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Absorption Enhancing Effect of Total Saponins derived from *Acanthopyllum squarrusom* and *Quillaja saponaria* on Nasal Permeation of Gentamicin Sulfate and Carboxyfluorescein.

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ABSTRACT

Nowadays, nasal route of drug administration is considered as a suitable delivery site due to avoid first-pass effect and less enzymatic activity in comparison with gastrointestinal tract. Several researches are made focusing on increasing absorption of drugs through nasal mucosa. Co-administration of absorption enhancing agents is one of the approaches to improve the uptake of poorly absorbable drugs. The objective of the study was to evaluate the influence of total saponins extracts from *Acanthopyllum squarrusom* (ATS) and *Quillaja saponaria* (QTS) as nasal permeation enhancers on the uptake of some water-soluble molecules such as gentamicin sulfate (GS) and 5(6)-carboxyfluorescein (CF). Two relatively potent surfactant; sodium lauryl sulfate (SLS) and benzalkonium chloride (BC) were utilized for comparison purpose. Nasal mucosa was excised from the nasal septum of rabbits and mounted on a specifically designed Franz-type diffusion cell. An aliquot of 100 µl of sample was withdrawn from the receptor compartment at 5, 10, 15, 20, 25 and 30 min and replaced with the same volume of phosphate buffer saline (PBS) to keep the volume constant. According to the results, the absorption enhancers increased the mucosal permeability of CF and GS across nasal membrane significantly. The results suggested that the saponins can potentially be used to increase the permeability of hydrophilic compounds through nasal epithelial membrane.

Keywords: saponin, nasal absorption, absorption enhancer, surfactant.

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INTRODUCTION

Nasal drug delivery is recognized as a very promising route for systemic effects, since the absorbed substances are avoided from liver first-pass metabolism and transported directly into the systemic circulation [1]. Intranasal route is a simple, economic and non-invasive route of drug administration, which its ease of consumption improves patient compliance [2]. Also, the nasal cavity has less enzymatic activity than the gastrointestinal tract [3] and has a vast absorption area (150 cm^2) with microvilli in epithelial cells [4]. In contrary to the skin, the nasal mucosa is not constructed with stratum corneum, but consists of epithelial cells with rich vascularity that provides direct entry of the drug into the systemic circulation [1]. Compared to polar drugs, lipophilic drugs are generally better absorbed from the nasal cavity. The most important factor limiting the nasal absorption of polar drugs is low membrane permeability [5]. To overcome this problem, different approaches have been attempted including employing absorption enhancers such as bile salts, surfactants, fusidic acid derivates and saponins to increase the passage of drugs with polar structure through nasal mucosa [4, 6]. Saponins are a highly heterogeneous group of glycosides which are common in plants, lower marine animals and some bacteria [7-9]. They have many medicinal applications including, anti-microbial, hepato-protective, anti-tumor, antioxidant, insecticide, hemolytic and anti-inflammatory activities [9-11]. In our study, the polar antibiotic gentamicin sulfate (GS) was utilized as a model drug in order to investigate improving the uptake of drugs through nasal membrane using permeation enhancers. It has been reported that gentamicin is slightly absorbed *via* the nasal route, due to its high polarity. While, the absorption was improved considerably by co-administration of bile salts [12]. In current study, 5(6)-carboxyfluorescein (CF), a hydrophilic anionic dye was also employed as a permeability marker due to its high aqueous solubility [13, 14]. The objective of the study was to evaluate the efficacy of total saponin of *Acanthopyllum squarrusom* (ATS) and *Quillaja saponaria* (QTS) as nasal absorption enhancers of polar drugs.

MATERIALS AND METHODS

QTS and gentamicin sulfate (GS) were purchased from Alfa Aesar, Germany and Alborz Daru Pharmaceutical Co., Iran respectively. Sodium lauryl sulfate (SLS) and benzalkonium chloride (BC) were purchased from Merck, Germany. 5(6) carboxyfluorescein (CF) was obtained from Sigma-Aldrich, Germany. All of the solvents were of the analytical grade.

