

Research Article

Analgesic, anti-inflammatory and anti-pyretic activities of *Caesalpinia decapetala*

Amna Parveen¹, Muhammad Sajid Hamid Akash^{2,3*}, Kanwal Rehman³, Qaisar Mahmood³, Muhammad Imran Qadir^{2*}

¹ College of Pharmacy, Chung-Ang University, Seoul, South Korea

² College of Pharmacy, Government College University Faisalabad, Faisalabad, Pakistan

³ Institute of Pharmacology, Toxicology and Biochemical Pharmaceutics, College of Pharmaceutical Sciences, Zhejiang University, Hangzhou, China

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Abstract

Introduction: In many pathological conditions, pain, inflammation and fever are interdependent to each other. Due to the use of synthetic drugs, many unwanted effects usually appear. Various studies have been conducted on *Caesalpinia decapetala* (*C. decapetala*) to evaluate its effects in the treatment of various diseases but no sufficient scientific literature is available online to prove its analgesic, anti-inflammatory and anti-pyretic activities.

Methods: The analgesic, anti-inflammatory and anti-pyretic activities of 70% aqueous methanolic and n-hexane extracts of *C. decapetala* was evaluated using Swiss albino mice (20-30 g).

Results: The results showed that aqueous methanolic extract of *C. decapetala* at the dose of 100 mg/kg exhibited significant ($p < 0.05$) activities in various pain models including acetic acid-induced writhing (18.4 ± 0.53), formalin-induced licking (275 ± 4.18) and hot plate method (2.3 ± 0.0328); whereas, n-hexane extract showed its effects in acetic acid-induced writhing (20 ± 0.31), formalin-induced licking (293 ± 1.20) and hot plate method (2.224 ± 0.029) compared to the effects observed in control group animals. Similarly, the aqueous methanolic extract of *C. decapetala* after 2 h of treatment exhibited more significant anti-inflammatory (0.66 ± 0.06) and anti-pyretic (38.81 ± 0.05) activities compared to the control group animals.

Conclusion: From the findings of our present study, we concluded that the aqueous methanolic extract of *C. decapetala* has stronger analgesic, anti-inflammatory and anti-pyretic effects than its n-hexane extract. Further studies are required to investigate the active constituents of *C. decapetala* that exhibit analgesic, anti-inflammatory and anti-pyretic activities.

Introduction

Herb- and plant-derived medicines have been used since ancient times and considered as part of our health remedies. The tendency of using natural products for the treatment of serious life-threatening diseases¹⁻⁵ has been increasing. It is stated that natural products are easily biodegradable, possess least environmental hazards, represent minimum side effects and are available at affordable prices. Although most of medicinal activities of the plants have been well-documented, the others are yet to be verified.⁶

Caesalpinia decapetala (*C. decapetala*) is commonly known as Roth.⁷ It is a pantropical genus which belongs to the family of Caesalpiniaceae having 120-150 species of trees, shrubs, and lianas.² The genus consists of several members of species that are used traditionally for the treatment of inflammation, hepatotoxicity as well as diabetes.^{8,9} *C. decapetala* is widely spread in subcontinent regions. It is thorny climber up to 25 m in height and its

leaves are 11-37.5 cm long. Flowers are yellow in color and 1.2-1.8 cm long. Its branches are hairy with hooked or straight prickles (Fig. 1). Traditionally, *C. decapetala* has had many medicinal properties. A bath with decoction of *C. decapetala* is valuable for the treatment of jaundice.¹⁰ Leaves are used for the treatment of burns, biliousness and stomach disorders. Leaves and roots are also used as a purgative and emmenagogue. Other uses of *C. decapetala* are as laxative, tonic, anti-pyretic and carminative.¹¹ The anti-oxidant, anti-tumor and anti-fertility activities of *C. decapetala* have been reported.¹⁰⁻¹³ Experimental study on gallic acid isolated from the *C. decapetala* is responsible for the antitumor and antioxidant activities.¹³ The leaves of *C. decapetala* contain several active constituents including cassane diterpenoid, squalene, caesaldecane, spathulenol, lupeol, resveratrol, quercetin, stigmasterol and astragalin.¹⁴ Presence of phenolic compounds in *C. decapetala* makes this plant valuable, but limited scientific

*Corresponding authors: Muhammad Sajid Hamid Akash, Email: [sajidakash\[at\]gmail.com](mailto:sajidakash[at]gmail.com)

