

Screening of *Ferula narthex* Boiss Crude Methanolic Extract for Analgesic, Gastrointestinal Motility and Insecticidal Activity

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Abstract: The crude methanolic extract of *Ferula narthex* (MeFn) was screened for analgesic, GIT motility and insecticidal activity. Acetic acid-induced writhing model was used for assessment of analgesic effect in mice, while charcoal meal model was used for GIT motility assessment. The crude MeFn showed dose dependent analgesic effect, the significant effect was observed with 100 and 200 mg/kg dose. The analgesic effect of diclofenac sodium (standard drug) was greater than crude MeFn. The crude MeFn extract reduced the motility of GIT in mice. The reduction in GIT motility is dose dependent and maximum effect was produced by 200 mg/kg dose. The insecticidal effect was not significant except n-Hexane fraction which possesses moderate activity against all tested insects (maximum 60% against *R. dominica*). These effects of crude MeFn extract justify its use in folkloric medicines for the management of pain and various gastro-intestinal disorders.

Key words: *Ferula narthex* • GIT motility • Analgesic • Insecticidal • Diclofenac sodium • Folkloric medicines

INTRODUCTION

Genus *Ferula* belongs to family umbelliferae, which comprises 275 genera and 2850 species, which are distributed throughout the world, especially in Afghanistan, Iran, India and Pakistan [1]. *Ferula narthex* Boiss, locally known as “raw” in Chitral and found in various localities of Pakistan like Gilgit, Chitral (Kamari, Damusar, Chillim, Gudai, Astore and also found at the hill of Majini Harai) [2]. Local people used this plant for cough, asthma, toothache, gastric problems and also in constipation, angina pectoris. Gum resin of *Ferula narthex* Boiss is used in hysteria, treatment of habitual abortion, whooping cough and scorpion sting [2-5]. Extract and pure compounds from this plant showed anticancer [6], antidiabetic [7] and anti-fertility effect [8]. A large number of active compounds have been isolated from *Ferula* genus. Mainly sesquiterpene, coumarins and sulfur containing compounds have been reported [9-12]. Based on folkloric use of this plant we want to explore it scientifically to justify its use in different ailments.

MATERIALS AND METHODS

Preparation of Crude Extract: In July 2010 *Ferula narthex* plant was collected from Chitral located in Khyber Pakhtunkhwa Pakistan. The plant was identified by Prof. Dr. Abdurashid, Department of Botany UOP and plant specimen was preserved in the Herbarium with voucher No. bot. 20002 (PUP). The collected plant of *Ferula narthex* was air dried in shade and then powdered. Dry powder of root (8.0 kg) was extracted by maceration method using methanol as an extraction solvent for 14 days at room temperature with daily shaking. After this it was filtered and the crude MeFn extract of roots (900 g) was obtained which was concentrated under vacuum at low temperature (45 °C).

Analgesic Activity: The below protocol was used to know about antinociceptive/ analgesic effect of *F. narthex* crude methanolic extract.

Acetic Acid Induced Writhing: The MeFn was screened for the presence of analgesic effect. For this purpose

BALB/C mice 18-22g body weights (either sex) were selected. These animals were divided into five different groups. Group I and group II were served as negative and positive control. Saline at a dose of 10 ml/kg was administered to Group I, while standard drug (Diclofenac sodium) at a dose of 10 mg/kg was administered to Group II through i.p. route. They were then fed according to the recommended guidelines, but food supply was stopped 2 h before the start of activity. The crude MeFn was administered to the remaining Groups i.e. III, IV and V at a dose of 50, 100 and 200 mg/kg respectively. After 30 min of the above mentioned treatments, 1% acetic acid was administered to all groups through intra-peritoneal route. Then after 5 min of acetic acid injection abdominal writhes (constrictions) were started to count for next 10 min. the percent writhing inhibition was calculated by formula given below:

$$\% \text{ writhing Inhibition} = \frac{\text{Mean No. of Writhes in control} - \text{Mean No. of Writhes in test}}{\text{Mean No. of Writhes in control}} \times 100$$

G.I.T Motility

Charcoal Meal Protocol: The MeFn was tested for its effect on G.I.T motility. BALB/C mice 25-30 g were selected for this purpose and were divided into four different groups. Group I served as negative control. Saline 10 mL/kg dose (i.p.) was administered to Group I. The crude MeFn was administered (i.p.) to the remaining Groups i.e. II, III and IV at a dose of 50, 100 and 200 mg/kg. After 15 min of the above mentioned treatments, charcoal suspension (aqueous) at a dose of 0.3 mL, p.o. was administered to each mouse. Then all animals were killed by cervical dislocation after 30 min of charcoal treatment [13]. Through dissection the small intestine was removed and movement of charcoal in small intestine was noted by calculating percent GIT motility with formula given below.

