ISSN 0976 – 3333

Available Online at www.ijpba.info

International Journal of Pharmaceutical & Biological Archives 2015; 6 (6): 32- 36

RESEARCH ARTICLE

Anti-arthritic Activity of Methanolic Extract and Various Fractions of *Trigonella foenum-graecum* Seed: An *In-vitro* Study

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Received 21 Aug 2015; Revised 28 Nov 2015; Accepted 08 Dec 2015

ABSTRACT

Inflammation is a complex biological response to harmful stimuli, pathogens, irritants characterized by redness, warmth, swelling and pain. It is the response of body to inactivate or destroy the invading organisms, to remove the irritants & set the stage for tissue repair. *Trigonella foenum-graecum* (TFG) is one of the oldest Ayurvedic medicinal plants, used in traditional Chinese medicine and as dietary spice. The aim of our study was to evaluate the *in-vitro* anti-arthritic activity of methanolic extract and their fractions. The anti-arthritic activity was evaluated by protein denaturation method and proteinase inhibitory activity. In protein denaturation method, the percentage inhibition for methanolic extract was found to be 39.70 %. The petroleum ether and n-butanolic fraction of methanolic extract were shown 44.11 & 50.0 % inhibition respectively. Similarly, in proteinase inhibitory activity, methanolic extract showed 41.50 % inhibition and petroleum ether and n-butanolic fraction of methanolic extract were shown 47.16 & 50.94 % inhibition respectively at 500µg/ml. The Diclofenac sodium was used as a standard drug in both models and shown greater activity as compared to petroleum ether and n-butanolic fraction. In conclusion, we found that TGF showed best activity in proteinase inhibitory model as compared to protein denaturation method.

Key words: TFG, Protein denaturation methods, Proteinase inhibitory activity, n-butanolic fraction.

INTRODUCTION

Inflammation is a complex biological response to harmful stimuli, pathogens, irritants characterized by redness, warmth, swelling and pain [1].It is the response of body to inactivate or destroy the invading organisms, to remove the irritants & set the stage for tissue repair [2].

Anti-inflammatory drugs like NSAIDs used to diminish the swelling and pain of inflammation, also these agents bear the risk of gastro-intestinal toxicity, cardiovascular and other toxicity for prolonged use [3].Protein denaturation is a process in which proteins drop their tertiary structure and secondary structure by appliance of external stress or compound, such as strong acid or base, a concentrated inorganic salt, an organic solvent or heat [4]. Previous researchers reported that denaturation of protein is one of the grounds of rheumatoid arthritis & chronic inflammation [5, 6]. The production of auto antigens in certain arthritic diseases may be owing to in-vivo denaturation of proteins [7]. The mechanism of denaturation most probable involves modification in electrostatic, hydrogen, hydrophobic and disulphide bonding [8]. Inflammation is triggered by the discharge of chemical mediators from injured tissue and migrating cells [9]. Proteinase is implicated in a broad variety of biological processes, including inflammation and tissue injury. Various studies have paying attention on the role of proteinase in tissue injury, and it was thought that the sense of balance between proteinase and proteinase inhibitors is a major determinant in maintaining tissue integrity [10, 11].

Trigonella foenum-graecum (TFG) Linn. is an annual herb belonging to the family *Leguminosae*, widely grown in India, Egypt, and Middle Eastern countries. The chemical constituents of *Trigonella* seed include volatile oils, alkaloids, Saponin, sapogenins, flavonoids and mucilage [12, 13]. It is

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one of the oldest Ayurvedic medicinal plants, used in traditional Chinese medicine and as dietary spice. It has been used for numerous indications, like lactation stimulation, aiding digestion, various types of inflammatory disorders and to generally improve metabolism and health [14]. *Trigonella foenum-graecum* seeds have reported therapeutic activities like Antidiabetic activity, Immunological activity, Anti-inflammatory and analgesic activity, Antioxidant activity [15]. In the absence of any scientific reports in previous literature survey, the present study was undertaken to evaluate *in-vitro* anti-arthritic activity of methanolic extract and its different fractions of *Trigonella foenum-graecum.*

MATERIALS AND METHODS

Procurement of material and extraction

The seeds of *Trigonella foenum-graecum* (Linn.) were purchased from the local market of Mandsaur, India. The seeds were ground into a uniform powder using a blender and stored in polythene bags at room temperature. The powder was loaded into soxhlet extractor and subjected to extraction with methanol. After extraction, the solvent was distilled off and the extract was concentrated on water bath to a dry residue. The dried extract was used for further fractionization. Preliminary phytochemical studies showed the presence of flavonoids, terpenoids, glycosides, alkaloids, and phenolic compounds.