Extraction of ATS

A. squarrusom roots were collected from Tabas, south Khorassan, Iran, and indentified in herbarium, Mashhad University of Medical Sciences. The roots of the plant were ground into powder, defatted in a soxhelet apparatus with petroleum ether (boiling range of 40-60 ºC) for removing lipids and phenolic compounds. The air-dried powder was extracted with methanol for 48 h. The solvent was removed under vacuum by rotary evaporator (Heidolph, Germany) and the resulting brownish residue was suspended in water, centrifuged at 2500 rpm for 45 min, and the supernatant was separated and extracted with water saturated n-butanol. The butanol phase was concentrated in rotary evaporator at 80ºC and then the dry residue was dissolved in the least methanol quantity (30 ml) and precipitated by addition of diethyl ether. Finally, total saponins were freeze-dried (Operon, Korea) and stored at room temperature [15-17].

To evaluate their foaming properties, 5 ml of each different concentrations of GTS, QTS, SLS and BC was added to three tubes and tubes were vortexed for 5 seconds and after one minute the foam height was measured. For emulsifying ability, 3 ml of liquid paraffin was added to tubes each containing 3 ml of aqueous solution of different concentrations of GTS, QTS, SLS and BC. Then they were vortexed at high rate for 2 min to form emulsion. The samples were stored at 25°C for 24 h and then thickness of emulsified layer was measured. Surface tensions of different concentrations of the compounds were also measured using Du-Nouy Ring Tensiometer at 25˚C [18]. For evaluating their hemolytic activity, 200 µl of red blood cell (RBC) suspension was incubated for 30 min with equal volume of the saponins and surfactants solution. Then the mixtures were spun in a micro-centrifuge at 3200 g for 15 s. Then 200 µl of supernatant was added to 3 ml Drabkins reagent to assay for the amount of haemoglobin released.

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Preparation of Nasal Mucosa

Rabbit nasal mucosa was used as model substrate for the study. Male rabbits (NWZ, 2-2.5 Kg) were anaesthetized by injection of methoxy fluorine and then were sacrificed. The nasal septum was carefully removed and placed in isotonic phosphate buffer (pH 7.4).

In Vitro Diffusion Studies

Nasal mucosa was mounted on a specially designed Franz-type diffusion cells with the apical and baso-lateral sides facing upward into the donor and receiver chamber, respectively. Throughout the studies, the samples were maintained at 37°C and stirred using a gas system (95% O_2 and 5% CO_2) in order to provide natural state of tissue. Uptake experiments were carried out using ATS, QTS (150 µg/ml), SLS and BC (100 µg/ml) as absorption enhancer and GS and CF as drug models (100 µg/ml). An aliquot of 100 µl of sample was withdrawn from the receptor compartment at 5, 10, 15, 20, 25 and 30 min and replaced with the same volume of phosphate buffer saline (PBS) to keep the volume constant. Concentration of GS and CF was determined using agar diffusion and spectrofluorimetry (Fluoro Max, SPEX Industries Inc., USA, excitation/ emission: 492/515 nm), respectively. Studies in this article have been carried out in accordance with the guideline and permission of the Animal Ethics Committee, Mashhad University of Medical Sciences, Mashhad, Iran. Each test was performed three times and the results were reported as mean ± standard error.