Muhammad Imran Qadir, Email: [mrimranqadir\[at\]hotmail.com](mailto:mrimranqadir[at]hotmail.com)





Fig. 1. *C. decapetala* (image was taken from flora of Pakistan)

literature is available online to prove its traditional usage. Although various studies have been conducted on *C. decapetala* to evaluate its effects in the treatment of various diseases, no sufficient scientific literature is available online to prove its analgesic, anti-inflammatory and antipyretic activities. The present study is aimed to focus on the evaluation of analgesic, anti-inflammatory and antipyretic activities of methanolic and n-hexane extracts of *C. decapetala* using rats as experimental animal models.

Materials and methods

Drugs and chemicals

Carrageenan suspension, formalin, acetic acid, aspirin, and tween 80 were purchased from a local pharmacy in Faisalabad, Pakistan. Brewer's yeast was purchased from Merck, Germany. Methanol, n-hexane, acetone and methyl cellulose were purchased from Sigma-Aldrich. All other chemicals and reagents used in this study were at least of analytical grade and used further without modification.

Plant collection

Leaves and branches of *C. decapetala* were used for this study. Collection of plant was done in Dir, Pakistan during the month of July 2012. The medicinal plant was identified and authenticated by plant taxonomist of department of Botany, University of Malakand, Khyber Pakhtunkhwa, Pakistan. Voucher specimen (voucher specimen No. 3015) was deposited at University of Malakand for future reference. The plant was dried under shade at temperature between 21 to 30 °C for 15 to 20 days and grounded to course uniformity.

Extraction of plant materials and sample preparation

The powdered plant was extracted by the cold maceration process using n-hexane and aqueous methanolic (70%) solvents. The powder was soaked for 1 week with irregular shaking and then passed through muslin cloth separately, filtered, and dried using rotary evaporator. The aqueous methanolic residue was dissolved in normal saline while the n-hexane residue was dissolved in 0.2% tween 80

before administration to animals according to the body weight of the experimental animals.

Experimental animals

Swiss albino mice (20-30 g) which were kept in propylene cages under controlled conditions were used in experiments. Temperature, humidity, and ventilation conditions were controlled. Clean and satisfactory food was given to animals with water which was given *ad libitum*. The experimental protocols were approved by the ethical review committee of Government College University, Faisalabad, Pakistan. The experimental animals were divided into four groups named as control, standard, aqueous methanolic and n-hexane extract group animals. Experimental animals belonging to standard groups were treated with aspirin (100 mg/kg), aqueous methanolic extract and n-hexane extract (orally) were administered with dose of 100 mg/kg, and control group received normal saline (2 ml/kg).

Determination of analgesic activity of *C. decapetala*

Acetic acid-induced writhing in mice

In accordance with the methods described previously¹⁵ with some modifications, analgesic activity of *C. decapetala* was assessed using acetic acid-induced writhing. Briefly, acetic acid which is noxious substance was injected in the abdominal cavity via intraperitoneal injection. The acetic acid was responsible for the cause of severe abdominal pain and contraction (writhing). The activity of drug was evaluated by reduction in the number of writhing and comparison with the control group.

Before experiments, the animals were kept on overnight fasting. Then they waited about half an hour after dosing for the indication of writhing. Each group was treated with 0.2 ml of 3% acetic acid solution intraperitoneally. Immediately after indication, the numbers of writhings were counted for 10 minutes. The response of aspirin and extracts of *C. decapetala* were compared with control group to assess the analgesic action accordingly.

Formalin-induced licking of paw in mice

In this method, analgesic activity was measured against licking of paw edema induced by formalin in mice in accordance to the methods prescribed previously with some modifications.^{16,17} We used 20 Swiss albino mice and divided them into four equal groups. All the animals were kept hungry for whole the night but water was given *ad libitum*.

After 1 h of treatment, each animal was injected subcutaneously the 25 µl of 2.5% of formalin solution under the surface of the left hind paw and the responses were observed at early phase after 5 min and then at late phase after 20 minutes. The time was noted of licking which was spent by each animal and used for the indication of pain. The first phase is generally known as neurogenic pain phase which was achieved after 5 min of formalin injection while the later phase which was after 20 minutes is known as inflammatory pain phase.¹⁶

Hot plate method

For evaluating the analgesic effects of aqueous methanolic and n-hexane extracts of *C. decapetala*, the hot plate analgesia meter was used according to the methods described previously with some modifications.^{17,18} For the selection of 20 Swiss albino mice, sensitivity test was performed by keeping the animals in hot plate at 55 °C. The animals which started licking and jumping within 5 s were selected because jumping and licking were considered the end point to pain response.

