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**Der Pharmacia Lettre, 2013, 5 (2):247-253**
***(http://scholarsresearchlibrary.com/archive.html)***

Der Pharmacia Lettre
Scholars Research Library
**ISSN 0975-5071**
**USA CODEN: DPLEB4**

# Pharmacological activities of saponin-containing fraction derived from *Gleditsia caspica* Desf. methanolic fruit extract

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**ABSTRACT**

*Phytochemical investigations of Gleditsia fruits have revealed the presence of triterpenoidal saponins. These saponins proved to possess anti-inflammatory and anti-allergic activities. The present study was undertaken to evaluate the anti-inflammatory, analgesic and antipyretic activities of the saponin fraction derived from the methanolic extract of Gleditsia caspica fruits. The fraction at 31.5 and 63 mg/kg b.wt. reduced significantly the oedema induced by carrageenan. The dose 63 mg/kg b.wt. showed higher effect than that of indomethacin (10 mg/kg b.wt.). Further, the saponin fraction showed significant protective effect against complete Freund's adjuvant - induced arthritis. At the dose 63 mg/kg b.wt. the percentage inhibition was 39.2 versus 22.2 for the standard drug prednisolone (4 mg/kg b.wt.), respectively, on day 5 and 53.2 versus 46.7, respectively, on day 12. The analgesic activity results revealed that the saponin fraction at doses 45 and 90 mg/kg b.wt. possessed significant central analgesic activity. In acetic acid-induced writhing model, the fraction showed significant peripheral analgesic activity characterized by double effect in suppressing the number of writhes at dose 90 mg/kg b.wt. compared to acetyl salicylic acid (100 mg/ kg b.wt.). The antipyretic activity results demonstrated that the fraction at the two dose levels 31.5 and 63 mg/kg b.wt. had no effect. Acute and chronic toxicity tests showed that the fraction may be safe for pharmacological uses. These observations suggest possible therapeutic potential of the saponin fraction in the treatment of inflammation and pain.*

**Keywords:** *Gleditsia caspica*, Fabaceae, analgesic, anti-inflammatory, antipyretic.

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## INTRODUCTION

Although a substantial progress in medicinal research have been made during the past decade, there is still a crucial need for discovering new drugs to treat inflammation diseases which represent one of the world's health problems [1]. Inflammation involves a complex array of enzyme activation, mediator release, extravasation of fluids, cell migration, tissue breakdown and repair [2]. Inflammation and pain conditions are currently managed by non-steroidal anti-inflammatory drugs (NSAID) and narcotics. As a result of the adverse effects associated with these drugs such as gastric lesions caused by NSAID, tolerance and dependence induced by opiates, the use of these drugs has not been successful. Therefore, alternative therapies lacking these side effects are necessary. Medicinal plants that have been the subject of intense research and represent a potential source of commercial drugs and lead compounds, could contribute to the discovery of effective, inexpensive and safe alternative anti-inflammatory and analgesic drugs.

Genus *Gleditsia* comprises 14 species of deciduous trees [3]. *Gleditsia* species have been widely used in traditional medicine. In China, the anomalous fruits and thorns are used for treating apoplexy, headache, productive cough, asthma and suppurative skin diseases [4].

Phytochemical studies carried out on fruits of *Gleditsia* members demonstrated that many triterpenoidal saponins and medicinal herbs rich in this kind of saponins, possess anti-inflammatory activities [5], [6], [7], [8], [9], [10], [11]. *Gleditsia caspica*, a tree that grows up to 12 meters, is cultivated in public gardens in Egypt for ornamental purpose due to its graceful habit, elegant form and delicate fern like foliage. Phytochemical investigations carried out by us revealed the occurrence of many saponins in *G. caspica* fruits and from the saponin- containing fraction (SFGC), seven triterpenoidal saponins namely gleditsia saponin E', gleditsioside I, gleditsia saponin C' and caspicaosides A-D, have been reported [12], [13]. Hence, the present study was undertaken to investigate the anti-inflammatory, analgesic and antipyretic effects of the saponin-containing fraction derived from *G. caspica* methanolic fruit extract.

## MATERIALS AND METHODS

### Laboratory animals

The animals used in this study were adult male albino Sprague-Dawley rats weighing between 130-150g and adult male albino mice weighing between 20 and 25g. Both rats and mice were obtained from the Animal House Colony of the National Research Centre (Giza, Egypt). They were fed on a standard laboratory diet and supplied with water source *ad libitum*.