Fractionization of extract

The dried methanolic extract (50 g) of *Trigonella foenum-graecum* (Linn.) was suspended in distilled water and filtered to remove the insoluble material. The water fraction was taken in separating funnel and fractionated by various organic solvents to get petroleum ether, chloroform, butanol and water soluble layer. Each fraction was dried under vacuum to obtain petroleum ether (2 g), chloroform (5.8 g), n-butanolic (6.4 g) and water fractions (2.8 g) [16].

Assessment of *in-vitro* anti-arthritic activity

Inhibition of albumin denaturation

The reaction mixture (5 ml) consisted of 0.2 ml of egg albumin (from fresh hen's egg), 2.8 ml of phosphate buffered saline (PBS, pH 6.4) and 2 ml of varying concentrations of TFG so that final concentrations become 100, 200, 300, 400, 500 µg/ml. Similar volume of double distilled water served as control. Then the mixtures were incubated at 37 ± 2ºC in a BOD incubator (for 15 min. and then heated at 70ºC for 5 min. After cooling, their absorbance was measured at 660 nm (SHIMADZU, UV) by using vehicle as blank. Diclofenac sodium at the final concentration of (100 µg/ml) was used as reference drug and treated similarly for determination of absorbance [2]. The percentage inhibition of protein denaturation was calculated by using the following formula:

% Inhibition = 100 × [Vt /Vc– 1]

Where; Vt = absorbance of test sample, Vc = absorbance of control.

Proteinase inhibitory activity

The reaction mixture (2ml) was containing 0.06 mg Trypsin, 2 ml 20mM Tris HCl buffer (pH 7.4) and 1 ml test sample of different concentrations (100-500 µg/ml).All the mixtures were incubated at 37° C for five minute and then 10 ml of 0.8 % w/v casein was added. The mixture was incubated for an additional 20 min. 2 ml of 70% perchloric acid was added to arrest the reaction. Cloudy suspension was centrifuged and the absorbance of the supernatant was read at 210 nm against buffer as blank. The experiment was performed as triplicate. The percentage inhibition of proteinase inhibitory activity was calculated [17].

Percentage inhibition = (Abs Control – Abs Sample) x 100 / Abs control.

RESULTS AND DISCUSSION

Protein Denaturation Methods

The methanolic extract of seeds showed 39.70 % inhibition. The petroleum ether and n-butanolic extract had shown the 44.11 and 50.00 % inhibition respectively. The Diclofenac sodium showed 55.88 % inhibition against denaturation of protein. The results are summarized in (**Table 1**).

Table 1: Effect of methanolic extract and various fractions of TGF on Protein Denaturation

S. No	Treatment	Concentration µg/ml	% Inhibition
1	Control	----	-----
2	Methanolic Extract of TFG	100	17.64
		200	20.58
		300	26.47
		400	32.35
		500	**39.70**
3		100	26.47
		200	30.88

	Petroleum ether Fraction of TFG	300	35.29
		400	41.17
		500	**44.11**
4	Chloroform Fraction of TFG	100	11.76
		200	14.70
		300	19.11
		400	25.00
		500	29.41
5	n-butanolic Fraction of TFG	100	30.88
		200	35.29
		300	39.70
		400	45.58
		500	**50.00**
6	Aqueous Fraction of TFG	100	14.70
		200	19.11
		300	23.52
		400	29.41
		500	35.29
7	Diclofenac Sodium	100	**55.88**

Proteinase inhibitory action:

The methanolic extract of seeds showed 41.50 % inhibition. The petroleum ether and n-butanolic extract had shown the 47.16 and 50.94 % inhibition respectively. The Diclofenac sodium showed 62.26 % inhibition against proteinase inhibitory activity. The results are summarized in (**Table 2**).

