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Original article

Anti-Inflammatory Effect and Skin Toxicity of Aqueous Extract *of Dorema ammoniacum* Gum in Experimental Animals

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Abstract

Background and objectives: *Dorema ammoniacum* gum resin is used in Iranian traditional medicine for different indications including inflammatory diseases which are on the rise. Considering the important role of inflammation in different diseases, in the present study, we aimed to investigate the in vivo anti-inflammatory activity and skin toxicity of *Dorema ammoniacum* gum extract (DAGE) in rats and rabbits. **Methods:** The systemic anti-inflammatory effect of DAGE (100, 200 and 300 mg/kg, i.p.) was assessed by carrageenan-induced paw edema method in 30 min, 1, 2, 3 and 4 h after the carrageenan injection to thirty male Wistar rats divided into five groups of six each. Control and standard groups received the vehicle and mefenamic acid (30 mg/kg, i.p.), respectively. To assess the topical anti-inflammatory effect of the gum, eighteen rats were divided into three groups of six: standard, vehicle, and test groups which received topical diclofenac gel, distilled water and DAGE, respectively. The acute dermal toxicity of DAGE was evaluated in nine white New Zealand rabbits. **Results:** The results showed significant anti-inflammatory effects of DAGE in systemic treatment. The findings also indicated that all doses of DAGE were more potent than mefenamic acid. However, the topical anti-inflammatory activity of DAGE (100 mg/kg) was comparable to that of diclofenac gel 2% and showed no skin toxicity. **Conclusion:** The results of the present study suggest that DAGE has significant anti-inflammatory effects without any erythema and edema in topical use. These effects might be partially or wholly due to possible inhibition of or interference with the production of some inflammatory mediators, especially prostaglandins, histamine, serotonin, and bradykinin.

Keywords: anti-inflammatory; carrageenan; *Dorema ammoniacum* gum; Draize test

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Introduction

Dorema ammoniacum D. Don. (Apiaceae), is a medicinal plant widely used in curing different types of diseases and has plenty of chemical constituents [1]. The genus *Dorema* has seven species in the flora of Iran, among which *D. aucheri* Boiss and *Dorema ammoniacum.* D. Don are endemic [2]. *Dorema ammoniacum* is a vulnerable species which is known by local Persian names of "Kandal", "Vasha" and "Koma-Kandal" grown in spring and early summer to a height of 1-2 m and contains a milky juice. It is one of the most important endemic medicinal plants in many semi-arid and arid regions of Iran, including Kerman, Tehran, Khorasan, Semnan, Isfahan, Sistan Baluchestan, Yazd, and Isfahan. Mohamadabad Ghaen, located in South Khorasan

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Province, Iran, is one of the main and most important natural habitats of *Dorema ammoniacum* for resin production [2-7].

Ammoniacum gum is a medical gum resin with a bitter, acrid and nauseous taste that is produced in the plant's stem cavities, petioles, and roots and is widely used in traditional medicine, cosmetic, food, and detergent industries [5-8]. It is produced in a large number of plants from the Apiaceae family such as asafetida [(the dried latex (gum oleoresin) exuded from the rhizome or tap root of several species of *Ferula)*], galbanum (an aromatic gum resin and a product of certain apiaceae Persian plant species in the genus *Ferula*, chiefly *Ferula gummosa* and *Ferula rubricaulis*), and ammonicum (a gum resin of *Dorema ammoniacum*) [9]. Ammoniacum is listed in the British Pharmacopoeia as an expectorant and antispasmodic plant and is still used in Western and Indian Medicine for chronic bronchitis and persistent coughs [8].

Inflammatory response, as a protective reaction of the microcirculation, is a highly coordinated sequence of events initiated after injury and/or infection and involves cellular, molecular and physiological alterations. It begins with the production of soluble mediators as cytokines, chemokines, vasoactive amines, eicosanoids and free radicals by lymphocytes, dendritic cells, endothelial cells, tissue macrophages, fibroblasts and mast cells in the injured or infected tissue. This well-characterized phase of the inflammatory response is routinely targeted by drugs such as pro-inflammatory cytokine-negating biologics (e.g. TNF-a-specific (tumor necrosis factor-a) antibodies) that inhibit the action of the mediators and non-steroidal anti-inflammatory drugs (NSAIDs) [10]. Considering the dose, duration of consumption and age of the patients, NSAIDs might have various side effects, including digestive (stomach pain or heartburn, black and tarry stool, nausea and vomiting), cardiovascular, renal, hematological and hepatic effects together with skin rashes, mouth lesions, fever and bruising [11,12].