### Drugs and chemicals

The chemicals used were carrageenan (Sigma-Aldrich, USA); yeast (44% suspension in normal saline, local market ); complete Freund's adjuvant (CFA) (Difco, USA); acetyl salicylic acid (ASA) (ADCO, Egypt); indomethacin (Merck, Germany); prednisolone (Sanofi- Aventis, Egypt); Diaion- HP20 (Mitsubishi, Japan), methanol and n-butanol (ADWIC, Egypt).

### Plant Material

Fruits of *G. caspica* were obtained from El-Orman public Garden, Giza,Egypt in November 2004 and identification was confirmed by Dr. Therese Labib senior specialist for plant identification.

### Preparation of SFGC

The methanolic extract (35 g) from the dried powdered fruits (1 kg) was suspended in water and partitioned with chloroform (3x250 ml) followed by n-butanol (3x250 ml). The n-butanol fraction (20 g) was dissolved in distilled water and the aqueous solution was passed through a column packed with the porous polymer gel Diaion HP-20 to remove phenolics and other water soluble constituents. The absorbed material was eluted with $H_2O$, 25, 50, 75 % and 100 % methanol. The material (15.2 g) from the combined 75 and 100 % methanol fractions as SFGC, was freeze dried and kept until use.

### Pharmacological studies

### Anti-inflammatory activity

### Carrageenan- induced rat paw oedema

The method described by Winter et al. [14] was employed. Four groups each comprising six rats were used. Group I received orally distilled water and kept as control. Groups II and III received orally 31.5 and 63 mg/kg b.wt. SFGC while group IV received orally 10 mg/kg b.wt. indomethacin and served as standard drug. Acute inflammation was induced in all groups by subcutaneous injection of 0.1 ml of 1% carrageenan in the left hind paw of the rats one hour after administration of drugs. Mean paw volume was measured immediately before carrageenan injection ($V_o$) and then every hour ( $V_t$) for four hours post administration of the test drug and control using a water displacement plethysmometer. The percentage oedema and the percentage inhibition were calculated as follows:

$$\% \text{ oedema (E)} = \frac{V_t - V_o}{V_o} \times 100$$

$$\% \text{ inhibition} = \frac{E_c - E_t}{E_c} \times 100$$

**Complete Freund's adjuvant (CFA)- induced arthritis in rats**

The method described by Pearson and Wood [15] was applied. Arthritis was induced by 0.1 ml injection of CFA (6 mg/ml Mycobacterium butyricium in mineral oil) into the sub-planter surface of the left hind paw. Drugs were administered orally once a day from the day of injection of CFA and continued up to 12$^{th}$day.The oedema component of inflammation ($V_t$) was quantified by measuring the difference in paw volume between the first day ($V_o$) and the volume recorded on day five and day twelve ($V_t$) using a water displacement plethysmometer. Twenty four rats were divided into four groups. Distilled water that served as control, was administered orally to group I. The doses 31.5 and 63mg/ kg b.wt. of SFGC were administered orally to group II and III, respectively. Group IV received the standard drug prednisolone at an oral dose of 4 mg/kg b.wt. The percentage oedema and percentage inhibition were calculated as shown in the previous method.

**Analgesic activity**

**Hot plate method**

The method described by Turner [16] was used. Twenty four mice were divided into four groups consisting of six animals in each group. Distilled water was orally administered to group animal I which served as control. Groups II and III were orally administered SFGC at doses 45 and 90 mg/ kg b.wt., respectively. Group IV served as reference group received ASA at dose of 100 mg/kg b.wt. The hot plate was maintained at 53 ± 0.5 °C and the reaction time between placing the animal on the hot plate until the animal jumped or licked its hind paw, was recorded 30, 60 and 90 minutes after the administration of the test and reference drugs.

**Acetic acid induced writhing response**

The method reported by Collier et al. [17] was applied. Twenty four mice were divided into four groups of six animals each. The animals were orally administered distilled water, SFGC (45 and 90 mg/kg b.wt.) and ASA (100 mg/kg b.wt.). Thirty minutes after treatment, mice received an intraperitoneal injection of 10 ml/kg b.wt. 0.7% acetic acid in distilled water. The mice were then placed in transparent boxes for observation. The number of writhes was counted for twenty minutes after acetic acid injection and the percentage protection was calculated.

