



In Vitro* Evaluation of Anti-inflammatory Activity in Seed and Callus Extract of *Trigonella foenum-graecum

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Abstract

Trigonella foenum-graecum is well known medicinal spice worldwide because of its alluring therapeutic properties. Investigations were executed to study *in vitro* anti-inflammatory activity in seed and callus extract of *Trigonella foenum-graecum*. Callus was induced from seed explants in MS medium supplemented with 2, 4-D. (2mg/l). Enough callus was obtained after 4 weeks of inoculation of explants. Crude extracts of seed and callus prepared in chloroform were evaluated for their anti-inflammatory activity by inhibition of Albumin denaturation and HRBC membrane stabilization method. Callus extract showed more inhibition of Albumin denaturation and heat induced hemolysis of HRBC membrane as compared to seed extract. The anti-inflammatory activity can be attributed to various phytochemicals such as flavonoids, polyphenols, triterpenoids, steroids and alkaloids. The finding suggests the potential therapeutic use of callus of *Trigonella foenum-graecum* for its anti-inflammatory activity.

Keywords

Anti-inflammatory activity, 2, 4-D, HRBC membrane, *in vitro*.

INTRODUCTION

India is hub to medicinal plants. Since ages people are using herbs and spices in their crude form for various curative purposes (1). Curative properties of plants can be attributed to their ability to synthesize a wide variety of chemical compounds (2). Phytochemical analysis of *Trigonella foenum-graecum* extract revealed the presence of various biochemical compounds such as alkaloids, flavonoids, glycosides, triterpenoids and saponins which exhibit a wide range of pharmacological activity. Inflammation is a body response to injury, infection or destruction characterized by heat, redness, pain, swelling and disturbed physiological

functions. It is triggered by the release of chemical mediators from injured tissue and migrating cells (3). Inflammation is a complex process, which involves various events such as: the increase of vascular permeability, increase of protein denaturation and membrane alteration. Denaturation of protein is a well- documented cause of inflammation (4, 5). Protein denaturation is a process in which proteins lose their tertiary structure and secondary structure by application of external stress or compounds, such as strong acid or base, a concentrated inorganic salt, an organic solvent or heat. Most biological proteins lose their biological function when denatured.

Membrane alteration of neutrophils leads to release of lysosomal enzymes which result in acute or chronic inflammation associated with certain pathological conditions as heart attacks, septic shocks and rheumatoid arthritis. Stabilization of lysosomal membrane is important in limiting the inflammatory response by inhibiting the release of lysosomal constituents of activated neutrophil which cause further tissue inflammation (6). Non-steroidal anti-inflammatory drugs (NSAIDs) constitute one of the most widely used classes of drugs (7, 8). In recent times, focus on plant research has widely increased due to multiple side effects of allelopathic drugs.

Smithia sensitiva belonging to the family Fabaceae is used as an anti-inflammatory and antioxidant drug by tribal peoples in Kerala. The effect is due to phytoconstituents present in the plant (9).

Flavonoids such as hesperidin, apigenin, luteolin and quercetin are found to be a potent anti-inflammatory constituent (10). High content of flavonoids and bioflavonoids in methanolic extract of *Cassia auriculata* flowers is reported for its anti-inflammatory activity (11). Anti-inflammatory activity of *Basella alba* has also been attributed to the presence of alkaloid and flavonoids (12).

Since triterpenoids and flavonoids have remarkable anti-inflammatory activity. *Centella asiatica*, widely used as anti-inflammatory plant revealed the presence of various biochemical compounds such as alkaloids, flavonoids, glycosides, triterpenoids, saponins and amino acids (13).

Trigonella foenum-graecum is medicinally used by the Indian tribes for a wide range of ailments, including constipation, kidney problems, gonorrhoea, spermatorrhoea, urinary troubles, diarrhea and hyperglycemia. In the present investigation, the *in vitro* anti-inflammatory activity of fenugreek seeds (*Trigonella foenum-graecum*) and callus has been reported.

Callus is defined as a coherent and amorphous tissue, formed by the disorganised multiplication of plant cells and consisting of meristematic and unspecialised parenchyma cells. Callus does not conform to any predictable organisational pattern, but localised centres of meristematic activity are present, sometimes accompanied by a simple cambial region with zones of vascular differentiation. Callus formation occurs in plants because of wounding, stress, and insect or microorganism attack, and is controlled by the endogenous hormones auxin and cytokinin. This response is associated with increased metabolic activity, including polyphenols to strengthen cell walls and

the synthesis of compounds to protect against pathogen attack (14).