Dorema species have been shown to own different biological activities. Some isolated compounds of *D. ammoniacum* gum have acetylcholinesterase inhibitory activity [13]. Previous studies indicated that *Dorema ammoniacum* gum has significant analgesic and anti-inflammatory effects on experimental animals. Moreover, the results of a research suggested that hydroalcoholic extract of the aerial parts of *D. aucheri*a showed significant anti-inflammatory effects [1,14].

The aim of this study was to investigate the anti-inflammatory effects of DAGE by systemic and topical uses on improving and treating the symptoms of inflammatory diseases.

Material and Methods

Ethical considerations

All experiments were performed in accordance with the NIH Guide for Care and Use of Laboratory Animals (NIH Publications No. 80-23) and were approved by Research and Ethics Committee of Kermanshah University of Medical Sciences, Kermanshah, Iran (IR.KUMS.REC.1395.505, 2016).

Chemicals

Mefenamic acid, Diclofenac, and Carrageenan were purchased from Sigma (USA).

Plant material

The dry gum of *Dorema ammoniacum* was purchased from a local herbal market in Tehran province, February 2016. A voucher specimen has been deposited at the Herbarium of the Department of Pharmacognosy, Pharmaceutical Sciences Branch, Islamic Azad University, Tehran, Iran, under code number 607_PMP/A.

Preparation of aqueous extract

The dry gum of *Dorema ammoniacum* was washed, chopped, air-dried under shade and then powdered. The powder was stored in an airtight container. About 100 g of the dried powder was soaked in 500 mL of water for 48 h with occasional shaking, and then it was passed through a muslin cloth and filtered through the paper filter. The extract was finally dried using a freeze dryer. In the present study, three doses of *Dorema ammoniacum* extract (100, 200 and 300 mg/kg) were selected based on previous preliminary studies [1,14].

Animals

The present experimental study was conducted on 45 male Wistar rats weighing 200-250 g and 9 white male adult New Zealand rabbits. All animals were kept under controlled conditions (22-24 °C and 12 h light/dark cycles) and were provided with standard rat food and water ad libitum [16-18].

Anti-inflammatory activity against carrageenan-induced rat paw edema

Carrageenan-induced paw edema in male Wistar rats was used to evaluate the anti-inflammatory effect of DAGE [15]. In order to evaluate the systemic anti-inflammatory effect of the extract, the rats were divided into five groups of six: 1) standard group which received mefenamic acid (30 mg/kg; i.p.); 2) vehicle (control) group which received saline (10 mL/kg; i. p.); and 3) three test groups which received DAGE (100, 200 and 300 mg/kg; i. p.). In addition, to evaluate the topical anti-inflammatory effect of DAGE, the rats were divided into three groups of five: 1) standard group which received topical diclofenac gel 2%; 2) vehicle (control) group which received topical normal saline; and 3) test group which received topical DAGE, a small quantity of normal saline was added to the freeze dried form of 100 mg/kg extract to dissolve it, and the resultant paste was applied one h before subcutaneous injection of 0.1% w/v aqueous solution of carrageenan into the subplantar region of the right hind paw. The paw volume was measured using a Plethysmometer (model PM 4500, Borj Sanat Co., Iran) prior to and 0.5, 1, 2, 3 and 4 h after the carrageenan administration. Anti-inflammatory activity was revealed as the inhibition percentage of the edema, compared to the control group. The inhibition percentage of the edema was calculated by the following equation:

% inhibition of edema = 100 (1-Vt/Vc)

Where Vc is the edema volume in the control group and Vt is the edema volume in test groups [14].