**Antipyretic activity**

Antipyretic activity was carried out according to the previously reported method [18]. Hyperthermia was induced in rats by intramuscular injection of 1 ml/ 100g b.wt. of 44 % yeast suspension in normal saline. After 18 hours of yeast injection, the animals that showed an increase of 0.3-0.5°C in rectal temperature, were divided into four groups six animal each. Group I was considered as control and received distilled water orally. Group II and group III were treated with SFGC at dose of 31.5 and 63 mg/kg b.wt., respectively, while group IV served as positive control and orally received ASA at a dose of 100mg/kg b.wt.. The rectal temperature was recorded by a multichannel electric thermometer 1, 2 and 3 hours after drug treatment, compared with control group.

**Acute toxicity test and $LD_{50}$**

The toxicological studies on SFGC were carried out for determination of the acute toxicity and $LD_{50}$ in mice according to the method of Karber [19]. Thirty six mice were divided into groups of six mice each. Graded doses of SFGC (0.25-2.5 g/ kg b.wt.) were separately administered to the mice of each group. The toxic symptoms and the mortality in each group were recorded 24 hours post administration.

**Chronic toxicity**

The effect of prolonged administration (2 months) of 63 mg/kg b.wt. of SFGC on blood criteria, liver, and kidney functions of rats was investigated.

**Statistical analysis**

The results were expressed as the mean ± S.E. The results obtained from the present study were analyzed using one way analysis of variance (ANOVA) followed by Dunnett's comparison test. P-values <0.05 were considered statistically significant. Statistical analysis was performed using SPSS analytical software version 11 , release (Aug, 23, 2008), Chicago, USA.

**Ethics**
All animal procedures were performed after approval from the Ethics Committee of the National Research Center-Egypt and in accordance with the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals.

## RESULTS

**Acute toxicity and $LD_{50}$**
Administration of SFGC up to 2.5 g/kg b.wt. produced no behavioral abnormalities in the animals. The oral $LD_{50}$ was found to be 900mg/kg b.wt. for mice equivalent to 630 mg/kg b.wt. for rats.

**Chronic toxicity**
Chronic administration of SFGC at a dose of 63 mg/kg b.wt. for two months, produced no significant physiological changes on kidney functions nor on liver functions nor on the blood picture of rats, when compared to normal control.

**Carrageenan-induced paw oedema test**
The two doses 31.5 and 63 mg/kg b.wt. of SFGC showed statistically significant (P< 0.05) oedema reduction at all the time intervals as shown in Table 1. The percentage inhibition values 57.00, 52.96, 57.21 and 54.16, observed after treatment with a dose of 63 mg/ kg b.wt. at time intervals 1h to 4h, were found higher than the corresponding percentage inhibition values 45.78, 47.43, 48.76 and 42.90 shown by the standard drug indomethacin at a dose 10 mg/kg b.wt. The lower dose 31.5 mg/kg b.wt., reduced the oedema significantly (P<0.05) with less percentage inhibition compared with that of the standard drug at all time intervals.

**Table 1. The effect of SFGC on carrageenan-induced paw oedema in rats**

| Treatment | Dose (mg/kg) | % Oedema | | | |
|---|---|---|---|---|---|
| | | $t_{1h}$ | $t_{2h}$ | $t_{3h}$ | $t_{4h}$ |
| Distilled water | -------- | 38.42 ± 1.53 | 55.80 ± 1.57 | 71.71 ± 4.54 | 72.09 ± 3.79 |
| Indomethacin | 10 | 20.83 ± 1.23 * (45.78) | 29.33 ± 2.05* (47.43) | 36.75 ± 2.60* (48.76) | 41.17 ± 1.75* (42.90) |
| SFGC | 31.5 | 24.24 ± 2.35* (36.90) | 47.20 ± 3.67* (15.41) | 49.26 ± 3.68* (31.31) | 53.13 ± 5.67* (19.55) |
| SFGC | 63 | 16.52 ± 1.19* (57.00) | 26.25 ± 1.51* (52.96) | 30.69 ± 2.05* (57.21) | 33.05 ± 2.71* (54.16) |

*Values represent the mean ±S.E., n = 6, each value in parenthesis indicates the percentage inhibition.*
**P < 0.05: Statistically significant from control (Dunnett's test).*

**CFA-induced arthritis test**
The results presented in Table 2 showed that SFGC significantly (P<0.05) decreased the oedema on day 5 and extended to day 12 at dose 63mg/kg b.wt. with percentage inhibition value 53.20 higher than that of the standard drug prednisolone (46.70%) given at a dose 4 mg/kg b.wt. The lower dose level of SFGC (31.5 mg/kg b.wt.) also significantly (P< 0.05) decreased the oedema on day 5 more effectively than prednisolone. The effect produced on day 12, was slightly less than that of prednisolone.

