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Anti-Inflammatory, analgesic and anti-pyretic activity of *Fagonia bruguieri* DC in rats

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Abstract: Traditional medicine has employed the plant *Fagonia bruguieri* DC. to alleviate inflammation, fever and pain. The goal of this study was to test the anti-inflammatory, analgesic and antipyretic properties of the methanol extract of whole plant of *Fagonia bruguieri* (*F. bruguieri*). The writhing test and Eddy's hot plate test were used to assess the analgesic potential of *F. bruguieri* at three different doses. Carrageenan-induced rat paw edema was applied to investigate anti-inflammatory activity, whereas antipyretic activity was estimated in Brewer's yeast induced pyrexia model. Flavonoids, alkaloids, saponins, tannins and glycosides were found in *F. bruguieri's* phytochemical analysis. *F. bruguieri* at 750 mg/kg reduced writhing count by 62.23 percent, while *F. bruguieri* enhanced latency in Eddy's hot plate test. In carrageenan-induced edema, *F. bruguieri* at 750 mg/kg exhibited considerable anti-inflammatory effect (41.11 percent) after 2nd, 3rd and 4th hours of therapy. *F. bruguieri* was also found to show antipyretic properties. The anti-inflammatory, analgesic and antipyretic properties of *F. bruguieri* were confirmed in this study, which might be attributable to the presence of several phyto-constituents.

Keywords: Analgesic, anti-pyretic, anti-inflammatory, *Fagonia bruguieri.*

INTRODUCTION

Inflammation is a complicated pathological process that is mediated by a multitude of chemical signals formed by leucocytes, macrophages and mast cells as these respond to diverse stimuli. This involves phagocytic cell uptake as well as the stimulation of inflammatory mediators such as nitric oxide, prostaglandin and tumor necrosis factor-α (TNF-α). Since inflammation and pain are involved in nearly all human and animal illnesses, these have been given significant attention in worldwide scientific investigation (Ahsan *et al*., 2019). Edema is caused by extravasation of liquids, proteins and the concentration of leucocytes at the site of inflammation as a result of stimuli. Furthermore, it is widely believed that cytokines released by immunological or CNS cells can directly activate peripheral receptors (Ahsan *et al*., 2019).

Bradykinins, TNF, interleukins (ILs) and prostaglandins (PGs) induce increased sensation to pain and distress by affecting the signal transduction activity nerves. Infection causes fever, which is induced by pyrogens such as ILs, TNF and interferon, that stimulate PGE2 production in the hypothalamus and elevates its temperature setting point. Fever, often known as pyrexia, is a complication of inflammation. Prostaglandin production is associated with inflammation, pain and fever. As a result, anti-inflammatory drugs are supposed to possess analgesic and antipyretic properties (Vaishnavi, 2020). The hunt for new medications with fewer or no side effects is in great demand because of the unwanted effects of non-steroidal anti-inflammatory drugs (NSAIDs) and opioids. In this respect, the present study focus has emerged toward medicinal plants due to their low cost and ease of availability, as well as the fact that these have less adverse effects (Ahsan *et al*., 2019).

Fagonia bruguieri DC. (*F. bruguieri*) is a perennial prickly weed or subshrub that grows on sandy plains and desert valleys (Dinarello, 2004). Amino acids, saponins, flavonoids, alkaloids, terpenoids, sterols, coumarins and trace elements, glycosides and flavonols, are all found in it (Alam, 2010). It's often used in medicine as a home cure for diabetes, asthma, stomach discomfort, tooth pain, hepatitis, abscesses, acne, common cold and kidney illness, as well as an anti-tumor, antioxidant and analgesic. This plant has shown anti-allergic, analgesic, anticoagulant and wound healing acceleration properties (Altameme 2021; Qureshi *et al*., 2010 and Hammad and Qari, 2010).

This study aimed to investigate the anti-inflammatory, anti-pyretic and analgesic potentials of *F. burguieri* in rodent model.

MATERIALS AND METHODS

Plant collection

Fresh plant was collected randomly from rigid areas along the mountains of village Ghundi, District Mianwali, Punjab, Pakistan and identified by GC University Lahore,

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Department of Botany (Herb.Bot.3663). The geographical coordinates are [32°41' 53" North, 71°39' 1" East], with the geographical location is Ghundi, Mianwali, Punjab, Pakistan, Asia.

Extraction of Plant

The plant was dried under shade for two weeks. The powdered plant (986 g) was soaked in absolute methanol and methanol extract was prepared by cold maceration as previously described (Ahsan *et al*., 2019). The percentage yield was 8.8%.