Then animals were divided into four equal groups for this experiment. All animals were kept fasting for whole the night but they had free access to drinking water. After 30, 60, and 90 min of treatments, each animal was kept on hot plate and the time lapse for the mouse to respond to the thermal pain was noted.

Anti-inflammatory effects of *C. decapetala*

Carrageenan-induced paw edema in mice

We investigated the anti-inflammatory effects of aqueous methanolic and n-hexane extracts of *C. decapetala* against carrageenan made paw edema using experimental animals according to the methods described previously.¹⁹ Twenty Swiss albino mice were divided into four equal groups. All the animals were kept on fasting for a night with free access to water. After 1 h treatment, we injected freshly prepared 0.1 ml carrageenan in 0.9% normal saline into the sub planter surface of the right hind paw of each animal. At 0, 1, 2, and 3 h of injection, the linear circumference was noted by the use of vernier caliper. The increase in paw circumference was used as measurement of inflammation.¹⁸

Anti-pyretic activity of *C. decapetala*

Yeast-induced pyrexia

For investigation of anti-pyretic activity of aqueous methanolic and n-hexane extracts of *C. decapetala* against yeast-induced pyrexia in Swiss albino mice, experiment was performed according to the methods described previously.²⁰ Before the start of experiment, 20 mice were divided into four equal groups and noted the initial anal temperature. Before 18 h treatment with the extracts, 15% yeast solution in 0.5% methyl cellulose was injected subcutaneously to induce pyrexia and animals were kept on fasting overnight in maintained conditions with free access to drinking water. Thereafter at 0, 1, 2 and 3 h of treatment, anal temperature was recorded.

Acute toxicity testing in mice

Acute toxicity was measured in mice. The animals received the extracts (n-hexane and methanolic) of *C. decapetala* with doses of 500, 1000, 1500, and 2000 mg/kg body weight and normal saline and measured the mortality for 2 days and their body weight was monitored per day for one week.

Statistical Analysis

The results were represented as mean \pm SD. Statistical

analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey–Kramer multiple comparison test using Graph Pad Prism 5 (Graph Pad, Software Inc., USA). The value of difference was considered significant at $p < 0.05$.

Result

Acetic acid-induced writhing in mice

The reduction in abdominal constriction and stretching of hind limbs induced by acetic acid was achieved with aqueous methanolic extract of *C. decapetala* (18.4 ± 0.53) compared with the control. However, the n-hexane extract did not show profound activity in comparison with the standard as well as aqueous methanolic extract (Table 1).

Formalin-induced licking of paw in mice

Pretreating the experimental animals with extract of plant as well as standard drug aspirin showed the significant reduction of paw licking as compared to control. However, the aqueous methanolic extract of *C. decapetala* (275 ± 4.18) showed more distinct activity than n-hexane extract (293.8 ± 1.20) as shown in Table 2.

Hot plate method

The result of hot plate revealed that the reaction time was significantly increased by treating the animal with extracts as compared to control (Table 3). At 60 min of reaction time, the aqueous methanolic and n-hexane extracts showed similar effects but at 90 min, the aqueous methanolic extract of *C. decapetala* found to be consistent in reducing the pain while, n-hexane extract could not show this effect.

Table 1. Effect of aqueous methanolic and n-hexane extracts of *C. decapetala* on writhing induced by acetic acid in mice

Treatment/dose	No. of writhings [#] Mean \pm SEM
Control (normal saline 2 ml/Kg)	22.6 \pm 0.51
Standard (aspirin 100 mg/kg)	17.6** \pm 0.51
Aqueous methanolic extract of <i>C. decapetala</i>	18.4** \pm 0.53
N-hexane extract of <i>C. decapetala</i>	20** \pm 0.31

[#] Values are Mean \pm SEM. Data was analyzed by one way ANOVA and $p < 0.05$ was considered significant. When data was compared with the control, it showed significant analgesic activity (* $p < 0.05$, ** $p < 0.005$).

