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Original Article

Preliminary phytochemical analysis and in vivo evaluation of antipyretic effects of hydro-methanolic extract of *Cleome scaposa* leaves

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ABSTRACT

Cleome scaposa has been associated with the prevention of many diseases as fever, abdominal complaints and cancer. But its antipyretic effect is not reported so far. The aim of this study was to assess the efficacy of *C. scaposa* in reducing temperature in Baker's yeast-induced fever model of rabbits. Rabbits were randomized into 4 groups (n = 24). Fever was induced in by *Saccharomyces cerevisae* (3 mL/kg of 10% suspension subcutaneous) in all study groups. Afterward, group 1, 2, 3 and 4 were orally administered with paracetamol 150 mg/kg b. wt., distilled water, *C. scaposa* 250 and 500 mg/kg b. wt. respectively. 500 mg/kg dosage was selected after dose fixation study. The standard control was paracetamol. Rectal temperature was recorded with the help of a digital thermometer. ANOVA followed by post hoc test was applied for statistical analysis of results. Results of the study indicate that *C. scaposa* possesses antipyretic activity comparable to that of standard drug paracetamol as it exhibited comparable antipyretic potential against baker's yeast-induced fever in rabbits. This study confirms the traditional use of *C. scaposa* in fever. So, it can be an alternative therapeutic choice in fever. However, specific constituents responsible for its antipyretic activity should be evaluated.

1. Introduction

The relationship between human health and plants exist from fossils history about 60,000 years ago.[1] About 215,000 to 500,000 species of higher plants subsist on earth. But only 6% of plants are being used for the biological activity. Almost 122 compounds have been extracted from 94 species of plants and 80% of these compounds have been used for the same intention or related purpose.[2]

Cleome scaposa DC (Capparaceae) is a useful traditional medicinal plant (weed). Traditionally the leaves of *C. scaposa* are used for their medicinal properties like analgesic, anti-inflammatory and anti-pyretic.[3,4] *C. scaposa* is also used to treat different abdominal complaints and cancers.[5,6] It is distributed as a common weed in Arabia, Egypt, India, Pakistan and throughout the tropics of the world. It is an annual, 10–30 cm tall weed. Flowers are 3–4 mm across, actinomorphic, white turning yellowish sometimes pinkish, leaves simple, capsule linear, 20–30 mm long, seeds about 0.6 mm in diameter and brown-black.[7]

The plant *C. scaposa* has several pharmacological activities and shows analgesic, anti-inflammatory and antiemetic potential.[7] Hence, present study was undertaken to evaluate the antipyretic potential of hydro-methanolic extract of *C. scaposa* leaves against baker's yeast-induced fever in rabbits as the drugs having anti-inflammatory activity can show antipyretic activity too.

2. Materials and methods

2.1. Animals used

Local strain, healthy male and female adult rabbits (1–1.5 kg) were used for the study and kept in departmental animal house, The Islamia University of Bahawalpur, Pakistan. Prior to study, rabbits were acclimatized in a controlled temperature of 22–25 °C and light/dark cycles for 12/12 h for one week. Animals were kept

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on standard diet and water *ad libitum*. They were fasted overnight before study however water was given in free access. The experiment was conducted in accordance with the guidelines 'Committee for the Purpose of Control and Supervision' and ethical approval was obtained from Pharmacy Research Ethics Committee (PREC) of Faculty of Pharmacy, The Islamia University of Bahawalpur, Pakistan (Approval No.: 88-2015/PREC).

2.2. Drugs, reagents and apparatus used

Paracetamol GlaxoSmithKline, Pakistan, Baker yeast (Rossmoor food products, Karachi, Pakistan), Normal Saline (Shazeb Pharmaceutical Industries Limited District Haripur, Pakistan), Distilled water (Faculty of Pharmacy and alternative medicine, IUB, Pakistan), Digital Thermometer (Medisign MANA & CO Pakistan).

2.3. Plant material

The leaves of *C. scaposa* were collected from Lal Suhanra, National Park Bahawalpur, Pakistan during the month of July 2015 and authenticated by the botanist, Dr. Sarwar, Lecturer Botany department, The Islamia University of Bahawalpur, Pakistan. The voucher specimen (2212/L.S) was deposited in Botany department, The Islamia University of Bahawalpur, Pakistan.