Animals used and Ethics

We employed adult male albino Wistar rats which were 8 weeks old and weighed 120-150 g. All rats were kept at a temperature of 25±2°C with a 12h light/dark cycle and provided with a regular meal and given water. The experimental techniques utilized in the study were approved by the Faculty of Pharmacy's Institutional Animal Ethics Committee (IREC#2020-02), The University of Lahore, Pakistan.

Drugs and Chemicals

Methanol of analytical grade (Sigma Aldrich) was used to prepare extract of plant. The drugs used were of high grade, diclofenac sodium (Voren injection, Continental Chemical Company (Pvt.) Ltd.), paracetamol (Provas injection, Sami Pharmaceuticals (Pvt.) Ltd.), pentazocine (Pentazogon injection, Indus Pharma (Pvt) Ltd.) were used with other agents like buffered formalin, aspirin, yeast, carrageenan, formalin, acetic acid and chloroform, which were of analytical grade and commercially available.

Determination of analgesic effect

Acetic acid induced writhing model

Analgesic activity of methanol extract of *F. burguieri* (MEOFB) was investigated in acetic acid-induced writhing model. The rats were divided into 6 groups, each group having five animals. In order to induce pain, a 1 percent acetic acid solution (20 ml/kg) intraperitoneal (i.p.) injection was used (Costa *et al*., 2019). The rats were treated as follows (table 1),

After study design, each rat was put in a clear viewing box and the number of writhes of each rat was counted for 20 minutes, beginning within 4-6 minutes of intraperitoneal injection of acetic acid. The percentage of writhing inhibition was then calculated as follows: % writhing inhibition = $\frac{C-T}{C} * 100$

Whereas C; Control group, T; Treated group

Eddy's hot plate

The hot plate was set at 55±2°C. The experimental rats were put on it and latency time was recorded. The rat with latency time greater than 50 seconds were excluded from the experiment. While a cut off time had been set as thirty seconds to avoid any damage to rats's paw. Group I (Control) was treated with 0.9% normal saline orally. Group II (Standard drug treated) received pentazocine (10 mg/kg i.p.). Group III, IV and V were administered with MEOFB (250, 500 and 750 mg/kg) respectively orally. The rats were placed on the plate one by one until the rat did either licking of paw or jumping, this time was set down by a stop-watch. The latency time was measured and recorded before at 0, 30, 60, 90, 120 and 150 min. after treatments for all groups. The prolongation or a significant difference between the latency times indicated the anti-nociceptive activity of that group (Sharma *et al*., 2019, Adnan *et al*., 2019).

Anti-inflammatory activity

Carrageenan induced paw edema model

The animals were divided into six groups, Group I (Control) was administered normal saline. Group II (Diseased); 1% carrageenan at the dose of 0.5 ml was injected into the planter tissue of the rat paw. The rats in Group III (Standard drug treated) were administered with the reference drug diclofenac sodium (15 mg/kg) prior to the administration of carrageenan. Group IV, V and VI received MEOFB at dose levels of (250, 500 and 750 mg/kg per oral) respectively. The plethysmometer was used to measure paw volume of rat, that is at "0" hour and then at successive time interval of 1, 2, 3 and 4th hour (Parra *et al*., 2019, Shoukry *et al*., 2020). Increase in thickness of paw volume was measured as the mean in paw thickness at "0h" and paw volume thickness at respective hours. The percentage inhibition of was calculated by following formula;

$$\% \text{ Of edema inhibition} = (V_i - V_t)/ V_i \times 100$$

Whereas Vi; volume of the paw at 1 hour, Vi; volume of paw at t time.

Antipyretic activity

Brewer's yeast antipyretic model

The rats were administered with brewer's yeast and left for 18 hours. The rats were free to drink water. The rectal temperature of rats was recorded by thermometer. Any rat which showed the rectal temperature more or less than 97.8°F-98.5°F was not included in study. The rats were divided into six groups, Group I (Control) was administered normal saline. Group II (Diseased) was being given 15% brewer's yeast (10 ml/kg) injected subcutaneously below the nape of the neck. The rats in Group III (Standard drug treated) were administered with the reference drug paracetamol (50 mg/kg). Group IV, V and VI were treated with MEOFB at dose levels of (250, 500 and 750 mg/kg) respectively.