Stability Studies

Results are shown as Mean±SE. ANOVA and student t-test was performed to compare the results together and also with control, and $P<0.05$ was assumed as significant difference**.**

RESULTS AND DISCUSSION

As shown Fig (1), at concentration of 10 mg/ml, SLS was a maximum foam height of 8.15±0.95 cm and there was a significant different between the two total saponin if compared with SLS solution ($P<0.001$). Also, foam producing abilities of QTS and ATS was significantly different ($P<0.01$). The results of emulsion stability showed that there was a significant correlation between the materials concentrations and formation of emulsions (Fig 2). A comparative analysis between the total saponins, SLS and BC showed that the onset of emulsification ability for the saponins was significantly slower than the synthetic surfactant ($P<0.01$), although, there was no significant different between QTS and ATS ($P>0.05$). It is apparent from Fig (3) that by the increasing the concentration of saponins and synthetic surfactants, the surface tension of the solutions was reduced. As illustrated in Fig (4), SLS and BC at concentrations of 100 µg/ml showed hemolytic effect via disruption of red blood cell membrane. While, ATS and QTS caused complete hemolysis only at concentration of 250 µg/ml.

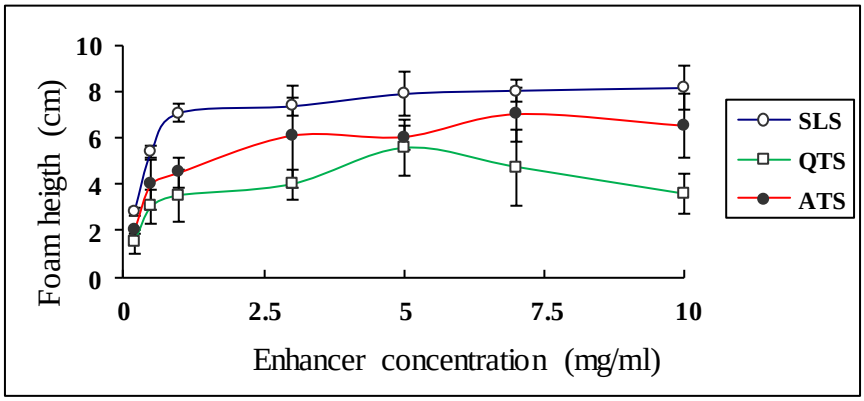

Fig (1): Foam formation of different concentrations of ATS, QTS, SLS and BC (mg/ml)

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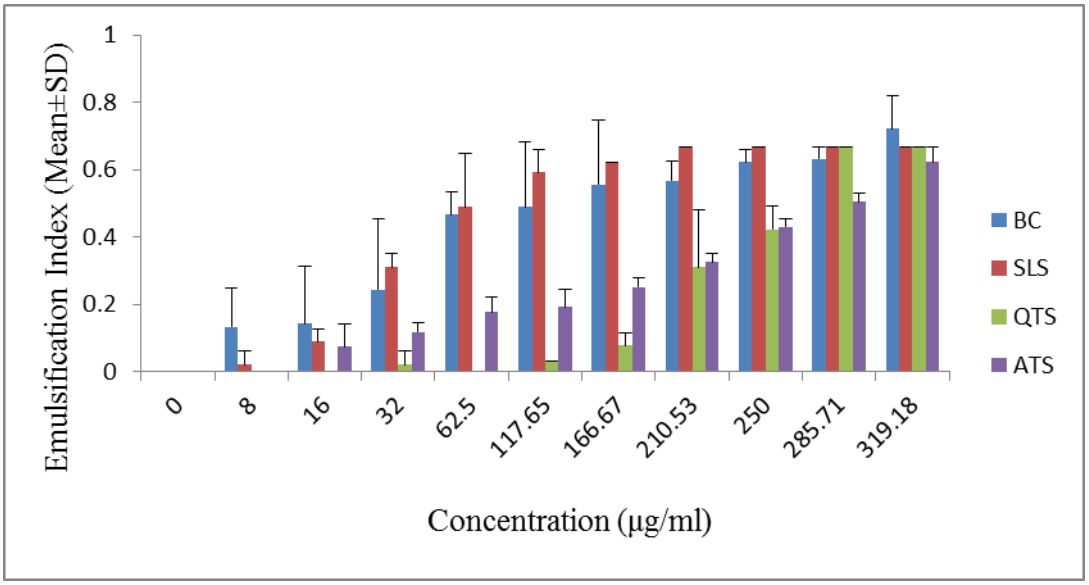

