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Research Article

Anti-inflammatory activity and the effect of *Trigonella foenum grecum* on some biochemical parameters in experimental rats

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Abstract: This study was carried out to investigate the anti-inflammatory activity of the methanolic extract of *Trigonella foenum grecum* seeds in carrageenan saline induced paw edema in rats, The methanolic extract of *Trigonella foenum grecum* showed significant anti-inflammatory efficacies against carrageenan induced paw edema. Rats that were ingested with 1000mg of extract/kg, showed a significant decrease in edema size in the second, fourth and twenty fourth hours and inhibition percentage at 13.49, 41.17, 41.17, 38.46 and 33.33 at the first, second, fourth, sixth and twenty fourth hours respectively. Hematological values of PCV, RBC and Hb showed no significant difference**.** *Trigonella foenum grecum* extract possesses an anti inflammatory activity. The extracts of this plant are safe at high doses.
Keywords: *Trigonella foenum grecum*; Anti-inflammatory; Carrageenan saline; Rats

INTRODUCTION

Sustainable management of traditional medicinal plant resources is important, not only because of their value as a potential source of new drugs, but due to reliance on traditional medicinal plants for health. The vast majority (70-80%) of people in Africa consult traditional medical practitioners (TMPs) for healthcare. The populations of developing countries worldwide continue to rely heavily on the use of traditional medicines as their primary source of healthcare. Ethnobotanical studies carried out throughout Africa confirm that native plants are the main constituent of traditional African medicines [1].

Trigonella is a member of family Fabaceae (Papilionaceae) is an ancient plant indigenous to Sudan. Recently the crop has attracted much interest as a cheap source of good protein for protein supplement. In Sudan *T. foenum grecum* is grown in the northern region and known as Helba, and traditionally used by lactating women as porridge (with sorghum as millet flour). It's also boiled with water and taken hot or cold drink to sooth stomach ailment[2]**.**

This study was carried out to investigate the anti-inflammatory activity of the methanolic extract of *Trigonella foenum grecum* seeds in carrageenan induced paw edema in rats.

MATERIALS AND EXPERIMENTAL DESIGNS

Twenty four Wistar white Albino rats from both sexes weighing between (60-155g) were obtained from the Medicinal and Aromatic Plants Research Institute (MAPRI), the National Research Center (NRC), Khartoum, Sudan. They were housed in cages and maintained in a room under standard environmental condition and controlled temperature (22 ± 2ºC), relative humidity 60% with free access to water and formula rats feed (2.5 mcal and 20% crude protein).

Animal were apparently healthy and they were identified by color tail marks. Three days were allowed as preliminary adaptive period. At the end of adaptive period the animals were weight- distributed and allotted depend on same or similar weight, to four groups, each of six rats. Rats in group 1were orally dosed with 1 ml/kg body Wt of normal saline and served as untreated control, the methanolic extract of the plant was concentrated by evaporating the solvent and the dried material was re-dissolved in distilled water and given orally in different doses, at 500 mg/kg body wt/rats to group 2, and at 1000 mg/kg body wt/rats to group 3, while group 4 received indomethacine 10 mg/kg body/wt orally. After half an hour all groups were injected subcutaneously with 0.1ml of 1% (W/V) carrageenan suspension (Sigma Chemicals Co; St Louis, Mo, USA), in the sub-planter region of the right hind paw as a local acute edema inducer.

Paw diameter was measured at 1, 2, 4, 6 and 24 hours post carrageenan injection using Hauptner Tuberculin Caliper (Hauptner, GmbH, Germany) to the nearest millimeter.

Blood samples were obtained from the ocular vein for hematological investigation and serum analysis. Hemoglobin concentration (Hb), packed cell

volume (PCV), red blood cells (RBC), white blood cells (WBC) counts and differential count were estimated.

Sera were analyzed for the activities of AST, ALT and also for concentration of total protein and albumin using the appropriate colorimetric methods.

Trigonella foenum grecum seeds were obtained from a local Market, Khartoum north, cleaned, shade dried and made into powder. The powder was extracted with methanol at 40-60º C using soxhelt apparatus, for about six hours. The solvent were evaporated under reduced pressure using Rota vapour apparatus then the extract was allowed to air in Petri dish to be completely dried before being ready for use.

The plant was identified, classified and authenticated by botanists in Medicinal and Aromatic Plants, Research Institute (MAPRI) The National Research Center (NRC), Khartoum, Sudan.

All procedures were carried on blood samples from the ocular veins of rats collected into clean dry bottles containing heparin as an anticoagulant.

