Original article

Total phenolic, flavonoid contents, and biological activities of stem extracts of *Astragalus spinosus* (Forssk.) Muschl. grown in Northern Border Province, Saudi Arabia

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Peer review under responsibility of King Saud University.

**Article info**

Article history:
Received 17 November 2021
Revised 9 December 2021
Accepted 12 December 2021
Available online 20 December 2021

Keywords:
Analgesic
Anti-inflammatory
*Astragalus spinosus*
Carrageenan
Hot plate

**Abstract**

Background and objective: Genus Astragalus belongs to the family Fabaceae and is one among the largest genera consisting of around 3000 species. The plants have been used traditionally in treatment of various ailments in folklore. The study was planned to assess the analgesic and inflammatory activity of *Astragulus spinosus* (Forssk.) Muschl extract of the stem.

Materials and methods: Course powder of stems of *Astragulus spinosus* was extracted using chloroform and methanol as solvents. Folin ciocalteu method was employed for determination of the phenolic acid content. Aluminum chloride colorimetric procedure was followed for estimating the flavonoid content. Both chloroform and methanolic extracts at 250 and 500 mg/kg, were tested for the analgesic activity, however, only methanolic extract was selected for anti-inflammatory property based on the results of analgesic activity. The analgesic effect was executed on male rats by the hot plate model. The anti-inflammatory effect was studied in the carrageenan rat paw edema model. The experimental information was interpreted statistically using one-way ANOVA and *p* < 0.05 was used to express importance of the results.

Results: The total phenolics of the methanol extract was 420 mg and that of chloroform extract was 265 mg while total flavonoid content in terms of quercetin was found to be 68 mg and 17.5 mg for methanol and chloroform extract respectively. Only methanolic extract exhibited significant (*p* < 0.001) analgesic activity by elevating the pain threshold starting from 15 min. The methanolic extract inhibited (*p* < 0.001) the edema in carrageenan induced model. The performance of higher dose (500 mg/kg) was better with reference to lower dose (250 mg/kg).

Conclusion: Outcome of the results show that the methanolic stem extracts exhibited significant analgesic and anti-inflammatory-like activity with reference to chloroform extract. Credit of which is given to flavonoids and phenolic content present in the methanolic extract. However, more research is suggested to establish the safety and effectiveness of the herbal drug to manage diseased states including pain and inflammation.

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1. Introduction

From ancient times plants are associated with the treatment of diseases; this is always accredited to the various biologically important phytoconstituents (Uddin et al., 2011). Several medical conditions are associated with pain. The International Association for the Study of Pain (IASP) defines pain as “an unpleasant sensory and emotional experience associated with actual or potential tissue damage”. It is primarily a protective and warning signal. Pain
is either acute and sometimes can be chronic. The biochemical mediators are usually prostaglandins and bradykinins (Merskey, 1979; Millan, 1999). Inflammation is referred to the protective response which involves various phases. The commonly used drugs to treat inflammation are NSAIDs and opiates. Administration of these drugs for extended period of time leads to side effects like gastric discomfort, hepatotoxicity and damage to the kidneys. Use of herbal therapy thus becomes an alternative and an interesting option. Herbal extracts having antioxidant properties are significant in the control of diseases related to inflammation as reported in the literature. (Menghini et al., 2016; Ferrante et al., 2017). The need to have research on medicinal plants has been acknowledged worldwide by practitioners in order to ameliorate these side effects while retaining the best efficacy. Hence consequently it is pertinent to explore and discover newer drugs from plants with lesser side effects (Junaid et al., 2011; Hijazi et al., 2017).

