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EVALUATION OF ANALGESIC AND ANTI-INFLAMMATORY ACTIVITY OF *DOREMA AMMONIACUM* GUM IN ANIMAL MODEL

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ABSTRACT: *Dorema ammoniacum* gum (DAG) has been used for different illnesses in Iranian traditional medicine. The main objective of the present study was to evaluate the analgesic and anti-inflammatory activities of aqueous solution of *Dorema ammoniacum* gum (DAG) in animal model. The anti-inflammatory and analgesic Effects of DAG were examined using carraginane-induced edema test, formalin test and acetic acid-induced writhing response. 42 male albino mice randomly assigned in different groups were pretreated randomly assigned 5 groups. Groups were pretreated either with normal saline, morphine or aspirin and different doses of DAG (125, 250, 500 mg/kg, i.p.) for determination of analgesic effect. The anti-inflammatory effect was evaluated by the rat paw carrageenan induced edema and was compared to positive controls. DAG exhibited the analgesic effect in chronic phase of formalin test in a dose-dependent manner ($P<0.05$). Also, application of different doses of DAG had significant analgesic effect in acetic acid writing test 30 min after its injection. DAG showed anti-inflammatory effect in a dose dependent manner. The anti-inflammatory effect of the extract was comparable to indomethacin in higher doses. Thus, our results demonstrate that ammoniacum gum has anti-inflammatory and analgesic effects in animal models and hence justify the traditional use of this plant in the treatment of pain and inflammatory conditions. Further studies seem necessary to clarify about the active components of this plant.

INTRODUCTION: Nonsteroidal anti-inflammatory drugs (NSAIDs) and opioids are the most commonly used drugs in treatment of inflammation and chronic painful conditions. Other than its beneficial effects, these drugs were found to have different side effects such as gastrointestinal disturbances, renal damage, respiratory depression, and possible dependence in patients. Therefore, there is a need to develop new more effective anti-inflammatory and analgesic agents with minimum side effects from natural sources. [1, 2] Iranian traditional medicine suggests some herbs and herbal preparation for reduction and management of inflammatory pain in joint disease.[3]

Dorema ammoniacum. Don (Umbelliferae), known as Vosha, is suggested for inflammatory and painful conditions in Iranian traditional medicine.[4] The oleo gum resin obtained from the stem and leaf of this plant has been used in traditional medicine. [5] Genus Dorema including six species in Iran, among which two are endemic *i.e. D. ammoniacum* and *D. aucheri*. *D. ammoniacum* grows to a height of 1-2 m in many arid and semi-arid regions of Iran such

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as Yazd, Isfahan and Semnan.[6, 7] Ammoniacum gum is a medicinal gum resin of *D.ammoniacum* which has medicinal and industrial applications. [8] In continuous to previous studies on anti-inflammatory and analgesic effect of Iranian traditional Medicinal plants. [9, 10] in the present study anti-inflammatory and analgesic effects of ammoniacum gum were evaluated in animal model.

Experimental:

Plant material: *D. ammoniacum* gum was purchased from a local herbal market in Tehran, Iran and identified by Dr G. Amin, member of the School of pharmacy, Tehran University of Medical Sciences, Tehran, Iran (DMP-819). The gum was dissolved in distilled water and aqueous solution was obtained which was further used in animal study.

Total phenolic content (TPC): The total phenolic content of the *Dorema ammoniacum gum* was determined according to the Folin–Ciocalteu method with minor modifications. [11]

For the preparation of calibration curve 1 ml aliquots of 75, 100, 150 and 200 μg/ml hydro-ethanolic (50:50) gallic acid solutions were mixed with 5 ml Folin–Ciocalteu reagent (diluted ten-fold) and 4 ml (75mg/ml) sodium carbonate. The absorption was read after 30min at 765 nm and the calibration curve was drawn. One ml of plant extract (1mg/ml) was mixed with the same reagents as described above, and the absorption was measured for the determination of plant phenolics. All determinations were performed in triplicate. Total content of phenolic compounds in *Dorema ammoniacum gum* was expressed as mean ± standard deviations of μg of gallic acid (GAE) equivalents/ mg extract.