Table 2: Effect of methanolic extract and various fractions of TGF on proteinase inhibitory activity

S. No	Treatment	Concentration µg/ml	% Inhibition
1	Control	----	-----
2	Methanolic Extract of TFG	100	16.98
		200	22.64
		300	28.30
		400	35.84
		500	**41.50**
3	Petroleum ether Fraction of TFG	100	24.52
		200	30.18
		300	37.73
		400	43.39
		500	**47.16**
4	Chloroform Fraction of TFG	100	5.66
		200	9.43
		300	16.98
		400	22.64
		500	26.41
5	n-butanolic Fraction of TFG	100	28.30
		200	33.96
		300	41.50
		400	47.16
		500	**50.94**
6	Aqueous Fraction of TFG	100	7.54
		200	13.20
		300	18.86
		400	24.52
		500	30.18
7	Diclofenac Sodium	100	**62.26**

DISCUSSION

Herbal drugs involve a major component in all the traditional system of medicine. Herbal medicine is a conquest of accepted therapeutic diversity [18]. The factors responsible for the sustained and widespread use of herbal remedies in India are their effectiveness, easy availability, low cost, comparatively less toxic effects and deficiency of practitioners of modern medicine in rural areas [19]. The remedial properties of medicinal plants are possibly due to the occurrence of a range of secondary metabolites such as alkaloids, flavonoids, phenols, saponins, sterols etc [20]. Earlier studies have proved that the chemical constituents such as flavonoids, bioflavonoids, alkaloids, tannins and terpenoids are promising agents in treatment of inflammation [21, 22].

The aim of our study was to evaluate the anti-arthritic activity of extract and various fraction of TGF by in-vitro models. The preliminary phytochemical studies of petroleum ether and n-butanolic fractions showed presence of terpenoids, steroids, phenolic compounds, glycosides and flavonoids respectively.

The arthritic disease progression was correlated with the fragility of the lysosomal membranes,

denaturation of proteins & release of inflammatory mediators. Denaturation of proteins is a well known cause of inflammation. The majority of biological proteins lose their biological functions when denatured. In vivo denaturation of proteins causes production of auto-antigens in arthritic disorders [7]. Agents that can prevent protein denaturation therefore would be worthwhile for anti-arthritic activity. As a component of the investigation, the ability of methanolic extract & its fractions to inhibit protein denaturation was studied. The methanolic extract as well as Petroleum ether & n-butanolic fractions were effective in inhibiting heat induced albumin denaturation at different concentrations. The inhibition of denaturation may be probable mechanism of extract and fractions.

Serine proteinases from inflammatory cells, including neutrophils, are implicated in various inflammatory disorders such as Rheumatoid arthritis and pulmonary emphysema. Neutrophils are known to be a rich source of serine proteinase and are localized at Lysosomes. Deficiency of protease inhibitors in circulation is the major risk factor for development of inflammatory disorder. Previous researchers reported that, leukocytes proteinase play an important role in the development of tissue damage during inflammatory reactions and significant level of protection was provided by proteinase inhibitors [23, 24].

In our study, we found that methanolic extract, petroleum ether and n-butanolic extract showed significant inhibitory activity against proteinase enzymes. Proteinase plays an important role in the development of tissue damage during inflammatory reactions. So the possible mechanism of our study was to inhibit the activation of neutrophils and proteinase enzymes that contributes to various cascade mechanism of inflammation.

CONCLUSION

In the present study, results indicate that the methanolic extract, petroleum ether and n-butanolic fractions possess anti-arthritic properties. The n-butanolic fraction had shown maximum activity in both models. These activities may be due to the presence of polyphenolics and flavonoids. Purification of further bioactive compound is much needed and the purified form of the compound can be used which may show increased activity. This study gives an idea that the phytoconstituents of the *Trigonella foenum-graecum* can be used as lead compound for designing a potent, anti-inflammatory drug which can be used for treatment of various diseases such as rheumatoid arthritis, cancer, neurological disorder, aging and inflammation.

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