Eighteen hours after yeast injection, rats showing 32.9°C± 33.8°C elevation in rectal temperature were grouped for respective oral treatments. After that, rectal temperature was taken periodically on hourly basis of for successive

Table 4: Effect of *Fagonia bruguieri* on brewer's yeast-induced pyrexia

Rat rectal temperature (°F)						
Time	Control	Diseased	Standard drug treated	MEOFB (250mg/kg)	MEOFB (500mg/kg)	MEOFB (750mg/kg)
Hrs.	Mean±SEM	Mean±SEM	Mean±SEM	Mean±SEM	Mean±SEM	Mean±SEM
0hr	98.4±0.07	98.3±0.05	98.3±0.03	98.2±0.04	98.2±0.02	98.4±0.03
18hr	98.2±0.04	102.5±0.08*	102.6±0.07	102.5±0.05	102.6±0.05	102.5±0.03
19hr	98.2±0.07	102.8±0.02*	98.6±0.06**	102.3±0.04	101.2±0.11	100.5±0.06
20hr	98.4±0.07	102.8±0.02*	98.6±0.08**	101.5±0.08	99.6±0.06**	98.6±0.06**
21hr	98.3±0.03	102.6±0.02*	98.2±0.05**	100.7±0.06	98.2±0.06**	98.2±0.04**
22hr	98.3±0.07	102.5±0.03*	98.3±0.03**	99.5±0.06**	98.6±0.05**	98.0±0.12**
23hr	98.3±0.05	102.3±0.08*	98.5±0.08**	98.6±0.08**	98.3±0.06**	98.3±0.07**

Anti-pyretic activity of *F. bruguieri* on brewer's yeast induced pyrexia model. Values are mean ±SEM, (n=5), whereas $P<0.05$ (*) compared to control and $P<0.05$(**) compared to Diseased group (MEOFB; methanol extract of *F. bruguieri)*

Table 5: Effect of *Fagonia bruguieri* on carrageenan-induced inflammation

Rat rectal temperature (°F)						
Time	Control	Diseased	Standard drug treated	MEOFB (250mg/kg)	MEOFB (500mg/kg)	MEOFB (750mg/kg)
Hrs.	Mean±SEM	Mean±SEM	Mean±SEM	Mean±SEM	Mean±SEM	Mean±SEM
0hr	0.45±0.008	0.45±0.010	0.44±0.012	0.45±0.008	0.46±0.005	0.47±0.007
1hr	0.45±0.009	0.95±0.028*	0.63±0.012*	0.95±0.005	0.75±0.014	0.64±0.015*
2hr	0.45±0.007	1.05 ±0.012*	0.58±0.015*	0.86±0.004	0.63±0.010**	0.56±0.006**
3hr	0.45±0.005	1.04±0.008*	0.51±0.010*	0.75±0.009	0.55±0.008**	0.51±0.005**
4hr	0.45±0.006	1.04±0.011*	0.45±0.011*	0.66±0.010*	0.46±0.008**	0.48±0.009**

Effect of *Fagonia bruguieri* on carrageenan-induced inflammation. Values are mean ±SEM, (n=5), whereas $P<0.05$ (*) compared to control and $P<0.05$(**) compared to Diseased group (MEOFB; methanol extract of *F. bruguieri*)

five hours. Percentage inhibition of anal temperature was calculated by formula (Shoukry *et al*., 2020)

$$\%\ \text{of rectal temperature inhibition} = \frac{(R_i - R_t)}{R_i} \times 100$$

Rí; Rectal temperature at the 18th hour after the Brewer's yeast administration, R_t; Temperature after the dose administration

Preliminary phytochemical screening

The MEOFB was subjected to qualitative phytochemical screening for alkaloids, cardiac glycoside, flavonoids, tannins, saponins, phlobatannins, terpenoids and anthraquinone (Shaikh and Patil, 2020).

STATISTICAL ANALYSIS

The data were presented in the form of mean±standard error of mean. To assess statistically significant differences between groups, a two-way analysis of variance (ANOVA) was utilized, followed by a Tukey post hoc test. The GraphPad Prism (version 8.0.2) had been used for data analysis. $P \leq 0.05$ was regarded to be the level of significance.

RESULTS

Analgesic activity

It was observed that the number of writhing movements was increased (64.6±1.2) in Diseased group when compared with Control and decreased (24.2±0.8) effectively by MEOFB (750 mg/kg) when compared with Diseased group in acetic acid induced writhing model (Table 2). Moreover, the latency time response was recorded for Control, Diseased, Standard drug and MEOFB (250, 500 and 750 mg/kg) treated groups at the different time intervals (0, 30, 60, 90, 120, 150 min) against hot plate method. When compared to the Control group, MEOFB (250, 500 and 750 mg/kg) treatment groups presented a statically significant delayed latency response (15.1±0.3), (16.3±0.3) and (22.0±0.3) respectively (Table 3).

Anti-pyretic activity

Table 4 showed a significant rise in rectal temperature after yeast treatment and decrease (98.3±0.07) in MEOFB (500 and 750 mg/kg) treated groups in comparison with Diseased rats.