Mean values of data were analyzed by the one way ANOVA. The efficacies were obtained by calculating the difference between the edema size in the treated and the control and the values were transformed into percentage using mean index using the formula:

(a-b)/a*100= efficacy

Where: (a) is the mean of the edema size in the control and (b) is the mean of edema size in the treated rats[3].

RESULTS

The anti-inflammatory effect of the methanolic extract of *Trigonella foenum grecum* seeds on rats is shown in Table (1), and the effect on edema size is shown in Figure (1) and on the inhibition percentage is shown in Figure (2). Rats of group 2 (500mg/kg) showed decrease on the edema size when compared to the control (carrageenan group) although it was not at the level of significance, and this group showed inhibition percentage of 7.9, 26.05, 26.05, 33.33 and 45.83 at the first, second, fourth, six and twenty fourth hours respectively.

Rats in group 3 (1000mg/kg) showed significant ($P<0.05$) decrease in edema size in the second, fourth and twenty fourth hours and inhibition percentage at 13.49, 41.17, 41.17, 38.46 and 33.33 at the first, second, fourth, sixth and twenty fourth hours respectively. Rats in group 4 (indomethacine) showed high decrease ($P<0.05$) in edema size when compared to the control (untreated group) at the first, second, fourth, sixth and twenty fourth hours and higher inhibition percentage at 54.47, 58.66, 43.69, 50.00 and 50.00 respectively.

Table 2 summarizes the hematological changes in blood of rats treated with *Trigonella foenum grecum* methanolic extract.

In all treated groups the values of PCV, RBC and Hb showed no significant difference when compared to the control group.

Table (3) summarizes the changes in WBCs count, neutrophil, eosinophil, lymphocyte, and monocyte percentages of rats treated with Trigonella methanolic extract. In group 2 (500mg/kg), nuetrophil, monocytes and eosinophil percentage, showed no significant difference when compared to the (carrageenan group), while there were significant ($P<0.05$) decrease in lymphocyte percentage and total white blood cell count. Group 3 (1000mg/kg) showed no significant differences in monocytes and eosinophil percentage or in white blood cells count, while neutrophil and lymphocytes were significantly decreased ($P<0.05$). The indomethacine group, recorded significantly ($P<0.05$) decreased percentage of neutrophils and total white blood cell count and no significant changes in lymphocytes, monocytes or eosinophil compared to the (carrageenan group) values.

Table (4) summarized the change in serum metabolites of rats treated with *Trigonella foenum grecum* methanolic extract. In all treated groups there were no significant changes in concentration of total protein and albumin and no significant change in the activities of ALT. AST activities significantly increased ($P<0.05$) in group 3 (1000mg/kg) and in the indomethacine group.

DISCUSSION

Inflammation is believed to be biphasic, the early phase (1 – 2 h) of the carrageenan model is mainly mediated by histamine, serotonin and increased synthesis of prostaglandins in the damage tissue surroundings and the late phase is sustained by prostaglandins release and mediated by bradykinin, leukotriens, polymorphonuclear cells and prostaglandins produced by tissue macrophages[4].

Also many studies reported that indomethacine is a standard anti-inflammatory drug in inhibiting response induced by various inflammagens in acute model of inflammation[5,6]. The reduction in the size of the induced edema indicates the anti-inflammatory activity of the plants, used. Morebise *et al* [7] (2001) reported an inhibition of the size of the induced edema within 3 hr by the plants known as *Chamanthera dependens* and Liu *et al* [8] (2001), on the other hand reported the inhibition of the induced edema but after only one hour by *Calligonum comosum.*

Table 1: Average (Mean ± SE) values of anti-inflammatory effects of *Trigonella foenum grecum* methanolic extract on carrageenan-induced paw edema in rats.

	Groups/dose	Edema size (mm)	Inhibition (%)
1hr	G1	1.26 ± 0.20^{a}	0.00
	G2	1.16 ± 0.29^{a}	7.90
	G3	1.09 ± 0.13^{ab}	13.49
	G4	0.47 ± 0.18^{b}	54.47
2hr	G1	1.19 ± 0.12^{a}	0.00
	G2	0.88 ± 0.13^{a}	26.05
	G3	0.70 ± 0.13^{b}	41.17
	G4	0.67 ± 0.07^{b}	43.69
4hr	G1	1.19 ± 0.12^{a}	0.00
	G2	0.88 ± 0.13^{a}	26.05
	G3	0.70 ± 0.13^{b}	41.17
	G4	0.67 ± 0.07^{b}	43.69
6hr	G1	0.78 ± 0.19^{a}	0.00
	G2	0.52 ± 0.21^{a}	33.33
	G3	0.48 ± 0.11^{a}	38.46
	G4	0.39 ± 0.08^{b}	50.00
24hr	G1	0.48 ± 0.10^{a}	0.00
	G2	0.40 ± 0.07^{b}	45.83
	G3	0.32 ± 0.07^{b}	33.33
	G4	0.24 ± 0.07^{b}	50.00

G1: (Control – Carrageenan)
G2: 500 mg/kg *Trigonella foenum grecum* + Carrageenan
G3: 1000 mg/kg *Trigonella foenum grecum* + Carrageenan
G4: 10 mg/kg Indomethacine + Carrageenan
Means in the same column with the same letter are not significantly different (P> 0.05).