MATERIAL AND METHODS

Plant material used

The seeds of *Trigonella foenum-graecum* were purchased from local market, Matunga. Seeds were washed thoroughly with running tap water and surface sterilized to remove the surface borne microorganisms.

Callus induction and establishment

Seeds were washed thoroughly in distilled water containing 2 to 3 drops of liquid detergent Teepol for 10-15 minutes. Seeds were transferred to lamina air flow and treated with 70% alcohol for 30 seconds followed by 2% sodium hypochlorite for 10 minutes and washed with autoclaved distilled water to remove all the traces of sterilant.

Sterilized seeds were inoculated on autoclaved Murashige and Skoog medium (15) solidified with 0.8% agar and adjusted to pH 5.8. Culture tubes were incubated in 16 h photoperiod and 8 hours dark period. Light intensity of 2500 lux was provided by white fluorescent lamps. Seven-day old seedlings served as source of cotyledonary explants which were inoculated onto MS medium supplemented with various concentrations of 2, 4-D (0.5-2 mg/l) and NAA (0.5 -2.0mg/l). After inoculation of cotyledons culture tubes were incubated in complete darkness at 27°C for 10- 12 days. Once the callus was induced, the culture tubes were transferred to 16 h photoperiod to allow the cultures to grow up to their maximum growth age i.e. 4 weeks which provided sufficient amount of callus to evaluate anti-inflammatory activity.

Data was subjected to ANOVA and results were expressed as mean \pm SD (16).

Preparation of crude seed and callus extract

Seeds were cleaned and grinded using motor and pestle. Extract was obtained with chloroform using Soxhlet apparatus. Callus was subjected to oven drying at 50°C then powdered and extracted with chloroform using Soxhlet apparatus.

Phytochemical screening of crude extract of seed and callus

Phytochemical screening of the both extracts was performed following the methodology of Sofowara, Harborne and Kokate (17, 18, 19) to determine the presence of various phytochemical constituents.

In vitro anti-inflammatory activity by inhibition of albumin denaturation

Protein denaturation is a process in which proteins lose their tertiary structure and secondary structure by application of external stress or compounds, such

as strong acid or base, a concentrated inorganic salt, an organic solvent or heat. Most biological proteins lose their biological function when denatured. Efficacy of Plant extract was evaluated in inhibiting heat induced denaturation (20).

Chemicals used: BSA (Bovine Serum Albumin)

2 ml of reaction mixture with 1ml of BSA was incubated at 37°C for 20 min and heated at 51°C for 20 min, after cooling the sample OD was taken at 660 nm.

% Inhibition = $\frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$

In-vitro anti-inflammatory activity by HRBC (Human Red Blood Cell) membrane test

HRBC or erythrocyte membrane is analogous to the lysosomal membrane and its stabilization implies that the extract may as well stabilize lysosomal membranes. When the RBCs are subjected to hypotonic stress, the release of hemoglobin from RBCs is prevented by anti-inflammatory agents because of membrane stabilization so the stabilization of HRBC membrane by drugs against hypo tonicity induced hemolysis serve as a useful *in vitro* method for assessing the anti-inflammatory activity of various compounds (21).

Chemical used: Alsever's solution, isosaline, hypo saline, phosphate buffer.

Equal volume of Alsever's solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% sodium chloride) and blood was centrifuged at 3000 rpm for 10 min. Packed cells were washed 3-4 times with equal volume of isosaline. 10% HRBC suspension with normal saline was prepared. Various aliquotes of plant extract (200 to 800 ug/ml) were prepared using distilled water. To each aliquot 1 ml of phosphate buffer, 2 ml of hypo saline and 0.5 ml of HRBC suspension was added. Reaction mixture was incubated at 37°C for 30 min. followed by centrifugation at 3000 rpm. Haemoglobin content was estimated spectrophotometrically at 560 nm. Diclofenac was used as standard and control was prepared by omitting extracts.

Percentage of HRBC membrane stabilization (% Protection) =

$\frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$.

RESULTS AND DISCUSSION

Callus induction

Callus induction and establishment was best reported on MS media supplemented with 2, 4-D (2mg/l) followed by NAA (1mg/l) using cotyledon explants. Callus was allowed to grow up to its maximum growth age i.e. 4 weeks.