The Draize primary skin irritation test

In this study, the toxicity level of DAGE was examined in the skin of white laboratory male adult rabbits [16]. The rabbits were divided into three groups of three: 1) test group which received topical DAGE; 2) control group which received topical normal saline; and 3) topical Selenium sulfide 2% shampoo as a positive irritant control group to assess the skin toxicity of DAGE [17]. Some parts of the back of animals' skin were shaved in dimensions of 2.5 × 2.5 cm and disinfected with alcohol. We used a small quantity of normal saline to dissolve 0.5 mg of the freeze-dried extract, and the resultant paste was applied topically. The degree of erythema and edema of the region was measured after 24, 48 and 72 h based on the Draize primary skin irritation test, where the extent of the developed inflammatory reaction was calculated by an index called Primary Irritation Index (PII). PII was the sum of the mean of the erythema and edema of each region during the above time periods. It should be noted that normal saline and selenium sulfate shampoo were respectively used as negative and positive controls.

Statistical analysis

The data at each time point were expressed as the mean ± standard error of the mean (SEM) and analyzed using Graph pad Prism Program, Version 6.0 (Graph Pad Software, Inc., La Jolla, USA). Comparisons between groups were made by Repeated measurement ANOVA analysis followed by the post hoc Tukey's test and $p<0.05$ was considered as the significant difference of means.

Results and Discussion

Repeated measures analysis of variance (ANOVA) showed a significant difference of the interaction between time and paw edema and so interaction between time and groups. The aqueous solution of *Dorema ammoniacum* gum (i.p.) showed anti-inflammatory activity in the carrageenan-induced paw edema in rats at different times. The anti-inflammatory activity of DAGE was comparable with that of mefenamic acid with a dose of 30 mg/ kg. The reduction percentages of rat paw edema in carrageenan test were also compared at different times and doses (table 1). DAGE (100, 200 and 300 mg/kg) as well as, mefenamic acid (30 mg/kg) significantly inhibited ($p<0.05$) the carrageenan-induced paw edema formation in rats (which was determined at the third hour of the experiment; peak of the edema formation) by 62.79, 98.45, 96.90 and 76.74%, respectively. At the fourth hour of the experiment, this edema inhibition reached 82.17, 98.45, 98.45 and 91.47 %, respectively (table 1).

The aqueous solution of topical *Dorema ammoniacum* gum showed slight anti-inflammatory activity in the carrageenan-induced edema in rats. The mean of the carrageenan-injected paw edema in test group (DAGE100 mg/kg) was from 0.23 ±0.01 in 30 min, to a minimum of 0.11±0.01 Cm^3 (mean±SEM) 1 h post induction. In contrast, paw edema in the control group showed no significant changes over time, in mean edema that ranged from 0.27 ±0.03 to 0.28±0.04 Cm^3.

Table 1. Effect of i.p. injection of DAGE on the inflammation induced by carrageenan in rats

Groups	Paw edema(cm^3) at various time intervals (%Inhibition)				
	30 min	**1 h**	**2 h**	**3 h**	**4 h**
N.S	0.3±0.06	0.3±0.06	0.29±0.05	0.22±0.03	0.22±0.03
Mefenamic acid (30 mg/kg)	0.11±0.02** (62.43%)	0.11±0.03** (63.69%)	0.10±0.02**** (67.24%)	0.08±0.02*** (62.79%)	0.04±0.01**** (82.17%)
DAGE (100 mg/kg)	0.14±0.02* (54.14%)	0.10±0.02** (66.48%)	0.01±0.00**** (98.85%)	0.01±0.00**** (98.45%)	0.01±0.00**** (98.45%)
DAGE (200 mg/kg)	0.09±0.03** (69.61%)	0.04±0.02**** (88.27%)	0.01±0.01**** (96.55%)	0.01±0.00**** (96.90%)	0.01±0.00**** (98.45%)
DAGE (300 mg/kg)	0.11±0.02** (65.19%)	0.06±0.01**** (80.45%)	0.05±0.02**** (83.91%)	0.05±0.03**** (76.74%)	0.02±0.01**** (91.47%)

Values are means±SEM (*p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001). Repeated measures ANOVA, with Tukey's test used for post hoc comparison versus control group rats; (n=6). N.S: Normal Saline; DAGE: *Dorema ammoniacum* gum extract