2.3.1. Preparation of plant extract

The leaves were dried in shade at room temperature for 7 days. The dried leaves were powdered with the help of grinder and powdered material was then subjected to maceration employing 70% methanol as solvent. A fine powder (1000 g) was soaked in 70% methanol (2 L) for 2 weeks with occasional shaking. The soaked material was filtered through muslin cloth. The process was repeated 3 times for collection of maximum contents.[8] The solvent was evaporated by using rotary evaporator. The residue was kept at 4 °C in refrigerator and used for antipyretic testing.

2.3.2. Phytochemical screening

The hydro-methanol extract was subjected to standard phytochemical screening for alkaloids (Dragondroff and Mayer's test), flavonoids (sodium hydroxide, ferric chloride and lead acetate test), saponins (foam test), tannins (ferric chloride test) and phenols (Ferric chloride test).

2.4. Antipyretic activity

The rabbits were randomly divided into four groups each consisting of six animals (n = 6). All the groups were first treated with baker yeast (*Saccharomyces cerevisiae*) (3 mL/kg of 10% suspension subcutaneous) for fever induction.[9,10] After 4 h of yeast administration, Group I animals were treated with distilled water and served as negative control. Group II animals were treated with paracetamol by oral administration and taken as positive control. Group III and IV animals were treated with plant extract 250 and 500 mg/kg body weight respectively. The dose of *C. scaposa* was selected by an effective dose fixation study method with slight modification.[11] Rectal temperature was measured with digital thermometer (Medisign MANA & CO Pakistan) coated with glycerin (as a lubricant). After baker yeast injection, rectal temperature was recorded one hourly. The animals showed rise in temperature of 0.5–1 °C during 4th h were included in study. After 4 h of yeast injection, all the tested samples were administered orally with the help of syringe. After medicine administration, rectal temperature was recorded one hourly for 6 h.

2.5. Statistical analysis

Results were expressed as the mean ± S.E.M (Standard error of mean). The data was analyzed by one-way analysis of variance (ANOVA) followed by LSD (Least significant difference) post hoc test. SPSS software version 20.0 was used for analysis. $p \leq 0.05$ was considered statistically significant.

3. Results

The preliminary phytochemical screening of the hydro-methanol extract of *C. scaposa* leaves showed positive test for alkaloids, flavonoids, saponins, tannins and phenols. Dose fixation studies showed non-toxic nature of *C. scaposa* leaves in the dose range between 1 and 5 g/kg body weight in normal rabbits. There were no mortality or side effects in the rabbits treated with the different dosages of extracts. 500 mg/kg body weight was selected as the highest dose (1/10th of highest dose of 5 g/kg) for antipyretic activity. The animals were also treated with 250 mg/kg body weight of *C. scaposa*.

After 4 h of *S. cerevisiae* administration, ≥ 0.5 °C increase in rectal temperature was observed in all the rabbits. Table 1 showed that maximum temperature in negative control group was attained during 6th to 7th h of *S. cerevisiae* administration.

The administration of hydro-methanol extract of *C. scaposa* (250 mg/kg and 500 mg/kg) and paracetamol reduced the rectal temperature significantly. The effects of extract at a dosage of 500 mg/kg were almost similar to that of standard drug paracetamol. The extract dose 250 mg/kg also reduced the temperature but the effect was slow and less significant as compared to higher dose. In all the groups except negative control, temperature became normal during 6 h of study (Table 1).

4. Discussion

Fever is part of the defense response known as the "acute phase reaction", that occur during inflammatory processes of several origins. In current study, baker's yeast-induced fever model of rabbits was effectively established after 4 h of *S. cerevisiae* administration. Previous studies had indicated that *S. cerevisiae*[12] and constituents of its cell wall, such as mannans, cause fever associated with an increase in the plasma levels of TNFα, IL-1b and interferon-c.[13] The innate immune cells (dendritic cells, macrophages, neutrophils,

Table 1
Antipyretic activity of leaves of *Cleome scaposa* hydromethanolic extract in rabbits.