**Table 2. The effect of SFGC on complete freund's adjuvant-induced arthritis in rats**

| Treatment | Dose (mg/kg) | day 5 | day 12 |
|---|---|---|---|
| Distilled water | -------- | 38.69 ± 3.40 | 58.51 ± 4.71 |
| Prednisolone | 4 | 30.11 ± 1.35* (22.20) | 31.19 ± 1.89* (46.70) |
| SFGC | 31.5 | 24.94 ± 1.25* (35.50) | 34.88 ± 1.79* (40.40) |
| SFGC | 63 | 23.52 ± 2.32* (39.20) | 27.41 ± 1.64* (53.20) |

*Values represent the mean ±S.E., n = 6, Each value in paranthesis indicates the percentage inhibition.*
**P < 0.05: Statistically significant from control (Dunnett's test).*

**Hot plate test**
The results recorded in Table 3 demonstrated that SFGC exhibited marked central analgesic activity as evidenced by significant increase in reaction time when compared to the control. The reaction time at doses 45 mg/kg and 90 mg/kg b.wt was comparable to that of the standard drug ASA at dose 100 mg/kg b.wt. through-out the experiment.

**Table 3. The effect of SFGC on hot plate-induced pain in mice**

| Treatment | Dose (mg/kg) | Reaction time (s) | | | |
|---|---|---|---|---|---|
| | | 0 min | 30 min | 60 min | 90 min |
| Distilled water | ------ | 6.36 ± 0.27 | 7.27 ± 0.27 | 8.87 ± 0.36 | 9.00 ± 0.52 |
| ASA | 100 | 6.75 ± 0.29 | 10.03 ± 0.19* | 12.8 ± 0.37* | 12.75 ± 0.44* |
| SFGC | 45 | 6.57 ± 0.25 | 9.97 ± 0.20* | 11.08 ± 0.26* | 11.90 ± 0.24* |
| SFGC | 90 | 7.00 ± 0.38 | 9.98 ± 0.17* | 11.27 ± 0.58* | 12.68 ± 0.43* |

*Values represent the mean ±S.E., n = 6, *P < 0.05: Statistically significant from control (Dunnett's test).*

## Mice writhing test

As shown in Table 4, intraperitoneal injection of acetic acid elicited the writhing syndrome in control mice with 44.3 ± 2.53 writhes counted in 20 minutes. SFGC produced a significant (P<0.05) reduction in the number of writhes with 100 % protection produced by the highest dose 90 mg/ kg b.wt. This effect was more than double that produced by 100 mg/kg ASA (46.6%). The effect produced by the lower dose of 45 mg/kg b.wt. (51.1 %) is also higher than that produced by ASA.

**Table 4. The effect of SFGC on acetic acid-induced writhing response in mice**

| Treatment | Dose (mg/kg) | Number of writhes | % protection |
|---|---|---|---|
| Distilled water | ------- | 44.3 ± 2.53 | ----- |
| ASA | 100 | 23.7 ± 0.88* | 46.6 |
| SFGC | 45 | 21.7 ± 2.32* | 51.1 |
| SFGC | 90 | 0.00* | 100.0 |

*Values represent the mean ±S.E., n = 6, *P < 0.05: Statistically significant from control (Dunnett's test).*

## Antipyretic activity

The intramuscular injection of yeast suspension markedly increased the rectal temperature in rats after 18 hours its administration. Treatment with SFGC at 31.5 and 63 mg/kg b.wt., respectively, showed no significant decrease in rectal temperature of the hyperthermic rats at different time interval compared with that of ASA at the dose of 100 mg/kg b.wt. (Table 5).

**Table 5. The effect of SFGC on yeast-induced hyperthermia in rats**

| Treatment | Dose (mg/kg) | Rectal Temperature at different time intervals | | | |
|---|---|---|---|---|---|
| | | Temperature after yeast injection | $t_{1hr}$ | $t_{2hr}$ | $t_{3hr}$ |
| Distilled water | ---------- | 39.1 ± 0.19 | 39.3 ± 0.21 | 39.0 ± 0.20 | 39.1 ± 0.18 |
| ASA | 100 | 39.2 ± 0.13 | 38.1 ± 0.23* | 37.5 ± 0.18* | 37.8 ± 0.14* |
| SFGC | 31.5 | 38.9 ± 0.12 | 38.8 ± 0.20 | 38.8 ± 0.24 | 38.8 ± 0.15 |
| SFGC | 63 | 38.8 ± 0.15 | 38.9 ± 0.20 | 38.8 ± 0.16 | 38.7 ± 0.14 |