Table 2. Effect of topical DAGE on the inflammation induced by carrageenan in rats

Groups	Paw edema (cm^3) at various time intervals (% Inhibition)				
	30 min	**1 h**	**2 h**	**3 h**	**4 h**
N.S	0.27±0.03	0.27±0.03	0.27±0.03	0.28 ±0.04	0.28 ±0.04
Diclofenac gel (2%)	0.23±0.05 (14.6%)	0.31±0.03 (-14.6%)	0.22±0.03 (18.0%)	0.21±0.02 (26.76%)	0.21±0.02 (26.24%)
DAGE (100mg/kg)	0.23±0.01 (15.3%)	0.11±0.01**## (59.85%)	0.21±0.08 (19.5%)	0.16±0.02 (42.25%)	0.16±0.02 (43.26%)

Each value represents the mean ± SEM (**p<0.01 compared to control, ##p<0.01 compared to diclofenac group), Repeated measures ANOVA, with Tukey's post hoc test used for comparison groups rat (n=5); N.S: Normal Saline; DAGE: *Dorema ammoniacum* gum extract

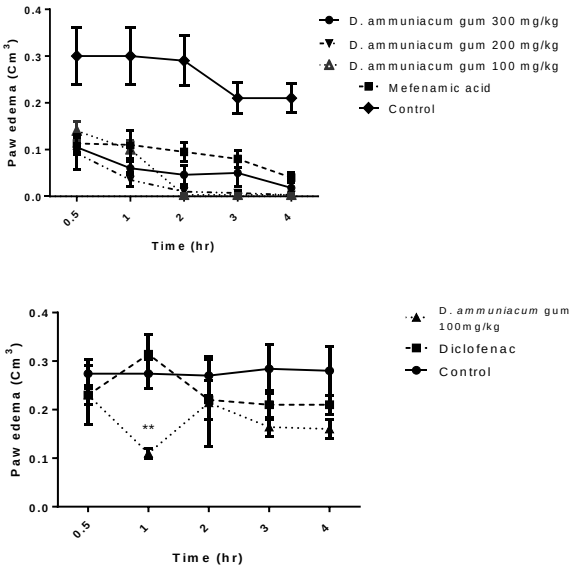

Figure1. Comparison of anti-inflammatory effect of different doses of i.p. (A) and topical (B) *D. ammoniacum* gum in carrageenan-induced paw edema in rats. Each value represents the mean ± SEM (**p<0.01 compared to the control group), Repeated measurement ANOVA followed by Tukey's post-test, (n=6); N.S: Normal Saline; DAGE: *Dorema ammoniacum* gum extract

The mean of paw edema in diclofenac gel (2%) group was from 0.23 ±0.05 on 30 min, to a minimum of 0.21±0.02 (mean±SEM) 3 and 4 h after induction (table 2). The anti-inflammatory activity of *Dorema ammoniacum* gum was comparable to that of topical diclofenac gel 2%. DAGE (100 mg/kg) inhibited the carrageenan-induced paw edema formation in rats (which was determined at the first hour of the experiment) by 59.85%. At the fourth hour of the experiment (peak of edema formation), this edema inhibition reached 26.24 and 43.26 % in DAGE and Diclofenac (2%) groups respectively (table 2). Repeated measures analysis of variance (ANOVA) showed no significant difference of the interaction between time and paw edema.

Draize primary skin irritation test in rabbits showed no toxicity in *D. ammoniacum* gum extract. As it was shown, *D. ammoniacum* induced very slight edema and erythema which are actually considered as negligible irritations (figure 2).

Due to various side effects related to the consumption of steroidal and non-steroidal anti-inflammatory drugs (NSAIDs), discovering new alternative drugs derived from natural sources has become increasingly important. In the present study, the anti-inflammatory activity of *Dorema ammoniacum* gum aqueous extract and its skin toxicity in experimental animals were assessed.

The present study is the first report describing the systemic and local anti-inflammatory activities of DAGE in acute inflammation in rats. The results indicated that i.p. and topical use of DAGE was effective in reducing the inflammation.