Fig (2): Emulsification indices of different concentrations of ATS, QTS, SLS and BC

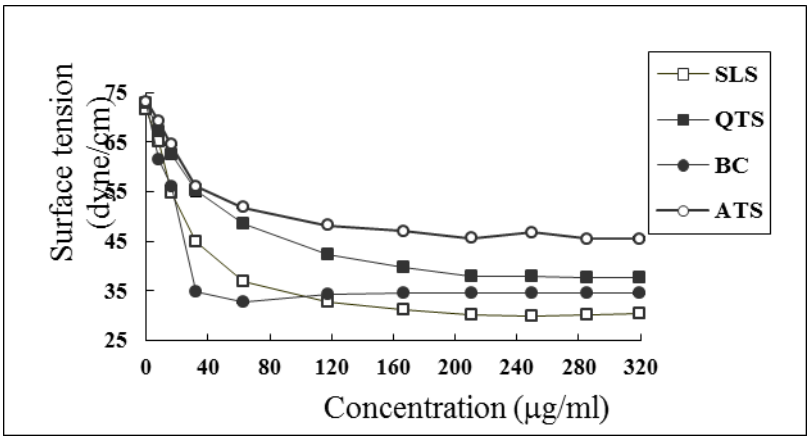

Fig (3): Surface tension lowering ability of different concentrations of ATS, QTS, SLS and BC

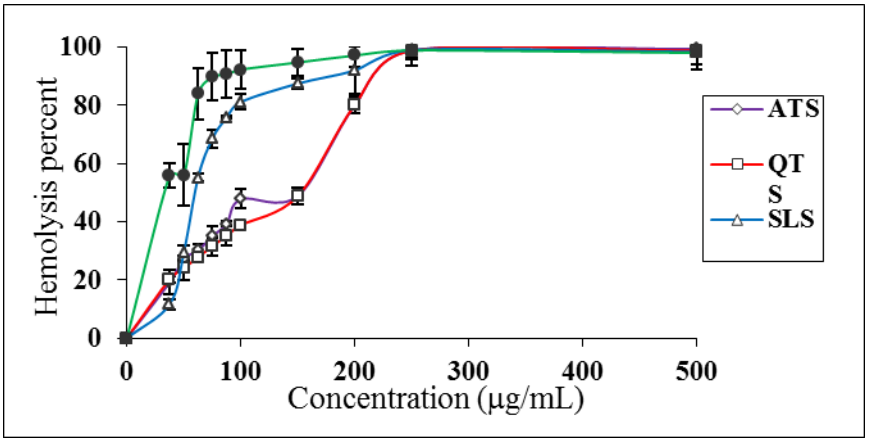

Fig (4): Hemolytic effect of ATS, QTS, SLS and BC

Nasal route can be useful for systemic delivery of low molecular weight polar drugs that are not easily administered *via* other routes including injection or GI. The nasal absorption of these drugs can be greatly improved if administered in combination with absorption promoting agents [19]. As shown in Fig (5), after 30 min, permeation of GS through nasal mucosa for control, QTS, ATS, SLS and BC was 3.25±0.03%, 19.63±0.02%,

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22.88±0.06%, 26.00±0.03% and 25.97±0.03%, respectively). In the other word, saponins and synthetic surfactants could increase permeation of GS *via* nasal mucosa. QTS in transport of GS was more potent than ATS, but its effect was lower than SLS and BC. Also, all of absorption enhancers were able to propmote CF permeation thruogh nasal mucosa (P>0.05). The cumulative permeated amount of CF for control, QTS, ATS, SLS and BC containing solutions after 30 min was 2.16±0.36%, 34±2.93%, 35.35±0.54%, 34.17±0.18% and 32.00±1.21%, respectively. Fig (6) shows the permeation profile of CF through the nasal mucosa. The effect of permeation enhancres in transpot of CF was more than GS.

