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The antinociceptive and anti-inflammatory activities of *ferula assafoetida* gum in rodent model

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ABSTRACT

The aqueous extract of the *ferula assafoetida* gum was chosen for pharmacological screening. Oral LD_{50} value greater than 5000 mg/kg was obtained indicating the safety of the gum extract for traditional use. In the acetic acid-induced writhing test, the extract showed 44.78 % inhibition of writhing. In radiant heat tail-flick method the gum extract produced 48.31 % elongation of tail flicking time 10 minutes after oral administration at the 250 mg/kg dose level. The oral administration of (250 mg/kg) gum extract caused maximum inhibition of 45.59 % in rat paw edema model induced by formaldehyde at the end of four hours (h). Diclofenac sodium at a dose of 50 mg/kg was used as a standard drug for comparing the antinociceptive as well as the anti-inflammatory activities of the *ferula assafoetida* gum. The gum extract of the *ferula assafoetida* had significant analgesic and anti-inflammatory effects supporting the traditional use of this plant in the treatment of different types of pains and inflammation.

INTRODUCTION

Plants have been a constant source of drugs and recently, much emphasis has been placed on finding novel therapeutic agents from medicinal plants. Today many people prefer to use medicinal plants rather than chemical drugs to avoid the side effects of chemicals. *Ferula asafetida* is follow the family of *Umbelliferae* has been found to be a rich source of oleo gum-resin obtained from the rhizome and root of plant[1]. This resin takes consideration as a class in folklore medicine[2]. The oleo-resin gum had many pharmacological actions as an antispasmodic digestive, expectorant, anthelminitic and antiseptic. In addition, Mahendra and Bisht[3] stated that it had aphrodisiac as it gave positive response in case of sexual impotency. *Ferula asafetida* extract induced significant reduction in blood glucose and increasing the serum insulin, indicating therapeutic protective effect against the pancreatic β- cells damage from alloxan-induced diabetes in rats[4]. *Ferula asafetida* showed high total phenol and flavonoid contents[5]. Also, this genus of plant presents interesting phytochemical features, such as the occurrence of sesquiterpenes and coumarins[6,7]. Studies have shown that increasing levels of flavonoids in the diet could decrease certain human diseases[8]. This study is designed to determine the therapeutic dose of *Ferula assafoetida* and consequently to investigate diverse ethno-pharmacological effects about the analgesic and anti-inflammatory properties of the gum extract of *Ferula asafetida*.

MATERIALS AND METHODS

Plant extract

Commercially available Asafoetida (oleo gum resin of *F. assafoetida*) was purchased from nation market, Egypt. The voucher specimen (A13) was authenticated at the herbarium of faculty of Science, Cairo University.

Animals

Albino Wistar female rats (150-170 g) and Swiss

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male mice (18–22 g) will be obtained from Laboratory Animal Colony, Helwan, Egypt. Animals will be maintained in the Animal House of Pharmacology Department, Faculty of Veterinary Medicine, Cairo University under controlled hygienic conditions.

Drugs and Chemicals The chemicals used in this study were formaldehyde (Sigma), - Diclofenac sodium (Voltarin®) from Novartis Pharma Co., Cairo, Egypt, under license from Novartis Pharma AG, Basle, Switzerland, tween 80 (Al-gomhoria Company, Cairo-Egypt).

Acute toxicity test

Lorke's[9] method will be used to ascertain the acute toxicity of the extracts of *Ferula assafoetida*. Three groups of 5 mice each will be administered 10, 100 and 1000 mg/kg of extract orally. The mice will be observed for 24 h for effects of toxicity and the number dying in each group within the period noted. When no deaths were recorded, another four groups of 5 mice each were administered 2000, 3000, 4000 and 5000 mg/kg of both extracts orally. The animals were observed for 48 h for effects of toxicity and the number dying in each group within the period will be recorded. The LD_{50} values were then calculated as the geometric mean of the highest non-lethal and the lowest lethal doses mathematically according to Kerber[10] method using the following formula:

$LD_{50} = LD_{100} - \Sigma (z \times d)/m$

Where z is a half of sum of animal quantity died from two next doses; d is the interval between two next doses and m is the number of animals/group.