Genus Astragalus consists of around 3000 species. They are traditionally used in native treatment of hypertension, stomach ulcer, bronchitis, diabetes, gynecological disorders etc (Labed et al., 2016). They have also been investigated for anti-inflammatory, antiviral, immunomodulatory, analgesic, antioxidant, anticancer, cardiotoxic activities using various screening models (Li et al., 2001; Huang et al., 2009; Hong et al., 2011; Nalbantsoy et al., 2011). Several reports are available in the literature for the analgesic and anti-inflammatory activity of various plants belonging to this genus. Hence Astragalus spinosus was an ideal candidate for investigation of these activities. Furthermore this plant is abundantly available in the Northern Province of Saudi Arabia. Hence it was found worthwhile to evaluate this plant for these activities.

Astragalus spinosus (family Fabaceae) is referred to as Kathad in Arabic. It is a terrestrial shrub having a height of around 60 cm. Leaves are imparipinnate or paripinnate with the rhachis ending in a spine. The flowers are in axillary racemes. The calyx is tubular, campanulate with 5 subequal teeth. Ovary is sessile. Fruit Pod is stipitate, while the seeds are kidney-shaped having a thread-like funicle. The plant is commonly found in the deserts of Middle-Eastern and African countries (Nalbantsoy et al., 2011). The various phytoconstituents reported from this plant are astraseiversianin tragalloside, trigoside, cycloastragenol, formonetin, spino coumarin, isoastragaloside I and trigoside I.sponio coumarin (Mohamed et al., 2007) rutin, quercetin,kamferpel, luteolin ,api-genin, gallic, chlorogenic acid, caffeic, ferulic, coumaric, cinnamic acids (Ashour, 2019). Traditionally, the plant is popular among tribal regions of middle-eastern countries. The extracts has been used in the treatment of leukemia, wound healing, allergic reactions, scorpion bite and other pain and inflammatory conditions (Fikria, 2002). Literature reports the antibacterial, antifungal, immunostimulant, anti-anxiety, antidepressant, and in treatment of hepatic, renal and cardiac toxicities. It has also been reported to modulate the neurotoxicity and DNA damage (Ashour, 2019; Fikria, 2002; Essawy et al., 2021; Abd Elkader et al., 2021; Afzar et al., 2015). We have earlier demonstrated the antioxidant activity of the leaves of this plant (Khaled and Nayeem, 2021). Oxidative stress is one of the contributing factors for inflammation and anti-oxidants prevent the damage of tissues and thereby prevent /protect tissue damage. Various plant extracts possessing antioxidant activities have also been reported to possess anti-inflammatory activities (Ofori-Baah et al., 2019; Ravipati et al., 2012; Borquaye et al., 2020).

Geographical variation has impact on quantity and quality of the secondary plant metabolites in plants. Environmental factors like temperature, altitude, humidity, light, etc influence the quality and quantity of the phytoconstituents which forms the basis for their medicinal activity [3]. Studies have been reported that environmental conditions such as temperature, stress, moisture content, type of soil, altitude etc can affect the secondary metabolites and other compounds that are produced by the plants which in turn can affect the biological activities (Arun et al., 2016). Literature cites several researchers that have demonstrated that plants collected from different geographical regions are diverse in the chemical composition (Cuneyt et al., 2014; Alok et al., 2014). Scientific reports indicating analgesic and anti-inflammatory activities of plant grown in Saudi Arabia are not found in the literature. Therefore, it was found worthwhile to assess the above-mentioned activities for the stems Astragalus spinosus grown in Saudi Arabia

2. Materials and methods

2.1. Collect of material and extract preparation

Astragalus spinosus was procured from desert areas of Rafha, Northern Border Province, Saudi Arabia. It was authenticated by Dr Heba Hamed Salem, Department of Natural Products and Alternative Medicine, Faculty of Pharmacy, Northern Border University. The specimen voucher of the plant has been stored in the Faculty of Pharmacy, Northern Border University. The stem was separated from the other parts, dried in the air and grinded to get a powder. Accurately weighed 75 g of coarse powder was macerated with 225 ml solvents of different polarities i.e. chloriform and methanol at normal temperature for 1 week with intermittent shaking. The filtered extracts were concentrated by using a rotary evaporator. The extracts thus obtained were stored in well closed container until further use. These extracts were subjected to preliminary phytochemical analysis using official methods.