Animals: 42 albino mice (20-25 g) were housed in animal unit of Tehran University of Medical Sciences under standard laboratory conditions (temperature 23 ± 2 °C) with 12 h dark and 12 h light cycle. All the animals were provided with free access to standard dry pellet diet and tap water *ad libitum.*

Anti-inflammatory activity:

Carrageenan-induced paw edema: Edema was induced by injection of carrageenan 1% (w/v) suspension into the subplanar region of the right hind paw of the mice. [12] The test groups were pretreated with Different doses of DAG (125-250, 500 mg/kg), indomethacin or normal saline 1 h before injection of carrageenan (20 μL).The paw size was measured by plethysmometer during 5 hours after carrageenan injection.

Analgesic activity:

Formalin test: Analgesic effect of DAG was evaluated by method of Hunskaar and Hole. [13] In this method, 25ml of formalin 2.5% was injected into the subcutaneous tissue on the plantar surface of the left hind paw of rats, 30 min after injection of DAG, aspirin, morphine or normal saline. The time (in sec) spent in licking and biting responses of the injected paw was taken as an indicator of pain response. The responses were observed as soon as the injection was given in acute phase (0-10 min) and 15-60 min after injection (chronic phase). The mean of the time spent on licking the injected paw in each group was determined.

Acetic acid-induced writhing test: The acetic-acid writhing test was performed using the procedure reported by Aoki *et al.,* 2006. [14]

Six Groups of rats(n=7), were administered with125, 250, 500 mg/kg of DAG or 300 mg/kg aspirin or morphine as positive control group and 1 mL distilled water as negative control group. After 30 minutes the animals were administered with interapritoneal injection of 0.1mL acetic acid (1%). Then the count of abdominal contractions of animals during 30 minutes after acetic acid injection was reported and the Percentage Analgesic Activity (PAA) was calculated using the following formula:

$$PAA = ((C- C_D)/C_D) \times 100$$

C = Mean of contractions' count in animals treated with different doses of *DAG*

C_D = Mean of contractions' count in negative control group

Statistical Analysis: The results are reported as mean ± S.E.M. The statistical analysis were performed using one way analysis of variance (ANOVA). Group differences were calculated by post hoc analysis using Tukey post hoc test. For all tests, differences with values of $P<0.05$ were considered significant.

RESULTS: TPC of ammoniacum gum was 28.54 ± 3.7 µg GAE/mg extract.

Formalin test: This study showed that the DAG at doses of 250, 500 mg/kg has significant analgesic effect compare to normal saline group (P<0.05) in acute phase of formalin test. In chronic phase of formalin test, DAG showed analgesic effect in all doses (125, 250, 500 mg/kg) compare to control groups (**Fig. 1** and **2**).

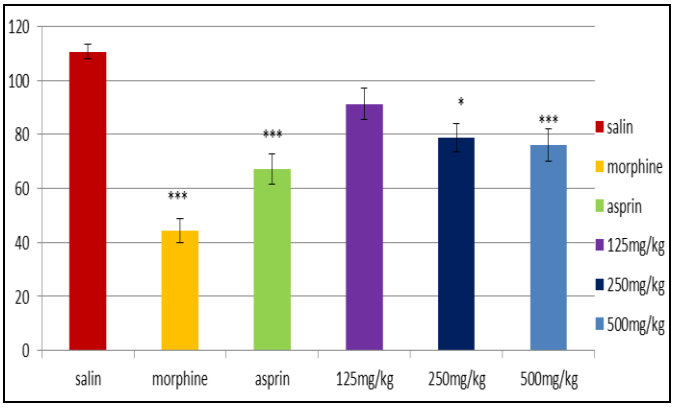

FIG. 1: ANALGESIC EFFECT OF DIFFERENT DOSES OF AMMONIACUM GUM EXTRACT IN ACUTE PHASE OF FORMALIN TEST COMPARE TO POSITIVE CONTROLS (MORPHINE AND ASPIRIN) AND NEGATIVE CONTROL (SALINE). Data are Means±SEM, p<0.05*, p<0.01**, p<0.001*** compare to control group.