$$\text{Percent Motility} = \frac{\text{Distance covered}}{\text{total length of intestine}} \times 100.$$

Insecticidal Activity: Crude MeFn extract of roots, aerial part and its subsequent fractions were tested for insecticidal activity. Three insects *viz.*, *Callosbruchus analis*, *Tribolium castaneum* and *Rhyzopertha dominica* were used in this assay. In 3 ml acetone 200 mg of crude MeFn extract and various fractions were dissolved to prepare stock solution. A 90 mm filter paper was taken and placed in petri dishes, with the help of micropipette it was loaded with test

sample (1019.10 $\mu\text{g}/\text{cm}^2$). It was then kept for 24 h to evaporate the solvent. Next day in above petri dishes 10 active insects were added and placed in growth chamber for 24 h at 27 °C. Standard drug (Permethrin 239.50 $\mu\text{g}/\text{cm}^2$) was considered as positive control along with 10 active insects. Petri dish containing acetone with 10 active insects was labeled as negative control. Mortality was calculated in term of percentage in comparison with positive and negative controls [14, 15].

Percentage mortality was determined by using formula:

$$\% \text{ Inhibition} = 100 - \frac{\text{Number of living insects in test}}{\text{Number of living insects in control}} \times 100$$

Statistical Analysis: The results were presented with mean and SEM (standard error of mean) for each group of animals. With ANOVA statistical analysis was performed and for multiple comparisons post hoc and Dunnetts test was used. The significance of P value was considered at $P < 0.05$.

RESULTS

Crude MeFn extract at different doses through i.p. route (50, 100 and 200 mg/kg) showed, decrease in mean number of writhing in different test groups as shown in Table 1. In saline treated group mean writhing were 57.00 ± 1.84 . The percent inhibitory effect produced by different test doses of MeFn was 9.86%, 23.19% and highest 50.10% was noted. The percent inhibition produced by Diclofenac sodium (standard drug) at 10 mg dose was 74.33%, which is greater than highest dose (200 mg/kg) of MeFn.

The crude MeFn decreased the percent GIT motility in dose dependent manner as results are presented in Table 2. The percent GIT motility was observed with 50 mg/kg test dose is 45.21%, at 100 mg dose 37.59% and at 200 mg dose 30.98%. The % GIT motility of normal saline treated group was 48.37%. From the results it is clear that crude MeFn exert its GIT motility reduction effect in dose dependent manner. The maximum decrease in GIT motility effect was produced by 200 mg/kg dose of MeFn.

The crude MeFn extract (A) of roots and aerial parts and its subsequent fractions which include (B) *n*-hexane, (C) chloroform, (D) ethyl acetate, (E) *n*-butanol and (F) aqueous were tested against three different insects *viz.*,

Table 1: Analgesic Activity of crude MeFn extract of *F. narthex*

S. No.	Treatments	Dose	No. of writhing (10 min) (Mean±SEM)	% inhibition of writhing
1	Control (Normal saline)	10 mL/kg	57.00 ± 1.84	0
2	Crude MeFn	50 mg/kg	51.33 ± 1.60	9.86
		100 mg/kg	43.67 ± 1.58	23.19**
		200 mg/kg	28.33 ± 1.40	50.10***
3	Diclofenac sod.	10 mg/kg	14.50 ± 1.20	74.33***

Table 2: G.I.T Motility of crude MeFn extract of *F. narthex*

Treatments	Dose	Mean Total length of intestine (cm)	Mean Charcoal movement (cm)	% GIT Motility
Normal saline	10 mL/Kg	56.50±1.118	27.33±0.8819	48.37 ± 1.259
Crude MeFn	50 mg/kg	55.00±1.211	24.83±0.6009	45.21 ± 1.145
	100 mg/kg	54.50± 0.718	20.50±0.6191	37.59 ± 0.855***
	200 mg/kg	57.67±0.988	17.83±0.7032	30.98 ± 1.372***

Table 3: Insecticidal activity of roots (A. Crude MeFn, B. *n*-hexane, C. Chloroform, D. Ethyl acetate, E. *n*-butanol, F. Aqueous)