2.2. Estimation of total phenolic content in methanolic and chloroform extract

Folin ciocalteu method was used to estimate the phenolic acid content for both methanol and chloroform extract (Sarvanakumar et al., 2009; Kamran et al., 2009). 25 mg of gallic acid was dissolved in 25 ml of methanol to obtain the standard solution of gallic acid. The concentration obtained was 1 mg/ml. The required quantity (10 mg) of the test extract were weighed and dissolved in 10 ml of solvent i.e. methanol. The concentrations of gallic acid for the estimation of phenolic acid were 100 μg/ml, 200 μg/ml, 300 μg/ml, 400 μg/ml, 500 μg/ml and 600 μg/ml. Standard flasks of 25 ml were taken and to this, 1 ml each of the concentrations of gallic acid was pipetted, followed by addition of 9 ml of distilled water. Same procedure was carried out for the samples and the blank. One ml of Folins ciocalteu solution and ten ml of 7% standard sodium carbonate solution was added to each flask. Distilled water was used to make the volume to 25 ml. After incubation for 90 mins, the absorbance was documented at 750 nm by UV spectrometer. A standard graph was plotted for gallic acid. The phenolic content was indicated as equivalent of gallic acid.

2.3. Determination of flavonoids in the in the methanol and chloroform extract

The flavonoid content of methanolic and chloroform extracts was evaluated by the Aluminum chloride colorimetric method (Sarvanakumar et al., 2009; Kamran et al., 2009). 25 mg quercetin was dissolved in 25 ml of methanol to get a standard solution containing 1 mg/ml. 1 ml from this was transferred to volumetric flask (10 ml) and methanol was added to get 100 μg/ml. 10 g of extract was accurately weighed and dissolved in 10 ml of the solvent methanol. Further dilution resulted in solution of concentration 100 μg/ml.
The extracts were mixed with 0.1 ml of 1 M potassium acetate, 1.5 ml methanol, 0.1 ml 10% aluminum chloride and 2.8 ml of water were incorporated into the extract. A standard curve for the flavonoid quercitin was plotted by using the concentrations 25 ml (lower), 50 ml, 75 ml and 100 ml (higher). 415 nm was the frequency used for measuring the absorbance. A standard calibration curve was plotted for quercetin i.e. Absorbance versus concentration. Total flavonoid content was determined as quercetin equivalent (Mabry et al., 1970).

2.4. Biological activities

Rats were weighed and housed in polypropylene cages on husk bedding. Each cage consisting of two animals. Ambient temperature of 25 ± 2 °C and 12 h light/dark cycle was maintained. The animals were fed with commercially available standard diet and water ad libitum. They were allowed to get adjusted to the lab environment prior to the experiment. They were allowed free access to food and water throughout the entire experimental period. The animals were handled as prescribed in the ethical guidelines for laboratory animals.

The methanolic extract was assessed for their analgesic and Anti-inflammatory activities (Afsar et al., 2015) while the chloroform extract was evaluated for its analgesic activity based on the results of analgesic activity. The experimental studies were conducted after ethical approval ((MCST/COP#25/2021) and during the entire course of action the instructions for experiment were strictly followed. In order to administer the crude extract at a lower dose of 250 mg/kg and a higher dose of 500 mg/kg body weight required quantity of extracts was measured and was triturated by gradual addition of small amounts of suspending agent Tween-80 and the volume was made with saline water. The extracts were administered orally.

To assess anti-inflammatory activity required quantity of methanolic extract was weighed and dissolved in normal saline (0.9% NaCl solution). The standard was prepared by dissolving 1.5 g Diclofenac sodium powder in 30 ml normal saline. 50 mg carrageenan was weighed and dissolved in normal saline by gentle heating in water bath and the volume was made up to obtain 1% solution. The final volume was made to 5 ml with the same solvent. This was introduced into sub-planter surface of the right hind paw after half an hour of test extract administration (Menghini et al., 2016).