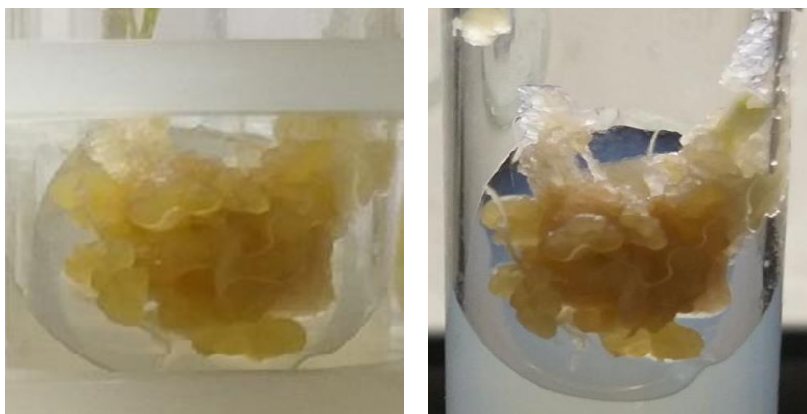


Fig 1. 4-6-week-old callus obtained from cotyledonary explants of *Trigonella foenum graecum*

Phytochemical screening of crude extract of seed and callus

Phytochemical constituents present in seed and callus extract are tabulated in Table 1.

Table 1. Preliminary phytochemical screening of seed and callus extract of *Trigonella foenum graecum*

S.No.	Phytoconstituent	Reagent/method used	Seed extract	Callus extract
1.	Alkaloids	Dragendroff's test Wagner's test	+	+
2.	Tannins	Ferric chloride test	+	+
3.	Glycosides	Keller-killiani test (for cardiac glycosides) Borntrager's Test (for anthraquinone glycosides)	+	+
4.	Flavonoids	Shinoda test	+	+

5.	Saponins	Haemolysis test	+	+
6.	Steroids	Salkowski test	+	+
7.	Resins	Acetic anhydride test	-	-

Inhibition of Albumin Denaturation

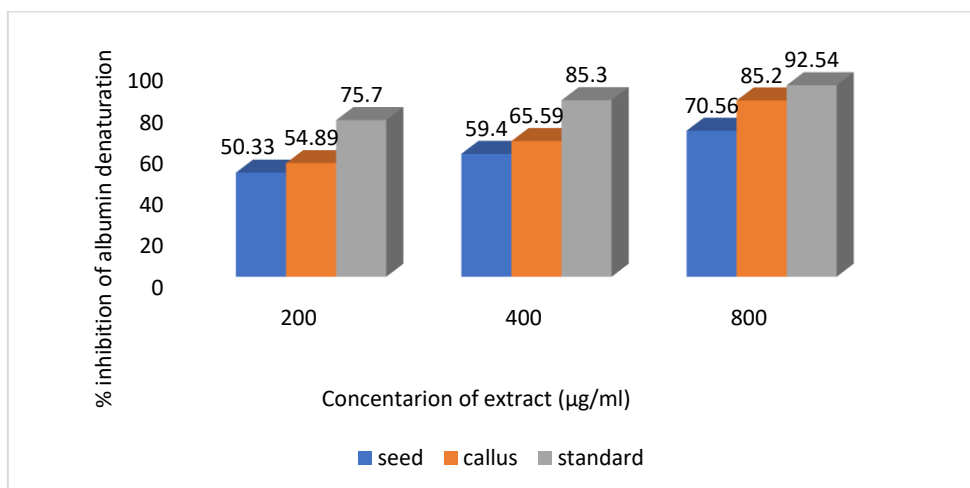
The effects of chloroform extract of seed and cotyledonary derived callus of fenugreek were effective in inhibiting heat induced albumin denaturation. Callus extract showed more

percentage inhibition (85.20%) as compared to seed extract (70.56%) at same concentration (Table 2). Diclofenac anti-inflammation drug showed the maximum inhibition.

Table 2. *In vitro* anti-inflammatory activity of chloroform extract of *Trigonella foenum-graecum* by Inhibition of albumin denaturation.

Extract	Concentrations ($\mu\text{g/ml}$)		
	200	400	800
	% inhibition of albumin denaturation		
Seed	50.33	59.40	70.56
Callus	54.89	65.59	85.20
Standard (Diclofenac sodium)	75.70	85.31	92.54

Graph 1. *In vitro* anti-inflammatory activity of chloroform extract of *Trigonella foenum-graecum* by Inhibition of albumin denaturation.