Evaluation the antinociceptive effect of *Ferula assafoetida*

1- Acetic acid induced writhing test

The peripheral analgesic activity of extracts of *Ferula assafoetida* will be measured by the acetic acid induced writhing test as described earlier[11]. Mice were fasted for 24 h with water given *ad libitum*. At the beginning of the experiment, Mice were treated orally with either 2% Tween-80, extracts of *Ferula assafoetida* (250 mg/kg) or diclofenac sodium (50 mg/kg). One hour later, animals were injected intraperitoneally with acetic acid (0.7%) at a dose of 0.1 ml/10g of body weight will be used to create typical stretching response. Animals were then placed in an observation box. The total number of writhes (abdominal constrictions) will be counted under a double blind observation for 10 min after the application of acetic acid.

2- Radiant heat tail-flick method

The central analgesic activity of extracts of *Ferula assafoetida* will be studied by measuring drug-induced changes in the sensitivity of the pre-screened (reaction time: 2-4 sec) mice to heat stress applied to their tails as described by Janssen *et al*.[12]. Mice were fasted for 24 h with water given ad libitum and were pretreated orally with either 2% Tween-80, extracts of *Ferula assafoetida* (250 mg/kg) or diclofenac sodium (50 mg/kg). After 30 min, 1–2 cm of the tail of mice will be immersed in water bath kept constant at 55 æ%C. The time taken by the mice to deflect their tails will be recorded as the reaction time. The cut-off reaction time will be fixed at 10 second to avoid any tissue damage.

Evaluation the anti-inflammatory effect of *Ferula assafoetida*:

In this experiment, formaldehyde-induced rat hind paw edema will be used as the animal model of acute inflammation according to described previously by Saha *et al*.[11]. Briefly, acute inflammation will be produced by subplantar injection of 0.2 ml formaldehyde (1%, w/v) into the rat hind paw, in the right hind paw of the rats one h after the oral administration of *Ferula assafoetida*. The paw volume will be measured by plethysmometer at 1, 2, 3, and 4 h after the formaldehyde injection. Diclofenac sodium (50 mg/kg body weight) will be used as standard anti-inflammatory agent.

The inhibition of inflammation will be calculated using the formula, % inhibition = 100 (1-Vt/Vc), Where 'Vc' represents edema volume in control and 'Vt' edema volume in group treated with test extracts.

STATISTICAL ANALYSIS

Data were analyzed by one-way ANOVA. *P* values <0.005 and *P* values <0.001 were considered statistically significant.

RESULTS

The safety of the *Ferula assafoetida* extract is evi-

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denced by the high LD_{50} value of the extract (>5g/kg). In addition, there were no significant modification in the general behaviour of the animals nor were there death after 72 hours at the highest administered dose (5g/kg) of the extract. Studies carried out to access the safety of this extract using mice revealed a wide margin of safety LD50 > 5 g/kg. Antinociceptive activity of *Ferula assafoetida* extract was determined using the acetic acid induced writhing response and tail flick models.

Diclofenac sodium caused significant reduction in writhing count, from 23 to 9.12 whereas *Ferula assafoetida* extract made it 12.7 from 23. The effect of extract and diclofenac sodium was analysed. The treatment of animals with gum extract of *Ferula assafoetida* and diclofenac sodium was found to be significant ($p<0.001$) compared with control group (TABLE 1).

TABLE 1 : Showing analgesic activity of *Ferula assafoetida* extract against writhing test (n =5)

Group	Mean ± S.E. of writhing number	Protection %
Control	23.00 ± 2.10	----
***Ferula assafoetida* (250 mg/kg)**	12.70 ±1.55*	44.78
Diclofenac sodium (50 mg/kg).	9.15 ±1.50**	60.22

***P<0.005 **P<0.001 as compared to control**

Ferula assafoetida extract exerted a good protective effect on chemical (acetic acid injection) and thermal (tail-flick) painful stimuli (TABLE 1&2). The central analgesic activity of extracts is more pronounced than the peripheral analgesic activity. The extract exerts a significant increase in the latency to response of tail to thermal stimulation.

TABLE 2 : Effect of *Ferula assafoetida* extract on the latency of the tail flick test in mice (n =5)

Group	Mean ± S.E. of Reaction time (sec)	Protection %
Control	4.45±0.16	0
Ferula assafoetida (250 mg/kg)	6.60 ±0.25**	48.31
Diclofenac sodium (50mg/kg).	7.25±0.20**	62.92

****P<0.001, compared to control**

Formaldehyde induced paw oedema model showed (TABLE 3) that subplantar injection of formaldehyde in rats caused a time-dependent increase in paw thickness where the maximal increase was observed at 4 h after formaldehyde administration to the control group. However, formaldehyde induced inflammation was significantly ($p<0.001$) reduced in all phases of the experiment for treatment with *Ferula assafoetida extract and* reference antiinflammatory drug diclofenac sodium.