2.4.1. Analgesic activity

Eddy’s hot plate model was utilized to assess the analgesic activity potential of the extract. Six male rats having average weight of 275 ± 10 g were used. The animals were separated into six groups. Each group comprising of six animals. The experimental rats were made to get acclimatized to lab surroundings for 24 h prior to the start of the experiment. Standard conditions were maintained, and the animals were fed with the available commercial diet. The first group was administered with normal saline and was marked as the control, the second group was treated with indomethacin which was the standard used, the third group was treated with the lower dose (250 mg/kg) of chloroform extract while fourth group was used for higher dose (500 mg/kg). Fifth and the sixth groups were utilized for the lower and higher dose of the methanolic extract. The temperature of the Eddy’s hot plate was around 55–56 °C (Kalkhambkar et al., 2008). The duration taken for flicking of hind paw, licking, or jumping was regarded as the reaction period. This was recorded for each of the experimental animal (Hijazi et al., 2017).

2.4.2. Anti-inflammatory activity

Anti-inflammatory activity of the extracts was assessed by Carrageenan induced paw edema model. Carrageenan (1% w/v) was introduced into the sub planter part of the left hind paws of the experimental rats to produce the inflammation. The animals were selected similar to the analgesic activity and were separated in 4 groups comprising of six animals. The first group was considered as a control and was administered normal saline, standard Diclofenac sodium (10 mg/kg body weight) (Afsar et al., 2015; Singh et al., 2010) was administered to group 2, and group 3 was administered lower dose (250 mg/kg) of methanolic extract while group 4 was used for higher dose (500 mg/kg). All the experimental rats were administered with Carrageenan (0.1 ml) into the sub planter area of the right hind paw. This was followed by measuring the volume of the paw at 1 to 5 h using plethysmograph. The decrease in volume of paw edema with administration of the extracts was an indication of anti-inflammatory effect. The percent of either decrease/increase in volume of the paw with time was documented and a comparison was made with the control group (Afsar et al., 2015). The formula used for the calculation of percentage of inhibition of edema was as follows:

\[
\% \text{ Inhibition of edema} = \left(\frac{V_c - V_t}{V_c}\right) \times 100
\]

Where Vt = Paw volume in test group animals.

Vc = Paw volume in control group animals.

2.5. Statistical analysis

Outcome of the studies were tabulated and the data obtained was expressed as mean ± SEM. Statistical analysis such as one-way ANOVA was used to determine the significance of results using GraphPad Prism 8.3.1 software. The data from the extract administered groups were compared with the control. If the p-value was seen to be less than 0.05 then it was taken to be of significance.

3. Results

The plant was extracted with solvents of different polarities i.e. methanol and chloroform. The percentage and the nature of the extract obtained is as depicted in the following table

3.1. Total phenolic content in the methanolic and chloroform extracts

The Folin ciocaltteu method measures the quantity of specimen that is required to inhibit the oxidation of the standard reagent. The methanolic extract showed an absorbance of 0.1446 while the absorbance of chloroform extract was 0.0854. A calibration curve was plotted using absorbance versus different concentrations of gallic acid. Total phenolics of the different extracts was obtained from the standard curve using the regression equation of \(Y = 0.0326x; R^2 = 0.9871\) and was expressed as µgram gallic acid equivalents with reference to dry weight. From the calibration curve of the standard the total phenolic content of the of chloroform extract was 265 µg and methanol extract was 420 µg.

3.2. Flavonoid contents in the chloroform and methanolic extracts

The methanolic extract showed absorbance of 0.670 while the chloroform extract absorbed at 0.252. From the calibration curve of quercetin the total flavonoid content in terms of quercetin was...
found to be 68 µg and 17.5 µg for methanol and chloroform extract respectively expressed as microgram of quercetin equivalents. This was calculated from the regression equation of the standard curve of quercetin (\(Y = 0.2084x; R^2 = 0.9915\)).