Table 2: Average (Mean ± SE) hematological values of rats treated with *Trigonella foenum grecum* seeds

Group/dose	PCV %	Hb (g/dl)	RBCs (10^6/mm^3)
G1	37.00 ± 3.33^{ab}	12.23 ± 0.53^{ab}	6.30 ± 0.35^{a}
G1	30.00 ± 0.76^{ab}	14.67 ± 1.52^{a}	5.43 ± 0.49^{a}
G1	32.00 ± 3.35^{a}	10.93 ± 0.17^{ab}	6.47 ± 0.79^{a}
G1	36.00 ± 1.58^{ab}	11.10 ± 0.64^{ab}	6.77 ± 0.09^{a}

G1: (Control – Carrageenan)
G2: 500 mg/kg *Trigonella foenum grecum* + Carrageenan
G3: 1000 mg/kg *Trigonella foenum grecum* + Carrageenan
G4: 10 mg/kg Indomethacine + Carrageenan
Means in the same column with the same letter are not significantly different (P> 0.05).

Table 3: Average (Mean ± SE) values of leukocytes count of rats treated with methanolic extract of *Trigonella foenum grecum*

Groups/dose	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)	TWBCs (10^3/mm^3)
G1	80.00 ± 0.57^{a}	27.33 ± 2.33^{a}	1.33 ± 0.33^{a}	1.33 ± 0.33^{a}	5.50 ± 0.76^{a}
G2	78.67 ± 0.33^{a}	18.00 ± 1.86^{b}	1.33 ± 0.33^{a}	1.00 ± 0.00^{a}	4.27 ± 0.94^{b}
G3	67.00 ± 0.33^{b}	18.00 ± 1.13^{b}	1.33 ± 0.33^{a}	1.00 ± 0.00^{a}	4.33 ± 0.44^{a}
G4	67.33 ± 0.33^{b}	22.00 ± 1.73^{a}	1.33 ± 0.33^{a}	1.00 ± 0.00^{a}	3.67 ± 0.29^{b}

G1: (Control – Carrageenan)
G2: 500 mg/kg *Trigonella foenum grecum* + Carrageenan
G3: 1000 mg/kg *Trigonella foenum grecum* + Carrageenan
G4: 10 mg/kg Indomethacine + Carrageenan
Means in the same column with the same letter are not significantly different (P> 0.05).

Table 4: Average (Mean ± SE) values of serum metabolites of rats treated with methanolic extract of *Trigonella foenum grecum*

Groups/dose	Total Protein (g/dl)	Albumin (g/dl)	AST (i.u/I)	ALT (i.u/I)
G1	5.70 ± 0.61[a]	3.5 ± 0.07[a]	113.00 ± 8.15[a]	67.00 ± 4.47[a]
G2	5.00 ± 0.25[a]	3.5 ± 0.12[a]	115.00 ± 5.86[a]	60.67 ±3.18[a]
G3	5.16 ± 0.27[a]	3.9 ± 0.23[a]	171.667 ± 6.90[b]	71.00 ± 8.74[a]
G4	5.33 ± 0.15[a]	3.5 ± 0.21[a]	121.667 ± 6.44[b]	59.67 ± 2.33[a]

G1: (Control – Carrageenan)
G2: 500 mg/kg *Trigonella foenum grecum* + Carrageenan
G3: 1000 mg/kg *Trigonella foenum grecum* + Carrageenan
G4: 10 mg/kg Indomethacine + Carrageenan
Means in the same column with the same letter are not significantly different (P> 0.05).