HRBCs Membrane Stabilization Test

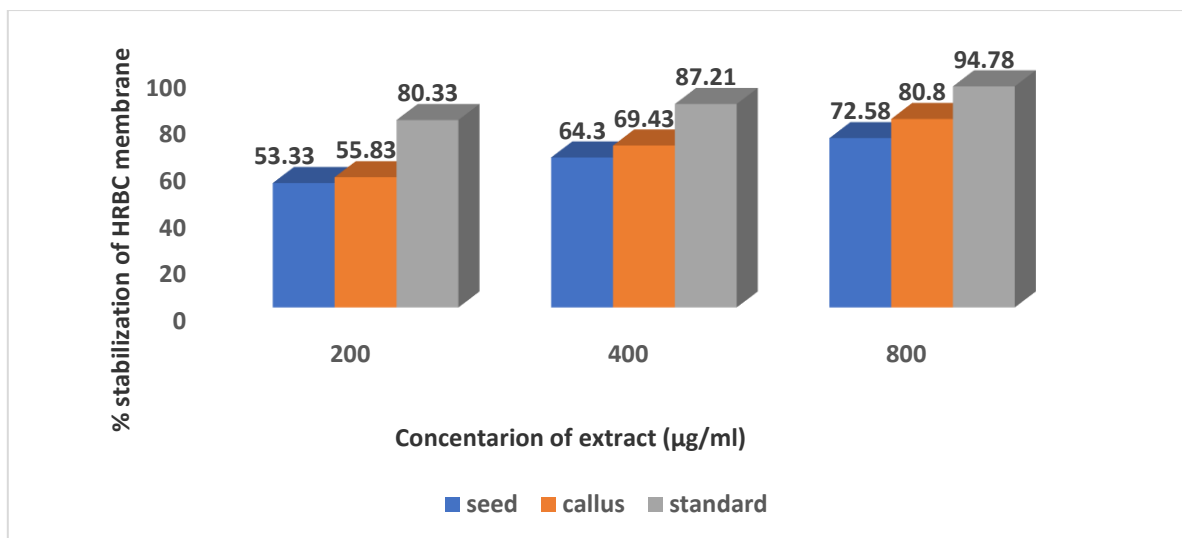
The extracts were effectively inhibiting the heat induced hemolysis of HRBCs membrane as shown in Table 3. This effect may possibly inhibit the release of lysosomal content of neutrophils at the site of

inflammation. The callus extract significantly inhibited the heat induced hemolysis of HRBCs membrane comparable with that of diclofenac sodium.

Table 3. *In vitro* anti-inflammatory activity of chloroform extract of *Trigonella foenum-graecum* by HRBCs membrane stabilization test.

Extract	Concentrations ($\mu\text{g/ml}$)		
	200	400	800
	% stabilization of HRBC membrane		
Seed	53.33	64.30	72.58
Callus	55.83	69.43	80.8
Standard (Diclofenac sodium)	80.33	87.21	94.78

Graph 2. *In-vitro* anti-inflammatory activity of seed and callus extract of *Trigonella foenum-graecum* by HRBCs membrane stabilization test.



CONCLUSION

This is the first comparative *in vitro* study on anti-inflammatory of callus and seed extract of *Trigonella foenum-graecum*. Results of present study revealed that chloroform extracts of *Trigonella foenum-graecum* (seed and callus) screened against HRBC membrane and protein denaturation showed significant anti-inflammatory activity at the increasing concentration. It may be due to the presence of chemical profile such as flavonoids, tri-terpenoids and phenols responsible for anti-inflammatory activity.

Seed and callus extracts exhibited HRBC membrane stabilization effect by inhibiting hypotonicity induced lysis of erythrocyte membrane. The erythrocyte membrane is analogous to the lysosomal membrane and its stabilization implies that the extract may as well stabilize lysosomal membrane. Stabilization of lysosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil such as bacterial enzymes and proteases which cause further tissue inflammation and damage. It is also concluded that callus extract is more effective in inhibition of protein denaturation and stabilization of HRBC membrane which could be attributed to the presence of secondary metabolites in more active and intense form as callus is established under well-defined chemical constituents and controlled environment. Synthesis of active constituents responsible for anti-inflammatory activity could be enhanced using elicitors in suspension culture. Proper isolation and purification of active constituents from *Trigonella foenum-graecum* might

help in finding the novel drug with potent activity and lesser side effects in the field of anti-inflammatory drug research.

CONFLICT OF INTEREST

Author declares no conflict of interest.

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