Table 2. Effect of aqueous methanolic and n-hexane extracts of *C. decapetala* on formalin-induced paw licking in mice

Treatment/dose	No. of paw lickings [#] Mean \pm SEM
Control (normal saline 2 ml/Kg)	307.8 \pm 5.23
Standard (aspirin 100 mg/kg)	265 ** \pm 5.00
Aqueous methanolic extract of <i>C. decapetala</i>	275** \pm 4.18
N-hexane extract of <i>C. decapetala</i>	293.8* \pm 1.20

[#] Values are Mean \pm SEM. Data was analyzed by one way ANOVA and $p < 0.05$ was considered significant. When data was compared with the control, it showed significant analgesic activity (* $p < 0.05$, ** $p < 0.005$).

Table 3. Effect of aqueous methanolic and n-hexane extracts of *C. decapetala* on pain induced by hot plate method in mice

Treatment/dose	Reaction time [#]			
	0 min	30 min	60 min	90 min
Control	2.04 ± 0.168	1.976 ± 0.164	1.922 ± 0.108	1.97 ± 0.0839
Standard	2.58 ± 0.141	3.03 ± 0.045	3.14* ± 0.022	3.22 ± 0.03
Aqueous methanolic extract of <i>C. decapetala</i>	2.086 ± 0.0314	2.19 ± 0.0409	2.3* ± 0.0328	2.35 ± 0.0198
N-hexane extract of <i>C. decapetala</i>	2.066 ± 0.025	2.13 ± 0.022	2.224* ± 0.029	2.176 ± 0.024

[#] Values are Mean ± SEM. Data was analyzed by two way ANOVA and $p < 0.05$ was considered significant. When data was compared with the control, it showed significant analgesic activity (* $p < 0.05$, ** $p < 0.005$).

Carrageenan-induced paw edema in mice

The anti-inflammatory activity at the dose of 100 mg/kg of extract was evaluated by measuring the average volume of the paw edema at different time intervals. The level of inflammation was increased gradually. The aqueous methanolic extract of *C. decapetala* was found to be little distinct at 2 h of interval which was almost equal to standard drug as well as n-hexane extract (Table 4).

Yeast-induced pyrexia

Pyrexia was induced by yeast and rectal temperature was noted. The result revealed that initially the temperature was high but after treating the experimental animals with extracts and aspirin, significant reduction in rectal temperature was achieved (Table 5). The aqueous methanolic extract of *C. decapetala* was found to be more distinct in comparison with other n-hexane extract of *C. decapetala*.

Acute toxicity testing in mice

The results achieved in measuring the acute toxicity represented no change in weight and/or behavior of the animals (data not shown). No animal was died during the acute toxicity studies.

Discussion

Body defense mechanism, commonly known as inflammation, is a response to many physiological conditions such as infection and thermal and/or physical injuries. Inflammatory response is necessary for the survival against environmental pathogens and harms.²¹ Inflammation is categorized into five cardinal signs which are known as redness, swelling, heat, pain and loss of function.²² Prostaglandins are produced by the cells which are involved in the production of pain, fever and

inflammation. Several enzymes such as cyclooxygenase including COX-1, COX-2, and COX-3 are responsible for the production of prostaglandin. COX-2 is responsible for promoting pain, inflammation and fever by producing the prostaglandin. Hence by inhibiting the cyclooxygenase enzyme, prostaglandin production can be blocked. Non-steroidal anti-inflammatory drugs (NSAIDs) are usually indicated in order to relieve the symptoms. By using the NSAIDs and other opioids, many side effects can occur such as gastric lesion; so the uses of these drugs are not successful. Inflammation plays a crucial role for the pathogenesis of many auto-immune diseases such as diabetes mellitus and many others.²³⁻²⁸ Instead of using traditional anti-inflammatory agents, naturally occurring anti-inflammatory agents such as interleukin-1 receptor antagonist have shown anti-inflammatory activities against various auto-immune diseases.²⁹⁻³⁷ Researchers are also trying to find medicinal plants that lack side and harmful effects. Natural medicine and herbs are considered to have minimum side effects and invite the scientists and researchers to find a natural medicine in order to treat pain, inflammation and pyrexia. Many plants such as *Fragaria vesca*,²¹ *Mimusops elengi* linn,²² and *Melanthera scandens* have recently been reported to possess analgesic, anti-inflammatory and antipyretic activities.³⁸ Preliminary phytochemical studies showed the presence of flavonoids and terpenoids which led to the investigation of analgesic, anti-inflammatory and antipyretic activities of *C. decapetala*.¹² The aqueous methanolic extract of *C. decapetala* exhibited strong analgesic activity. For assessment of analgesic activity, acetic-acid induced abdominal writhings, formalin-induced paw licking and hot plate method were used, (Tables 1-3) respectively. The acetic acid, formalin and hot plate are responsible for the release of endogenous