Name of insects	% Mortality (samples)						% Mortality Permethrin (STD) Acetone
	A	B	C	D	E	F	
<i>Tribolium castaneum</i>	20	40	-	-	-	-	100 0
<i>Rhyzopertha dominica</i>	-	60	-	-	-	-	100 0
<i>Callosbruchus analis</i>	-	20	-	-	-	-	100 0

Table 4: Insecticidal activity of aerial part (1. Crude MeFn, 2. *n*-hexane, 3. Chloroform, 4. Ethyl acetate, 5. Aqueous)

Name of insects	% Mortality (samples)					% Mortality Permethrin (STD) Acetone
	1	2	3	4	5	
<i>Tribolium castaneum</i>	-	-	-	-	-	100 0
<i>Rhyzopertha dominica</i>	-	-	-	-	-	100 0
<i>Callosbruchus analis</i>	-	-	20	-	-	100 0

T. castaneum, *R. dominica* and *C. analis* as given in Table 3 and 4 respectively. In case of testes samples of roots, the crude methanolic extract and *n*-hexane fraction showed moderate insecticidal effect against *T. castaneum* which caused percent mortality of 20 and 40% respectively. The *n*-hexane fraction was observed with good insecticidal activity against *R. dominica*. The percent mortality was 60%. The remaining fractions were inactive against *R. dominica*. Against *Callosbruchus analis* only *n*-hexane fraction showed low grade insecticidal effect and percent mortality was 20%. In case of aerial parts only chloroform fraction showed mild insecticidal effect with percent mortality of 20%. The remaining fractions were inactive against all the tested insects. Only *n*-hexane fraction of roots showed insecticidal activity against all three tested insects.

DISCUSSION

The experiments showed that the crude MeFn extract posses analgesic effect and also decreased the GIT motility. The acetic acid induced pain model is a common, rapid, sensitive and easy method for the determination of

peripheral analgesic effect of plant extracts and other drugs as well [16]. Abdominal constriction occurrence in mice is considered to be involvement of the local receptors (peritoneal) [17]. It is also reported that there is an increase sensitization of peritoneal receptors (nociceptive) to prostaglandins. Generally it is assumed that with acetic acid induced pain model, the synthesis (production) of prostanoids like PGF_{2α} and PGE₂ and lipooxygenase derivatives increased in the peritoneal fluids and served as pain mediators [18]. These substances are produced by cyclo-oxygenase (COX) pathway derived from arachidonic acid, which is liberated from phospholids of inflamed abdominal tissue [19]. These chemicals produced in peritoneal fluids are responsible for pain which may appear in the form of abdominal constrictions. Writhing inhibition by various substances may be involved in the decreased production or inhibition of prostanoids, which is considered for pain inhibition through peripheral mechanism [18].

It is suggested that the crude MeFn extracts exert its peripheral analgesic effect through inhibition of abdominal receptors (nociceptive) and also in decreased synthesis or inhibition of prostanoids production.

The active chemical(s) present in crude MeFn produced analgesic effect in the form of reduction in abdominal constrictions, suggesting the mechanism of action is linked to its interference with pain mediators.

The charcoal meal protocol is one of the best and well studied procedures for the determination of GIT motility [20]. The test doses of plant showed significant reduction in GIT motility with increase in dose of crude MeFn. The different plants belong to genus *Ferula* is famous for antispasmodic effect and this study also supports this effect [21]. The crude MeFn may cause relaxation of intestine by interaction with M₃ receptor (muscarinic) found in small intestine, where acetylcholine can act and activation of M₃ receptors will lead to increase contraction [22]. The plant extract reduces GIT motility and can be used for the management of diarrhea and in abdominal spasm.

Writhing numbers (mean ± SEM) given for test doses of crude MeFn (50, 100 and 200 mg/kg), standard drug (Diclofenac sodium 10 mg/kg) and for saline groups. Values of % GIT motility were also presented by mean ± SEM. ANOVA (Post Hoc Dunnett's test) was used for the analysis of data. The statistical significant values were labeled with asterisks, **P < 0.01, ***P < 0.0001.

The insecticidal activity of test samples is not significant. Only *n*-Hexane fraction showed moderate effect against all insects.

CONCLUSIONS

From this study we all inferred that the MeFn extract contains some bioactive agents, which comprise of analgesic, anti-diarrheal and spasmolytic effects. Furthermore it also supports the folkloric use of this plant for the management of pain and gastrointestinal disorders.

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