Groups	Before treatment (Temperature °C)		After treatment (Temperature °C)					
	0 h	4 h	1st h	2nd h	3rd h	4th h	5th h	6th h^
Paracetamol 150 mg/kg	38.8 ± 0.2	39.7 ± 0.1	38.7 ± 0.2$^{¥}$	38.5 ± 0.3$^{¥}$	38.4 ± 0.3^{Ψ}	38.2 ± 0.5^{Ψ}	38.5 ± 0.4$^{¥}$	38.5 ± 0.1$^{¥}$
Negative control	38.8 ± 0.5	39.4 ± 0.4	39.5 ± 0.9	39.8 ± 0.4	39.8 ± 0.5	39.7 ± 0.4	39.6 ± 0.4	39.2 ± 0.4
C. scaposa 500 mg/kg	38.7 ± 0.4	39.4 ± 0.5	38.7 ± 0.2$^{¥}$	38.6 ± 0.1$^{¥}$	38.6 ± 0.1$^{¥}$	38.6 ± 0.2$^{¥}$	38.7 ± 0.2*	38.7 ± 0.2*
C. scaposa 250 mg/kg	38.9 ± 0.9	39.6 ± 0.3	39.1 ± 0.6	39 ± 0.4*	39 ± 0.5*	38.8 ± 0.6*	39 ± 0.5*	38.9 ± 0.5*

Note: ^ h – hour.
$^{*}p \leq 0.05$, $^{¥}p \leq 0.01$, $^{Ψ}p \leq 0.001$ compared to control. Values are expressed as mean ± S.E.M N = 6, 0 h reading is normal temperature of rabbits before yeast induction, 'before treatment' 4th hour reading is after fever induction reading. 1st to 6th h readings are after medicine administration.

and lymphocytes) recognize pathogen and produce inflammatory cytokines such as IL-1b and TNFα. These soluble mediators coordinate the local and systemic inflammatory response against microbial products, increase vascular permeability and migrate immune cells to the infected area. Through the vagal and humoral pathways, cytokines signal to the brain and cause PGE2 production and fever.[14] The data is supported by the findings of Okawa et al.[15]

In this study, orally administered paracetamol at 150 mg/kg significantly attenuated baker's yeast-induced fever in rabbits. Our study results are matching to other studies that have also shown reduction of temperature in rabbits by paracetamol at same dose.[10,16] Antipyretics and non-steroidal anti-inflammatory drugs (NSAIDS) reduce temperature by inflammation reduction in the peripheral and CNS thermoregulatory sites.[17]

In current study, *C. scaposa* hydro-methanolic leaf extract reduced baker's yeast-induced fever in rabbits significantly. Preliminary phytochemical study indicated that extract contains alkaloids, flavonoids, tannins and phenolic compounds. The presence of these bioactive compounds may be responsible for the antipyretic activity of this extract as flavonoids like baicilin have antipyretic effect by suppression of TNFα[18] and alkaloids like bolidine have the ability to reduce the elevated temperature by inhibition of prostaglandin E2 synthesis.[19]

Cleome species have various types of flavonoids such as kaempferol 7-rhamnoside, quercetin 3-rutinoside, quercetin 7-rhamnoside, apigenin[20] and pinocembrin.[21] A study reported the inhibitory effects of kaempferol and quercetin against fever causing inflammatory mediators.[22] Moreover, pinocembrin has shown the reduction of TNFα, IL-6, and IL-1β in lipopolysaccharide (LPS)-induced endotoxemia.[23] It could be assumed that *C. scaposa* has antipyretic effect due to presence of kaempferol, quercetin and pinocembrin that inhibit inflammatory mediators and pyrogenic cytokines. Although the actual mechanism of antipyretic activity of the bioactive composites is yet to be proven. The report made by Hämäläinen M and coauthors that anti-inflammatory activity of flavonoids (kaempferol and quercetin) occur through the inhibitory effect on iNOS expression and NO production in activated macrophages[22] could be used to support our findings. Moreover, triterpenoids are also present in *Cleome* species like brachycarpone, cabralealactone, deacetoxybrachycarpone and ursolic acid.[7] In vivo anti-inflammatory potential of ursolic acid[24] and *C. scaposa*[7] also intimates that the antipyretic action of extract may be due to the inhibition of prostaglandin synthesis.

5. Conclusion

It is concluded that hydro-methanolic extract of *C. scaposa* leaves has significant antipyretic activity. So, traditional use of *C. scaposa* in fever is supported by this study and it would encourage its use in fever with greater degree of assurance of its efficacy. However, in future specific constituents of extract with antipyretic activity should be evaluated.

Conflict of interest

Authors declare that they have no conflict of interest.

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