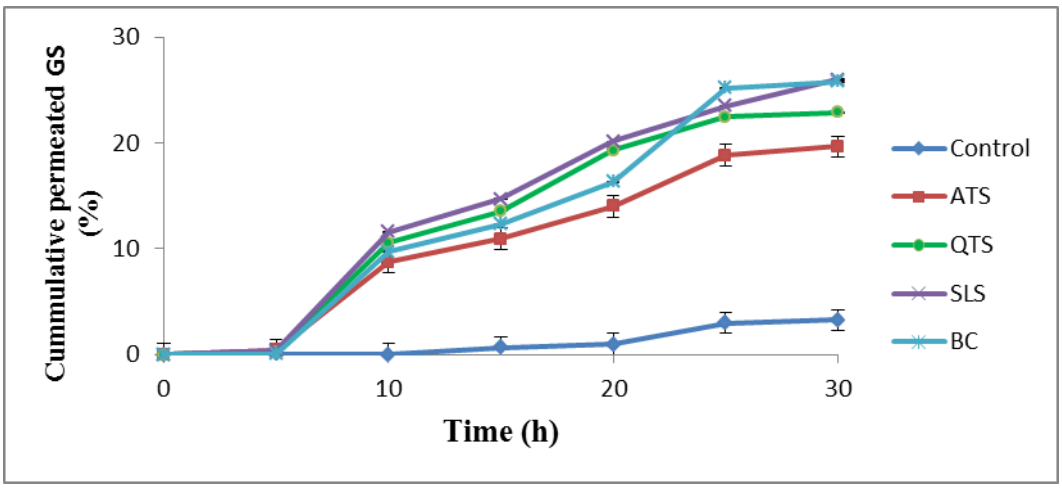

Fig (5): Release profile of GS using different absorption enhancers

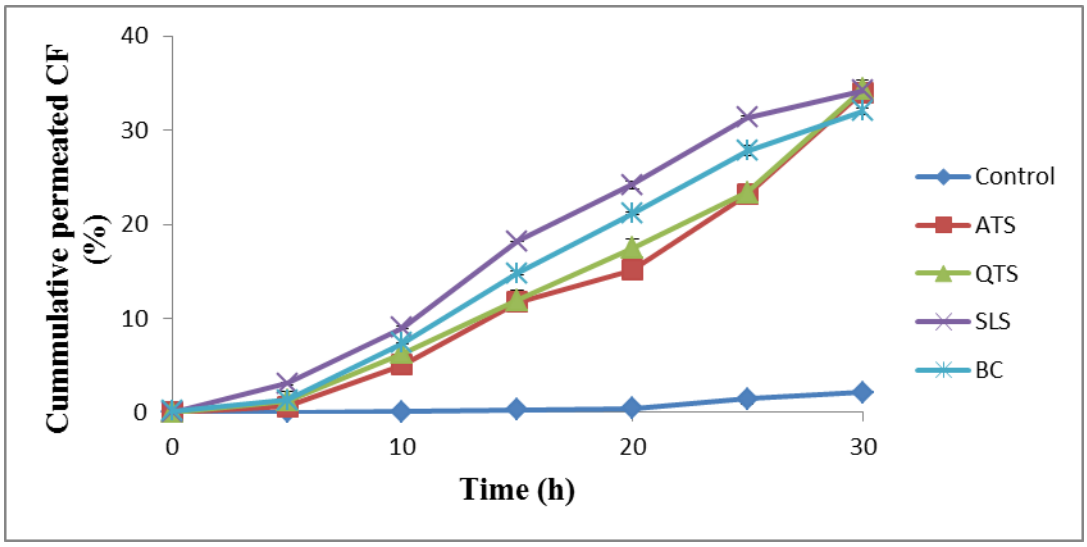

Fig (6): Release profile of CF using different absorption enhancers

It is unclear which of the pathways for absorption, is the dominant route of transporting. It has been previously reported that polar drugs with molecular weights less than 1000 Da will generally pass the membrane through paracellular route [19]. Therefore, CF and GS which have molecular weight of 376.32 and 477.596 Da, respectively, may flux through paracellular routes. However, for transporting drugs *via* the transcellular route, it is likely that the enhancers work by micellar formation and interact with the membrane that resulting transcellular absorption [20]. In previous studies, it was reported that the CMC value of ATS, QTS and SLS was approximately 50, 160 and 150 µg/ml, respectively [21]. Since the concentration of QTS (150 µg/ml) and SLS (100 µg/ml) in the study was under their CMC, so it is believed that these excipients enhance membrane permeability of GS and CF *via* paracellular rather than transcellular route. While, the utilized concentration of ATS (150 µg/ml) was above its CMC, it is suggested that the mechanism of action might involve the formation of micelle to facilitate transepithelial absorption. The findings agree with the Sajadi Tabassi *et al*. (2007) findings that investigated the enhancing affects of ATS on nasal absorption of insulin. The

concentration of ATS in their study was above its CMC. They were demonstrated that the ATS may form micelle that can interact with cell membrane and increase insulin uptake through transcellular pathway [21]. According to the preliminary results of the current study, it is possible to enhance the absorption of hydrophilic molecules through nasal mucosa using permeation enhancers. However, in some cases increased drug absorption may be accompanied by toxic effects and damage to the mucosal epithelia. As mentioned earlier, SLS and BC showed the most effective increase in uptake of GS across nasal mucosa. BC is a cationic surfactant [22] and its biological activity is based on the interaction with proteins, lipids and guanine nucleotide triphosphate binding proteins (G proteins) in biological membranes [23]. Besides, it is a quaternary ammonium compound that is commonly used to