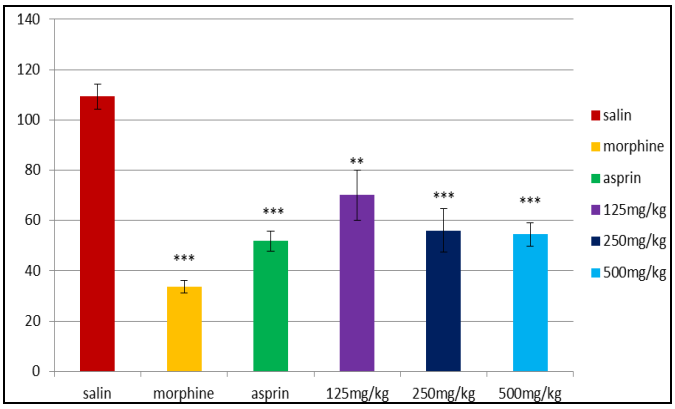

FIG. 2: ANALGESIC EFFECT OF DIFFERENT DOSES OF AMMONIACUM GUM EXTRACT IN CHRONIC PHASE OF FORMALIN TEST COMPARE TO POSITIVE CONTROLS (MORPHINE AND ASPIRIN) AND NEGATIVE CONTROL (SALINE). Data are Means±SEM, p<0.05*, p<0.01**, p<0.001*** compare to control group, (n=7).

Acetic acid-induced writhing test: Application of different doses of DAG showed significant analgesic effects in the animals in 30 min after acetic acid injection (**Fig. 3**).

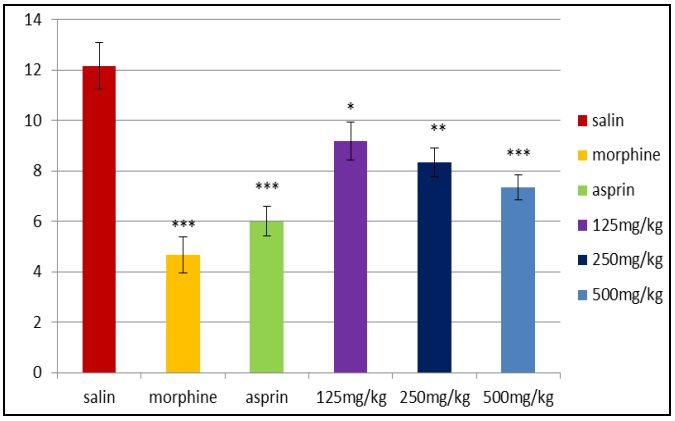

FIG. 3: THE WRITHING RESPONSE IN 30 MINUTES AFTER ACETIC-ACID INJECTION IN THE PRESENCE OF DIFFERENT DOSES OF THE AQUEOUS SOLUTION OF *DOREMA AMMONIACUM* GUM IN EXPERIMENTAL ANIMALS. Data are Means±SEM, p<0.05*, p<0.01**, p<0.001*** compare to control group, (n=7).

Carrageenan-induced paw edema: The aqueous solution of ammoniacum gum showed anti-inflammatory activity in a dose-dependent manner in the carrageenan-induced edema in mice. Anti-inflammatory activity of DAG was comparable to indomethacin in dose 500 mg/kg (**Fig. 4**).

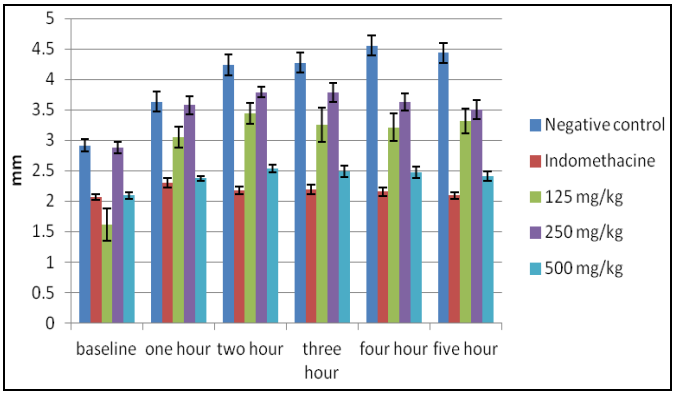

FIG. 4: ANTI-INFLAMMATORY EFFECT OF DIFFERENT DOSES OF *DOREMA AMMONIACUM* GUM IN CARRAGINANE-INDUCED EDEMA. Data are Means±SEM, n=6

DISCUSSION: Due to various side effects associated with the use of analgesic and anti-inflammatory drugs, an interest has been increased in discovering new alternative medicines from natural sources. [1, 2] In traditional medicine of Iran, several natural products have been used for inflammation and painful conditions like joint pains. *Dorema ammoniacum* is a medicinal plant which has been used in Iranian traditional medicine for epilepsy, inflammation and in treatment of various other clinical conditions for many years. [15]

The main aim of this study was to uncover the anti-inflammatory and analgesic activities of the aqueous solution of *D.ammoniacum* gum in animal models using carrageenan-induced rat paw edema, acetic acid-induced writhing test and formalin test. The results from our study revealed that *D.ammoniacum* gum showed significant anti-inflammatory and analgesic activity in a dose dependent manner. The highest analgesic and anti-inflammatory activity of DAG was found at dose of 500 mg/kg which was comparable to positive controls.