### 3.3. Analgesic effect of the methanolic and chloroform extracts

The data from the analgesic activity indicated that administration of methanolic extract significantly \(p < 0.05\) increased at 15 min interval compared to control. Both the tested doses of test extract increased the time at 30, 45 and 60 min. The highest dose (500 mg/kg) increased the time more than 250 mg/kg at all the intervals of time. The standard drug (diclofenac – 10 mg/kg) (Afsar et al., 2015) also increased the time significantly \(p < 0.001\) in all time intervals with reference to the control. However, administration of chloroform extract of the plant did not produce significant effect at either of the doses in all tested time intervals (Table 1).

### 3.4. Anti-inflammatory activity effect of the methanolic extracts

The anti-inflammatory property of the plant was assessed in the methanolic extract, and the outcome is as represented in Table 2. The administration of the extract at 500 mg/kg significantly \(p < 0.001\) reduced paw volume after 1 h of carrageenan administration when compared to the control. Further, when the subsequent durations of time intervals such as 2, 3, 4 and 5 h were tested, both the tested dose (250 and 500 mg/kg) inhibited significantly \(p < 0.001\) the paw volume. The standard drug exhibited significant \(p < 0.001\) inhibitory effect at all the tested time duration in comparison to control (see Table 3).

### 4. Discussion

This study was planned to assess the analgesic and anti-inflammatory activity of the extracts of the stems of *Astragalus spinosus* in well-established and validated experimental methods. Analgesic activities were evaluated by hot plate method and anti-inflammatory potential by Carrageenan induced paw edema respectively. Only male rats were utilized for the experiment as it has been reported that the pain sensitivity changes during the menstrual cycle and also the fact the estrogen exhibits anti-inflammatory activity (Vegeto et al., 2008).

The hot plate test is one of the frequently used methods specially to test central-mediated anti-nociceptive effects and analgesic effect. This method was chosen as it is sensitive to strong analogesics and has a cutoff period of 15 sec. Furthermore less time is required and measurements are generally precise. The antinociceptive activity was evaluated by introducing the rats on a temperature maintained hot plate. Withdrawal or licking of paws was considered as the reaction time. Time taken for this response was noted. It has been postulated that the cyclooxygenase pathway advances the inflammatory pain through conversion of arachidonic acid to prostaglandin E2 by cyclooxygenase-2, which is a crucial for inflammation (Riccio1 and FitzGerald, 2011; Badole et al., 2012; Tadiwos et al., 2017). During the course of the experiment the analgesic activity of extracts was dose dependent. The time taken by the samples extracts was 45 mts (maximum effect). The extracts showed significant analgesic activity by elevating the pain threshold starting from 15 min in comparison the control. It was noted that the extracts exhibited the analgesic activity in a dose dependent manner. Higher dose of methanolic extracts exhibited better activity at 45 min when compared to the chloroform extract. However the highest analgesic effect was seen for the methanolic extracts at all times of observation (Table 1). This could be due to the combined effect of the various polyphytoconstituents. The possible mechanism of central analgesic activity can be via activating the release of endogenous peptides like endorphin/enkephalin. The endogenous peptides descend the spinal cord and inhibit the pain impulse that are transmitted via the synapse through peripheral mechanisms and involve the inhibition of the endogenous substances responsible for the central pain transmission (Zhao et al., 2018).