Table 4. Effect of aqueous methanolic and n-hexane extracts of *C. decapetala* on carrageenan-induced edema in mice

Treatment/dose	Level of inflammation [#]			
	0 h	1 h	2 h	3h
Control	0.52 ± .09	0.74 ± 0.05	0.8 ± 0.03	0.78 ± 0.04
Standard	0.38 ± 0.04	0.54* ± 0.04	0.64** ± 0.04	0.52* ± 0.03
Aqueous methanolic extract of <i>C. decapetala</i>	0.54 ± 0.05	0.64 ± 0.02	0.66* ± 0.06	0.56 ± 0.02
N-hexane extract of <i>C. decapetala</i>	0.58 ± 0.03	0.66 ± 0.05	0.68** ± 0.03	0.58 ± 0.05

[#] Values are Mean ± SEM. Data was analyzed by two way ANOVA and $p < 0.05$ was considered significant. When data was compared with the control, it showed significant anti-inflammatory activity (* $p < 0.05$, ** $p < 0.005$).

Table 5. Effect of aqueous methanolic and n-hexane extracts of *C. decapetala* on yeast-induced pyrexia in mice

Treatment /dose	Rectal Temperature in °C after 18 h of Yeast Injection [#]			
	0 h	1 h	2 h	3 h
Control	41.31 ± 0.18	40.91 ± 0.02	40.47 ± 0.15	39.38 ± 0.16
Standard	40.73 ± 0.22	38.32 ± 0.09	37.67** ± 0.12	37.44* ± 0.08
Aqueous methanolic extract of <i>C. decapetala</i>	40.83 ± 0.15	40.06 ± 0.17	38.81* ± 0.05	37.86* ± 0.02
N-hexane extract of <i>C. decapetala</i>	40.96 ± 0.27	39.32 ± 0.03	39.02 ± 0.21	37.85* ± 0.05

Initial rectal temperature of all groups was 37.5 ± 0.18. [#] Values are Mean ± SEM. Data was analyzed by two way ANOVA and $p < 0.05$ was considered significant. When data was compared with the control, it showed significant analgesic activity (* $p < 0.05$, ** $p < 0.005$).

mediators such as bradykinins, serotonin, histamine, substance P, prostaglandins (PGE2 α , PGF2 α) and some cytokines like TNF- α , IL-1 β , and IL-8.^{39,40}

In carrageenan-induced edema, both extracts of *C. decapetala* were observed (Table 4). The data revealed that at early stage of inflammation, significant effect was noted which might be due to the involvement of histamine, kinins and serotonin release; while later, there was further reduction in paw edema which might be due to the release of prostaglandin. Involvement of flavonoids in the reduction of inflammation is reported.³⁸ Flavonoids have also been found in the extract of *C. decapetala*.¹²

Anti-pyretic activity is a common characteristic of drugs which inhibit the synthesis of prostaglandins. The yeast-induced hyperthermia was employed for the investigation of anti-pyretic activity of *C. decapetala*. The results showed that the aqueous methanolic extract of *C. decapetala* significantly exhibited the anti-pyretic activity as compared to n-hexane extract of *C. decapetala* (Table 5). The anti-pyretic effect of *C. decapetala* was because of inhibition of prostaglandin biosynthesis which is a regulator of body temperature.

Conclusion

To conclude, the results of present study revealed that aqueous methanolic extract of *C. decapetala* represented analgesic, anti-inflammatory and anti-pyretic activities with minimum toxicity. Therefore, our results support the claim of traditional use of *C. decapetala* for the treatment of pain, inflammation and hyperpyretic convulsions. Future studies may focus on the isolation and chemical characterization of phytoconstituents of *C. decapetala* and clarification of their mechanism of action against various disease conditions.

Competing interests

Authors declare no conflict of interests.

Ethical issues

All the procedures involving the animals were in accordance with the approved protocol of the Ethics Committee on animal experimentation of Government College University Faisalabad, Faisalabad, Pakistan.

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