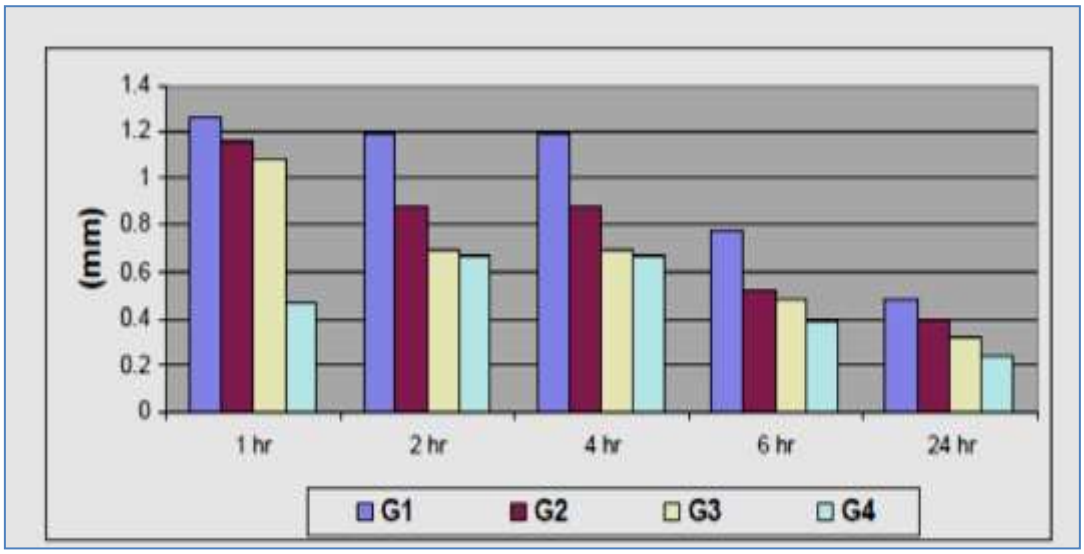

Fig-1: Comparison of edema size in rats treated with methanolic extract of *Trigonella foenum grecum* (G1: (Control – Carrageenan); G2: 500 mg/kg *Trigonella foenum grecum* + Carrageenan; G3: 1000 mg/kg *Trigonella foenum grecum* + Carrageenan; G4: 10 mg/kg Indomethacine + Carrageenan)

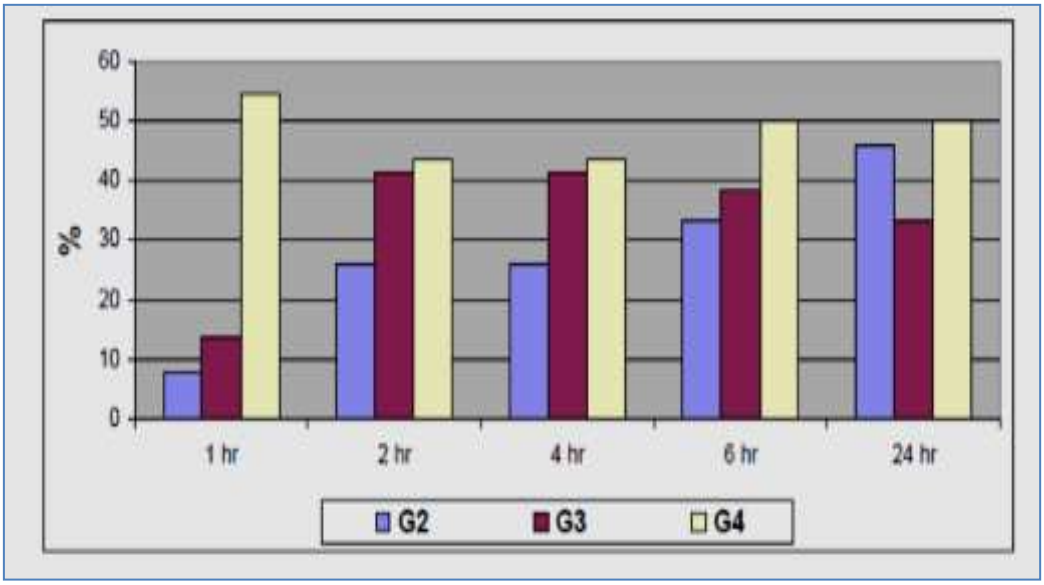

Fig-2: Comparison of inhibition percentage of edema in rats treated with methanolic extract of *Trigonella foenum grecum* (G1: (Control – Carrageenan); G2: 500 mg/kg *Trigonella foenum grecum* + Carrageenan; G3: 1000 mg/kg *Trigonella foenum grecum* + Carrageenan; G4: 10 mg/kg Indomethacine + Carrageenan)

Many previous studies tried to explain the antidiabetic potential of this plant. In addition, one study investigated the inhibitory potential of ethyl acetate and water extract of *T. foenum-graecum* on enzymes α-amylase and α-glucosidase. Different concentrations of extracts were used to study inhibition of enzymatic activity of α-amylase and α-glucosidase. A dose dependent inhibitory effect on enzymes was observed and α-amylase and α-glucosidase inhibitory potential of *T. foenum-graecum* was revealed[9].