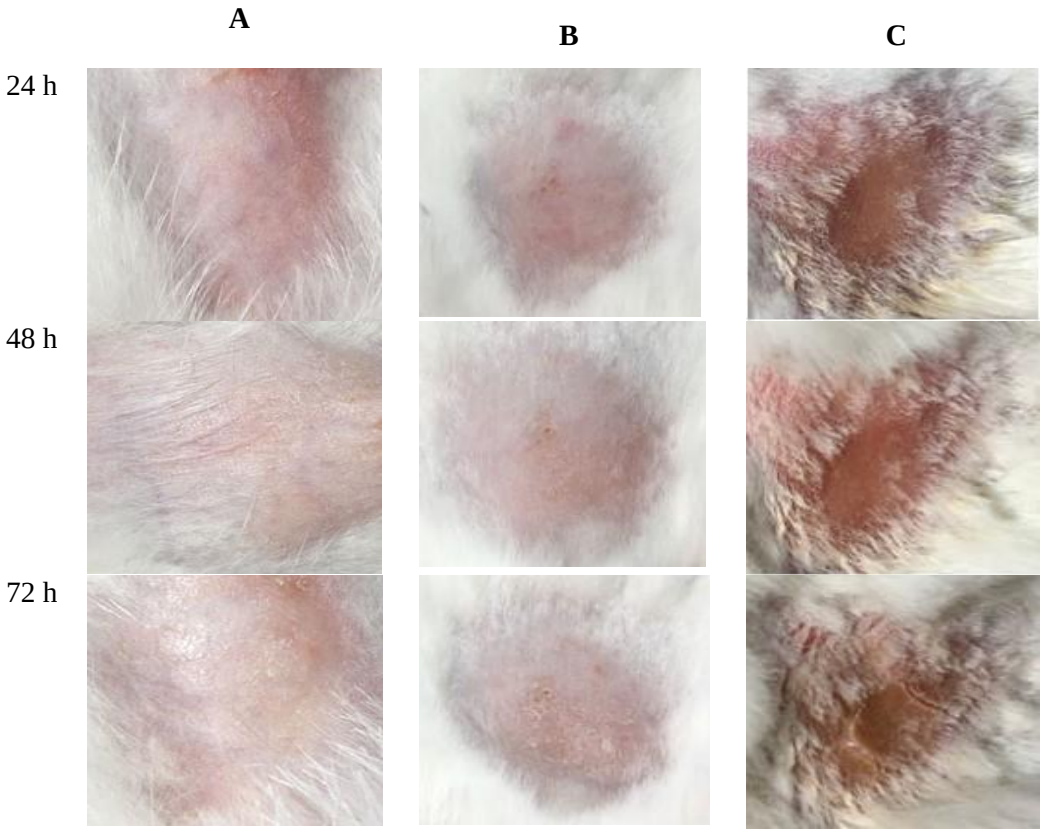

Figure 2. Draize skin irritation test for *D. ammoniacum* gum aqueous extract by topical use in white New Zealand rabbits. (A) Normal saline, (B) DAGE and (C) selenium sulfide 2% shampoo within 24, 48 and 72 h following the topical use (n=3)

Table 3. Draize skin irritation test. Topical use of Normal saline, DAGE and selenium sulfide 2% shampoo within 24, 48, and 72 h followed by evaluating the existence of erythema or edema

Rabbit		24, 48, 72 h (N.S)	24, 48, 72 h (DAGE)	24, 48, 72 h (S.S)
1	Erythema	-	-	+++
	Edema	-	-	+++
2	Erythema	-	-	+++
	Edema	-	-	+++
3	Erythema	-	-	+++
	Edema	-	-	+++

N.S: Normal saline; S.S: Selenium sulfide 2% shampoo (n=9)

The anti-inflammatory activity of i.p. DAGE was comparable to that of mefenamic acid at all doses (100, 200, and 300 mg/kg) and all times (30, 60, 120, 180, and 240 min).We used the 100 mg/kg dose of DAGE to assess its topical anti-inflammatory effect. The reasons for choosing this dose was the increasing trend of anti-inflammatory effect at all different time courses of i.p. injection, and the highest percentage inhibition of edema in the three final time courses, which according to the chronicity of inflammation in various diseases is of great importance. On the other hand, considering probable complications of skin toxicity at higher doses in humans, priority is always given to using a more effective but lower topical dose. As could be observed, one hour after the topical application of the gum, it indicated significant anti-inflammatory effect. Moreover, its topical application of 0.5 mg (prepared freeze-dried resultant paste with normal saline) revealed no skin toxicity in rabbit, which was assessed by Draize primary skin irritation test.