*Values represent mean ±S.E., n = 6, *P< 0.05: Statistically significant decrease from the hyperthermic control (Dunnett's test).*

## DISCUSSION

Inflammation is a pathophysiological response of living tissue that leads to local accumulation of plasmatic fluid and blood cells. Although it is a defense mechanism that helps body to protect itself against infection burns, toxic chemicals or other noxious stimuli. The complex events and mediators associated with inflammation can induce, maintain and aggravate many diseases [20]. Carrageenan oedema has been commonly used as experimental animal model for evaluation of acute inflammation and is believed to be biphasic. The early phase (1h-2h) of the carrageenan model is mainly mediated by histamine, serotonin and increase synthesis of prostaglandins. The late phase (3h-4h) is sustained by prostaglandin release and mediated by bradykinins, leukotrienes, polymorphonuclear cells and prostaglandins produced by tissue macrophages [21]. SFGC at doses 31.5 and 63 mg/kg b.wt., produced significant inhibition of carrageenan induced paw oedema at all time intervals (1h-4h). Therefore, it can be inferred that the inhibitory effect of SFGC on carrageenan-induced inflammation could be due to inhibition of the enzyme cyclo-oxygenase and subsequent inhibition of prostaglandin synthesis. Significant inhibition of paw oedema in the early hours of study could be attributed to the inhibition of histamine and/or serotonin.

The model of adjuvant induced inflammation in rats has been extensively used in the study of the inflammatory processes. Freund adjuvant is an antigen solution emulsified in mineral oil that is used as an immune-potentiator. The complete Freund's adjuvant (CFA) is composed of inactivated and dried mycobacterium butyricium and is

effective in stimulating cell mediated immunity and may lead to the potentiation of the production of certain immunoglobulins. Shortly after the administration of CFA into the hind paw, pronounced swelling appears in the hind paw which persists for several days. After that, the contra lateral paw as well as front paw also becomes swollen and inflammatory nodules will be observed. In the present study, it was observed that SFGC could significantly ($P< 0.05$) inhibit the progression of the inflammation in the treated animals and the percentage oedema was decreased on day five at a dose of 63 mg/ kg b.wt. and extended till day twelve. The effect was higher than that of the standard drug prednisolone at a dose of 4 mg/ kg b.wt.

The analgesic activity of SFGC was studied using the hot plate and the mice writhing tests. The hot plate test is one of the most common tests for evaluating the analgesic efficacy of drugs. The paws of mice are very sensitive to heat at temperatures which are not damaging the skin. The responses are jumping, withdrawal or licking the paws. The time until these responses occur is prolonged after administration of centrally acting analgesics. The results of the analgesic activity of SFGC showed that it possessed significant ($P< 0.05$) activity. The reaction time for the animal group received SFGC at dose of 90 mg/kg b. wt. was almost equal to that of the standard drug ASA at dose level of 100 mg/kg b.wt. The analgesic activity of SFGC was also evaluated by using acetic acid induced writhing in mice. The injection of acetic acid into the peritoneal cavity of mice induces writhing (contraction followed by extension of the hind limbs). The writhing test which is a visceral pain model was found simple, reliable and rapid for investigating analgesics. The inhibition of writhing by using SFGC suggests peripherally mediated analgesic activity based on the association of the model with stimulation of peripheral receptors especially the local peritoneal receptors at the surface of cells lining the peritoneal cavity [22], [23]. The results of this study demonstrated that SFGC significantly ($P < 0.05$) inhibited the acetic acid induced pain in the low and high dose levels. The animal group that received SFGC at dose of 90 mg/kg b.wt., showed more than double the effect observed for the reference group treated with 100 mg/kg b.wt.of ASA.

It is worthy to note that many saponins have been reported to exhibit significant anti-inflammatory, antinociceptive and antipyretic activites as well as many other diverse activities such antiallergic, analgesic and others [24], [25], [26], [27]. Moreover, a variety of triterpenoidal saponin-rich extracts such as *Polyscias fruticosa* [28], *Kalopanax pictus* [29], [30], *Akebia quinata* [31] and *Mimusops elengi* [32], have been found to display anti-inflammatory, analgesic and antipyretic activities.

## CONCLUSION

From the experimental findings in this study, we can conclude that the saponin-containing fraction from the methanolic extract of *Gleditsia caspica* fruits, showed anti-inflammatory and analgesic activities in the tested models. The analgesic activity was found to be the most effective at higher dose employed. The anti-inflammatory and the analgesic activities of SFGC are correlated and might share common mechanism through inhibition of the prostaglandins pathways.

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