In order to determine the anti-inflammatory response of DAG, carrageenan-induced paw edema test was performed. When carrageenan was injected into the paw of mice various cardinal signs like edema and hyperalgesia was developed. Previous research shows that this test aggravates the formation of various proinflammatory cytokines like histamines, bradykinins and tachykinins. Also, various cells like neutrophils can infiltrate into the site of injection making the paw enlarged 5 h later due to edema formation. [16] it clearly revealed that the in carrageenan test, DAG in a dose of 250, 500 mg/kg has significantly decreased the inflammation in comparison to negative control and was nearly comparable to positive control. Hence, this result justifies that DAG has promising anti-inflammatory effect and active ingredient.

Formalin test is another valid model for the evaluation of clinical pain and inflammation. Experimentally this test has two phases including acute and chronic or late phases. The acute or early phase of pain is achieved upon the activation of C-fiber by peripheral stimulation while late phase is as a result of inflammatory response. [17] It has been postulated that drugs such as morphine which acts mostly in the CNS can prevent both acute and chronic phase. While drugs which are reported to inhibit the pain peripherally is usually attributed by halting the activity of cyclooxygenase (COX). Our results are in line with the later stage as DAG inhibited the pain at the peripheral level. [18] It has been shown that drugs such as morphine inhibited both acute and chronic phase of formalin induced pain while aspirin or hydrocortisone which are peripherally acting only inhibits the late phase of pain. [19, 20] As shown in **Fig. 1** and **2,** The DAG in a dose of 500 mg/kg significantly reduced the pain in acute ($p<0.001$***) and chronic phase ($p<0.001$***) compared to control group in formalin test. The paw licking activity was significantly reduced. Hence, it is clear that the analgesic effect of DAG is mediated peripherally.

Writhing test is another test used as a useful tool for the investigation of peripheral antinociceptive activity of drugs and is considered as a chemical induced pain model. [21, 22] In short, in this test the irritable compound such as acetic acid or phenylquinone is injected into the mice. [23] Our results showed that ammoniacum gum has anti-inflammatory effect since, the peripheral analgesic effect was significantly reduced ($p<0.001$*** compare to control group) by DAG (500 mg/kg) in acetic acid induced writhing test. Most of the peripheral pain is associated with the formation of inflammatory cytokines and autacoids like interleukins and bradykinins.

Hence, taking into consideration the above facts it is clear that DAG has significant analgesic and anti-inflammatory activity. This activity is achieved by inhibiting the pain at peripheral level which might be due to inhibition of proinflammatory cytokines. However, it needs further studies to clarify the mechanism.

Different compounds of dorema species showed varieties of biological activities. Some Dorema species showed anti-inflammatory and anti-nociceptive activity in animal models. In a study by Mokhtari *et al*., hydro alcoholic extract of aerial parts of *D.aucheri*a showed significant anti-inflammatory and analgesic effect especially with a dose 400 mg/kg. [24] Some isolated compounds from *D.ammoniacum* gum have acetyl cholinesterase Inhibitory Activity. [25] Phenolic compounds isolated from *D.glabrum* showed geno/cytotoxicty and apoptotic properties. [26] According to the present study, *D.ammoniacum* gum contains phenolic compounds which may play an important role in biological activity of the gum. Although further studies are the subject of need to determine the active principle of ammoniacum gum which was responsible for anti-inflammatory and analgesic activities of this plant.

CONCLUSION: The results of this study demonstrated that ammoniacum gum showed anti-inflammatory and analgesic effects in animal models and hence justify the traditional use of this plant in the treatment of pain and inflammatory conditions. Further studies seem necessary to clarify about the active components and mechanisms of action of this plant.

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CONFLICT OF INTEREST: Authors have no conflict of interest.

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