Carrageenan-induced paw edema has been usually offered as an acute inflammation model in the experimental animals. It is well known that carrageenan-induced paw edema is regarded as a biphasic episode with the involvement of inflammatory mediators [15,18,19]. Since carrageenan-induced inflammation model is a significant predictive test for anti-inflammatory agents acting by the mediators of acute

inflammation [15,20], the results of this study suggest that aqueous extracts of *Dorema ammoniacum* gum can be effective in acute inflammatory disorders. It effectively suppressed the edema produced by carrageenan and thus, found to be effective in acute inflammatory conditions. This shows the extract's efficacy in the possible inhibition of the synthesis, release or action of inflammatory mediators.

The present study demonstrated that curative topical treatment of rats using the aqueous extract of *D. ammoniacum* has anti-inflammatory properties similar to that of diclofenac as a reference drug. The anti-inflammatory effect of diclofenac is mediated mainly through inhibition of cyclo-oxygenase and prostaglandin production [21]. Regarding the results of skin irritation test, topical administration of the extract to healthy rabbits showed a comparable effect between the extract-treated and control groups. Phytochemical screening of the essential oil of *D. ammoniacum* has shown the presences of α-gurjunene (49.5 %), β-gurjunene (19.0 %) and α-selinene (4.6%) in leaves [3] and oxygenated monoterpenes (58.4%), sesquiterpene hydrocarbons (31.7%), (Z)-Ocimenone (22.3%), (E)-Ocimenone (18.1%), β-cyclocitral (9.9%) and arcurcumene (6.4%) in fruit [3] and β-bisabolene (15.1%), hexadecanal (13.2%) and (E)-nerolidol (11.3%) in roots were characterized as the major components [22]. Free salicylic acid, ammoresinol, dshamirone and some sesquiterpene chromandiones have also been isolated and identified from D. *ammoniacum* gum [23,24]. Additionally, *D. ammoniacum* ethyl acetate and chloroform extracts of the roots demonstrated strong antibacterial activity against *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* [22]. The fruits oil of *D. ammoniacum* have been shown to possess potent cytotoxic activity on SW-480 and MCF-7 cells as well as, high antimicrobial effects against *Bacillus subtilis* and *Staphylococcus epidermidis* [3,25].

The topical usage of DAGE in the present study showed anti-inflammatory effects, compared to the control and diclofenac groups. Skin as an extremely effective barrier avoids the permeation of most drugs applied for therapeutic goals [26, 27]. Most of the topical dosage forms available on the market have poor penetration which leads to poor therapeutic benefit [28,29]. However, it might be the reason that i.p. DAGE is more effective than its topical administration. Therefore, novel topical drug delivery systems are recommended to maximize possible paths of absorption available for this aim.

Consumption of some topical drugs can also result in dermatological damages including the development of different types of scars or lesions. This can develop as a result of single-use or repeated use in the form of development of inflammation (erythroderma). The skin irritation test is among the methods employed to examine the damages caused by medications to the skin cells and vascular system. Draize primary skin irritation test in rabbits showed no toxicity in *D. ammoniacum* gum extract. As it was shown, *D. ammoniacum* induced very slight edema and erythema which were actually considered as negligible irritations.

The results of the present study suggest that *D. ammoniacum* gum aqueous extract in the studied doses has significant anti-inflammatory effects without producing erythema and edema in topical use. Studying the active ingredients of this gum extract to understand the mechanism of its anti-inflammatory effect is also of great importance.

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Author contributions

Amir Kiani designed all the animal studies, Marziyeh Pandpazir and Sajad Fakhri have done all tests, analyzed the data and wrote the manuscript. Zahra Mousavi edited the manuscript. All the authors have read and approved the final manuscript.

Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the content of the paper.

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Abbreviations

DAGE: *Dorema ammoniacum* gum extract; N.S: normal saline