In rat paw edema model induced by formaldehyde, *Ferula assafoetida* extract at the 250 mg/kg dose level showed inhibition of edema volume at the end of 4h. The oral administration of (250 mg/kg) gum extract caused maximum inhibition of 45.59 % that was nearly close to diclofenac sodium (47.06 %) at a dose of 50 mg/kg (TABLE 4).

DISCUSSION

Medicinal plants indeed have been an indispensable arm in ameliorating common inflammation, pain sensation as well as nonciception[13]. The constriction response of abdomen produced by acetic acid is a sensitive procedure for peripheral analgesic agents. This response is believed to be mediated by the prostaglandin pathways because the abdominal injection of acetic acid in mice has been attributed to the release of arachidonic acid metabolite, which results the synthesis of prostaglandin via the cyclooxygenase (COX) enzyme[14,15]. The special nerve endings that sense pain is very sensitive to prostaglandin. When prostaglandin is released, the nerve endings respond to it through prostaglandin E2 (PGE2) receptor by picking up and transmitting the pain and injury messages to the brain and cause visceral writhing stimuli in mice[16]. Therefore, it has been suggested that the inhibition of prostaglandin synthesis is remarkably an efficient antinociceptive mechanism in visceral pain[17]. Since the gum extract produced antinociceptive activity and thus indicates the presence of analgesic components that might influence the prostaglandin pathways. In the radiant heat tail-flick test, the plant extract prolonged the stress tolerance capacity of the mice, indicating the possible involvement of a higher center[18]. The oral administration of (250 mg/kg) gum extract caused maximum inhibition of 45.59 % n rat paw edema model induced by formaldehyde at the end of four hours (h). The extract exerted a

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TABLE 3 : Mean ± S.E. of paw thickness of *Ferula assafoetida* extract by formaldehyde induced rat paw edema (n =5)

Group	Mean ± S.E. of paw thickness in mm				
	Dose in mg/kg	1 h	2 h	3 h	4 h
Control negative	----	0.30±0.02	0.30±0.01	0.30±0.01	0.30±0.01
Control positive (formaldhyde)	----	0.58±0.03	0.66±0.03	0.74±0.05	0.68±0.04
Ferula assafoetida	250	0.46±0.03*	0.50±0.03*	0.42±0. 01**	0.37±0.02**
Diclofenac sodium	50	0.40±0.02**	0.45±0.03**	0.40±0.03**	0.36±0.02**

TABLE 4 : Anti-inflammatory activity of *Ferula assafoetida* extract by formaldehyde induced rat paw edema (n =5)

Group	% of inhibition				
	Dose in mg/kg	1 h	2 h	3 h	4 h
Control (form)		-----	-----	-----	-----
Ferula assafoetida	250	20.68	24.24	43.24	45.59
Diclofenac sodium	50	31.00	31.18	45.94	47.06

good protective effect on chemical (acetic acid injection) and thermal (tail-flick) painful stimuli. Such an efficacy on these two stimuli is characteristic of central analgesics, such as morphine, which inhibits inflammatory and non-inflammatory pain[19]. Therefore, the extract appears to has morphine like effects, which would explain the antinociceptive effects on CNS observed in this study.

The extract was then tested against other model of experimental pain. It was assayed on the first phase of formaldehyde induced pain known as neurogenic pain. The anti inflammatory activity of the same extract was estimated volumetrically by measuring the mean increase in hind paw volume of formaldehyde induced oedema in rats with the help of plethysmometer. Development of edema in the paw of rat after injection of carra geenen is indeed a biphasic, of which the initial phase observed during the first hour is attributed to the release of histamine and serotonin where as the second one of edema is due to the release of prostaglandins, protease, and lysosome[20].

This leads to a dilation of the a rterioles and venules and to an increased vascular permeability. As a consequence, fluid and plasma proteins are extravagated, and edema forms[21].

The gum contains sesquiterpenes and flavonoids that have been proved to possess analgesic anti-inflammatory activities[8]. Therefore, it can be suggested that the pharmacological effects of the gum extract may be due to their content of the preceding active constituents.

CONCLUSION

We concluded that gum extract of the *ferula assafoetida* had significant analgesic and anti-inflammatory effects supporting the traditional use of this plant in the treatment of different types of pains and inflammation. However, further studies are still essential to confirm the above results in other experimental models to conclude whether the effect observed is actually valid for analgesic and antiinflammatory effect. Pharmacodynamic studies should be undertaken to establish the mechanism of action of the plant extract. Phytochemical investigation is also proposed in order to isolate the active fraction and finally the pure compound.

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