The present studies establish the anti-inflammatory activity of the methanolic extract of *Trigonella foenum grecum* seeds. Some biochemical assays were recorded including AST, ALT, total protein and albumin as well as some hematological parameters like Hb, PCV and RBC count so as to reveal no toxicity evidence of the doses used and hence indicate effectiveness and safety action for the treatment of conditions associated with inflammation.

The methanolic extract of *Trigonella foenum grecum* seed showed significant inhibition of edema size at the high doses 1000 mg/kg after 2, 4 hr and 24 hr of treatment and showed inhibition percentage of 41.17, 41.17 and 33.33% respectively and this may be due to the insufficient absorption of the extract which was also reported by Khairalla (2002) in the study of anti-inflammatory acting of *Haplophyllum tuberclatum* [10].

Anti-inflammatory and antipyretic effects of the *Trigonella foenum-graecum* (TFG) leaves extract were examined by Ahmadiani *et al* (2001). For anti-inflammatory activity, the formalin-induced edema model was used. Hyperthermia was induced by intraperitoneal injection of 20% (w/v) aqueous suspension of brewer's yeast. Sodium salicylate (SS) was used as a positive control. Both TFG and SS significantly reduced formalin-induced edema in single dose (TFG 1000 and 2000 mg/kg, SS 300 mg/kg) and chronic administration (TFG 1000 mg/kg and SS 300 mg/kg). TFG and SS also significantly reduced hyperthermia induced by brewer's yeast in 1 and 2 h after their administration. The results indicate that the TFG leaves extract possess anti-inflammatory as well as antipyretic properties in both i.p. and p.o. administration. Phytochemical studies indicate that alkaloids, cardiac glycosides, and phenols are the major component in the extract. Although existence of three anti-inflammatory, analgesic and antipyretic effects in this extract suggest a NSAID-like mechanism for it, but the presence of alkaloids, the absence of other effective compounds such as flavonoids, saponins, steroids, etc., and also its analgesic effect on tail-flick test that usually is not produced by NSAIDs, suggest another mechanism for the extract. So the possibility of alkaloids as effective compounds, in this extract, increases [11].

Results obtained in the rats paw edema showed that extracts of *Trigonella foenum grecum* significantly reduced the size of the paw edema, suggesting that the active ingredients present in the extracts may act as anti-inflammatory. It is well known that some plants constituents can significantly inhibit the biosynthetic pathway of these mediators such as prostaglandins, histamine, serotonin and bradykinin[12].

In addition, as was mentioned that flavonoids and terepenoids had been reported to have anti-inflammatory effects. A number of previous studies suggested that flavonoids may interact directly with the prostaglandins system in the same way as non-steroidal anti-inflammatory drugs[13,14]. Among the above facts flavonoids can be responsible for the anti-inflammatory effects of *Trigonella foenum grecum* extracts in this study.

The chemical compositions of the hydroalcholic extract of *Trigonella foenum grecum* seed were investigated using Perkin-Elmer Chromatography–Mass Spectrometry (GC/MS) by Priya et al (2011), while the mass spectra of the compounds found in the extract was matched with the National Institute of Standards and Technology (NIST) library. GC/MS analysis of ethanol extract of *Trigonella foenum grecum* seed revealed the existence of á-D-Glucopyranoside, methyl (74.54.00%),3-OMethyl- d-glucose (16.11%)2-Propen-1-amine, N-ethyl- (3.43%), Aziridine, 1,2,3-trimethyl-,trans- (2.41%),. The results of this study offer a platform of using *Trigonella foenum graecum* seed as herbal alternative for the current synthetic antimicrobial agents[15].

Neutrophils participate in the endogenous control of the inflammatory pain in rats[16] *Trigonella foenum grecum* extract caused a significant decrease in white blood cells, lymphocytes and neutrophils percentage. This may be due to anti-inflammatory activity of the extract of this plant. Whereas, neturophils and lymphocytes recorded the highest count of white blood cells. This was explained in the study of USEPA[17]that the animal body may point out some chemicals and their metabolites as invading hemotoxicants. No change in monocytes and eosinophil count put in consideration that eosinophils share in parasitic inflammation and monocytes share in viral inflammation.

CONCLUSION AND RECOMMENDATIONS:

Trigonella foenum grecum extract possesses an anti inflammatory activity. The extracts of this plant are safe at the doses used. Investigation of anti inflammatory activity of this plant, by using higher doses and its range of safety when used topically on the skin as well as analysis of its effective ingredients is highly recommended.

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