Carrageenan induced paw edema is a commonly used method in research for detecting the orally active inflammatory agents. Inflammation involves two phases i.e. The initial phase i.e. 0–2 h releases histamine, serotonin and bradykinin which are responsible for the occurrence of paw edema because of blood vessel expansion and increase in permeability. In the next phase i.e. 2–6 h, releases prostaglandins which is mediated by leukotriene and bradykinin (Sengar et al., 2013; Marzouk et al., 2010). Carrageenan being non-antigenic is free from systemic effect. It has been reported that this model has a high degree of reproducibility making it a most common and frequently use model for screening of inflammatory activity of plant extracts. The characteristics of edema are swelling, pain and redness. The various signs of inflammation and erythema were seen instantly after injecting the carrageenan as a result of the activity of numerous pro-inflammatory agents such as reactive oxygen and nitrogen species bradykinin, etc. The inflammatory response is gauged by analyzing the change in paw size. Decrease of edema caused by carrageenan can therefore be used for quantification of anti-inflammatory property of extracts (Pishgahzadeh et al., 2019; Mazid et al., 2010; Yimer et al., 2020).

The total phenolic acids and flavonoids content were evaluated by using standard methods. From the results it was evident that the content of both flavonoids and phenolic acids was less in chloroform extract when compared to the methanolic extracts (Figs. 1 and 2). Hence only the methanolic extract was subjected to the anti-inflammatory activity. Administration of carrageenan induced an acute localized inflammation. In our study the methanolic extracts at the lower and higher doses produced significant inhibition of edema. The effect of the extracts began from 1st phase and seen until second phase of inflammation (1-4 hrs). The methanolic extract at a higher dose (500 mg/kg) exhibited the highest activity at 4th hr, with inhibition of paw edema at (37.28%) and (45.17%) for the lower dose (250 mg/kg) and higher dose (500 mg/kg) respectively. Edema inhibitory effect exhibited by the higher dose was as good as the standard drug (47.36%) respectively (Table 2). This could be attributed to the various phytoconstituents that may be present in the plants; which may be interfering with the activity of the chemical mediators and thereby suppressing the stages of inflammation by hindering with the liberation of the chemical mediators (Riccio1 and FitzGerald, 2011). It was noted that the extract inhibited edema from the very first hour and dampened the inflammatory response in all phases of inflammation hinting that these extracts might have reduced the inflammatory mediators.

The extracts were found to contain phytoconstituents such as flavonoids and phenolic compounds (Figs. 1 and 2). Review reports that polyphenolics like flavonoids and phenolic acids are known to exhibit analgesic and anti-inflammatory effect by several mechanisms (Rao et al., 2005; Rios et al., 2000). Reports on flavonoids possessing various biological activities such as anti-inflammatory, wound healing, antitulcer,

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### Table 1

| Sl.no | Solvent   | Nature            | % yield |
|-------|-----------|-------------------|---------|
| 1.    | Methanolic| Chocolate brown   | 7.4%    |
| 2.    | Chloroform| Light brown mass  | 3.1%    |
hepatoprotective, anticancer, neuroprotective, antibacterial, antidiabetic, and antithrombotic are available in the literature. Oxidative stress and antioxidant defense mechanism may also be a contributing as they are linked with inflammatory conditions (Pietta, 2000; Santos et al., 2017). Hence, it can be suggested that the activities of the extracts maybe related to the existence of the flavonoids and phenolic compounds.

5. Conclusion

The outcome of this investigation advocates that the methanolic extract of *A. spinosus* has shown significant dose related analgesic and anti-inflammatory activity; this may be due to alleviation of the mediators of inflammation by phenolic compounds and flavonoids. Being popular as the traditional folk medicine, the plant extract could become a useful nature-derived drug for pain and inflammatory conditions. However, more research is required to identify and isolate specific bioactive compounds to find out the exact detailed underling molecular mechanism of action of the phytoconstituents that may be directly or indirectly behind the biological activities and also to determine the safety and efficacy.

Funding

The author Majid Alhomrani supported by Taif University with number (TURSP 2020/257), Taif University, Taif, Saudi Arabia.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.
Acknowledgement

The authors would like to express his gratitude to AlMaarefa University, Riyadh, Saudi Arabia, for providing funding (MA-004) to carry out this study. The two authors NN and MI are thankful to Northern border university for providing support for this work.

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