prevent bacterial contamination [24, 25]. Numerous *in vivo* studies suggested that even prolonged use of topical nasal preparations containing BC causes no significant damage to the nasal mucosa [25]. While, Deutschle *et al*. in 2006 reported that BC can induce DNA changes in respiratory epithelial cells at concentrations commonly employed in commercially available nasal preparations [23]. SLS is an anionic surfactant that acts as permeation enhancers by perturbation of intercellular lipid, protein domain integrity and disorder of membrane [5]. Our results indicated that saponins may be good candidates for nasal delivery of poorly absorbable drugs such as GS. It should be considered that *Q. saponaria* and *A. squarrosum* saponins are mixture and it can be expected that the absorption enhancing effect can be improved by fractionating the more efficacious component from these saponins. Recchia *et al.* in 1995, investigated the utility of a purified semi-synthetic saponin, DS-1, prepared by deacylation of a naturally occurring saponin from the bark of the *Q. saponaria*, as a permeation enhancer for nasal delivery of gentamicin. They observed significant transport of antibiotic across mucous membranes due to the presence of DS-1. Also, nasal irritation was not observed in groups of rats that receiving DS-1 [26]. More studies are needed to evaluate their efficacy in drug absorption across the nasal membrane in animal models and their safety on the mucosal structure. Moreover, pathological situation, inflammation and obstruction in nasal cavity can greatly influence the rate of drug delivery *via* nasal route, so the next steps may be evaluation of absorption-enhancing effect of these ingredients in such conditions.

CONCLUSION

According to the results, co-administration of saponins and synthetic surfactants led to absorption enhancement of the hydrophilic drug models through nasal mucosa. By means of these ingredients, CF and GS may cross the epithelial cell membrane either by transcellular route or by paracellular route. Further investigations are needed for evaluation of safe and effective of absorption enhancers for a promising future in the area of nasal drug delivery. By use of a suitable absorption enhancer, in the near future it is expected to see a range of novel nasal products reaching the market.

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