 1: Mean weight of saliva in continuous 7-min intervals before and after injection of different types of extracts

	Mean weight of saliva(mg) in continuous 7-min intervals		Standard deviation	Number of rat
A	Before extract injection	2.84	.716	7
	After extract injection			
	1-7min	2.64	.932	7
	8-14min	2.07	.415	7
	15- 21min	2.45	.718	7
	22-28min	2.78	1.889	7
B	Before extract injection	2.95	.830	7
	After extract injection			
	1-7 min	2.30	.632	7
	8-14min	1.94	.645	7
	15-21min	1.97	.518	7
	22-28min	1.92	.394	7
C	Before extract injection	2.65	.609	6*
	After extract injection			
	1-7 min	2.68	.923	6*
	8-14min	1.75	1.144	6
	15-21min	1.83	.568	6
	22-28min	1.88	.790	6
D	Before extract injection	3.14	1.325	7
	After extract injection			
	1-7 min	2.20	.365	7
	8-14min	1.67	.555	7
	15-21min	1.87	.243	7
	22-28min	1.65	.287	7
E	Before extract injection	3.28	1.380	7
	After extract injection			
	1-7 min	2.10	.369	7
	8-14min	1.84	.599	7
	15-21min	1.80	.525	7
	22-28min	1.74	.769	7
F	Before extract injection	2.58	1.002	7
	After extract injection			
	1-7 min	2.11	.524	7
	8-14min	1.82	.994	7
	15-21min	1.62	.464	7
	22-28min	1.51	.575	7
G	Before extract injection	2.74	.427	7
	After extract injection			
	1-7 min	2.32	.596	7
	8-14min	1.64	.519	7
	15-21min	1.88	.433	7
	22-28min	1.51	.418	7
H	Before extract injection	2.67	.540	7
	After extract injection			
	1-7 min	2.27	.502	7
	8-14min	1.70	.476	7
	15-21min	1.64	.472	7
	22-28min	1.50	.447	7
N	Before extract injection	2.21	.442	7
	After extract injection			
	1-7 min	2.06	.434	7
	8-14min	1.85	.239	7
	15-21min	2.10	.845	7
	22-28min	2.10	.789	7

**Since salivation in one rate was unreasonably so above, it was eliminated from the analysis*

A: Total methanolicextract, B: Watery, C: Di ethyl ether, D: Petroleum ether, E: Chloroform, F: Methanolic, G: Ethyl acetate, H: Di chlorometan, N: Negative control(CMC and tween80)

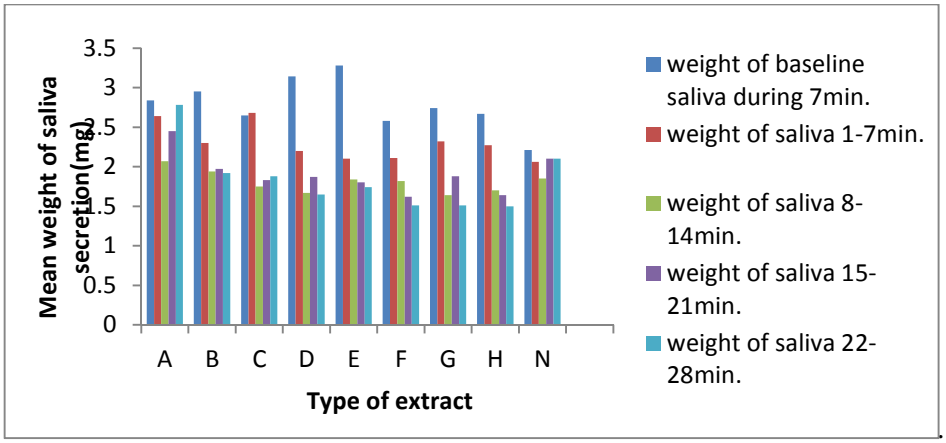

Fig. 1.mean salivary secretion during 5 minute intervals for the studied extracts and negative and positive control
**Mean of saliva secretion in positive control group after injection was very much, so preferred to omit this group from the figure*

Since100 mg/kg concentration of ginger did notstimulate saliva secretion; the effect of total extract of ginger with 1000 mg/kg concentration on salivation was evaluated. Animals were administered different concentration of ginger extract using the gavage route, but there was no significant increase in salivation after administering the extract through the latter route.

DISCUSSION

Systemic injection of ginger extract and each of the seven fractions did not result in an increase in saliva secretion. Previous studies have demonstrated that ginger extract has cholinergic effect and might lead to increased saliva secretion[25-28]. However, an anti-cholinergic effect has been attributed to ginger in some other studies [21,25,]. Though the exact mechanism is not clear yet, but it seems that ginger has a dual effect on cholinergic transmission and there might be some alterations in its effect upon the change in extraction method or the specific ginger strain used for extraction procedure.

Chamani et al. (2011) evaluated the effect of different herbal preparations on saliva secretion and demonstrated that ginger administration leads to an increased salivation. Though they did not evaluate the exact mechanisms involved, but their results are not consistent with the current study which might be due to the altered extraction procedure and use of different fractions in the present study [28].

Ginger is comprised of several different components including phenols, which are believed to have a cholinergic action, but there are also other substances present in the whole extract which might possess anti-cholinergic properties[21,25-27]. Extracting different fractions from the ginger extract using different solvents might justify the current findings, since each of the active components present in the fractions might have different and even opposing effect on salivation.

Another reason for the current findings and its contrast with previous publication from the same authors might be the different ginger preparations used and the possibility of different composition of the extracts used[18,19].

CONCLUSION

In summary, results of the current study did not demonstrate a stimulatory effect of ginger fractions on salivation. Further studies using a dose-response protocol and determining the optimum dose are warranted. Furthermore, it is suggested that further studies specifically determine the composition of ginger extracts used so that its effect on salivation might be attributed to a specific component in the extracts. Studies on the effect of phenolic compounds like gingerol and shogaol on salivation is also recommended due to their established cholinergic effects[18,19].

Conflict of interest
The authors declare no conflict of interest.

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