Anti-inflammatory

The inflammation of rat paw edema was measured for Control, Diseased, Standard drug and MEOFB (250, 500 and 750 mg/kg) treated groups. A rise in volume of paw was observed (1.05±0.01) in Diseased group when compared with the Control while a fall (0.48±0.009) in paw edema was found in rats treated with MEOFB (750 mg/kg) (table 5).

Preliminary phytochemical screening

Phytochemical screening of MEOFB revealed the presence of various phytochemicals which include

alkaloids, flavonoids, terpenoids, saponin and cardiac glycosides.

DISCUSSION

It is clear that traditional medicines are associated with a number of side effects that vary from person to person in different doses; Limited use in medical settings because of unwanted effects of analgesic, antipyretic and anti-inflammatory drugs such as NSAIDS and opioids including heart failure, peptic ulcer, prolonged bleeding, liver failure and other side effects. Due to the limitations of these traditional medicines and other related issues, it is now increasingly important to find new medicines from medicinal plants that have analgesic, antiseptic and anti-inflammatory activity in traditional medicine systems (Ahsan *et al*., 2019).

In this study, we presented the findings for the estimation of analgesic, antipyretic and anti-inflammatory potential of MEOFB in Wistar albino rats. To test the analgesic activity of MEOFB acetic acid pain test and hot plate test were used. Acetic acid-induced pain test is being employed to test peripheral action of the analgesic drugs.

In this study, MEOFB presented analgesic activity by decreasing the number of acetic acid-induced abdominal pains in rats after treatment with MEOFB, 30 minutes after the test period, MEOFB showed the most analgesic activity by reducing (62.23%) the writhing count with 750 mg/kg when compared to Standard drug treated (61.92%). Pretreatment with different doses of MEOFB significantly reduced discomfort. This explains its ability to alter the amplitude of the inflammatory response, which may explain its both anti-inflammatory and analgesic effects (Sharma *et al*., 2019).

On the other hand, rats given MEOFB in the hot plate group showed significantly different values than Control group. Statistics presented that the significant differences in the Control group and treated groups were observed at 60 minutes (39.56%), 90 minutes (43.80%) 120 minutes (28.29%) and 150 minutes (27.95%). Therefore, these results appeared to indicate that MEOFB has significant analgesic activity. MEOFB significantly inhibited abdominal contractions in a dose-dependent fashion as compared significantly to Control and Standard drug treated at given doses 250mg/kg, 500mg/kg and 750 mg/kg of MEOFB. This suggests that MEOFB exerts peripheral as well as central analgesic activity (Sharma *et al*., 2019).

The pyrexia by yeast was produced by increased synthesis of PGs. This is considered a practical test for antipyretic effects in testing plant materials as well as synthetic drugs. When MEOFB was administered, it significantly decreased the rectal temperature of the rat.

Thus, proving that MEOFB is responsible for the antipyretic activity, which may result from the presence of pharmacologically active principle(s) that interferes with the release of prostaglandins (Cordaro *et al*., 2020). In this study, MEOFB produced dose-dependent effects over time. A dose of 75 0mg/kg showed the significant antipyretic activity in the last hours. MEOFB has antipyretic properties attributable to the presence of flavonoids and terpenoids as both have the ability to inhibit prostaglandin synthesis (Ferede *et al*., 2021).

Injection of carrageenan into the hind paw of the rat induces a biphasic edema. In the carrageenan inflammatory model, the main inflammatory mediators found in early phase (1 hour) are serotonin and histamine and in the second or late phase, major mediators include prostaglandins and cyclooxyginase-2. MEOFB exhibited a marked anti-inflammatory activity on the carrageenan-induced hind paw edema in albino rats in both phases. These results were similar with previous studies (Cordaro *et al*., 2020; Ahsan *et al*., 2021).

Phytochemical screening of methanol extract of *Fagonia bruguieri* revealed the presence of various phytochemicals which may be responsible for its anti-inflammatory, analgesic and antipyretic activities. These phytochemicals include flavonoids, triterpenoids, saponins and cardiac glycosides. Among these phytochemicals, flavonoids may reduce prostaglandin synthetase activity. Therefore, the reported content indicates that flavonoids have potential anti-inflammatory activity. Glycosides, more specifically cardiac glycosides, which have the activity of suppressing hypersecretion of IL-8, a protein involved in lung inflammation and thus responsible for de-activation of NF-β signaling pathway (Mbiri, 2017, Furst *et al*., 2017).

CONCLUSION

The data obtained in this study presented the important anti-nociceptive, antipyretic and anti-inflammatory properties of *Fagonia bruguieri* which might be due to the presence of phytochemical components with pharmacological potentials.

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