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Raphanus sativus L. Var. *caudatus* as an Analgesic and Antipyretic Agent in Animal Models

Ishrat Younus[1,2] and Afshan Siddiq[1]*

[1]*Department of Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi, Karachi*
[2]*Department of Pharmacology, Faculty of Pharmacy, Hamdard University, Karachi.*

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IY carried out research, compiled the data and wrote manuscript. AS designed the study, supervised and reviewed the manuscript.

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ABSTRACT

Raphanus sativus L. Var. *caudatus* belongs to Radish plant has been reported for analgesic potential thus it might have antipyretic potential. The present investigation was undertaken to evaluate analgesic and antipyretic activities of ethanol extract of *Raphanus sativus* var. *caudatus* in albino mice. The analgesic and antipyretic activities were determined at three different doses (50, 100 and 200 mg/kg) using different pain models (writhing induced, tail flick) and yeast induced pyrexia tests, respectively. The extract exhibited significant ($p < 0.05$) *in vivo* analgesic and antipyretic potential in term of % writhes inhibition, increased latency time and reduced rectal temperature when compared with control. The extract displayed noteworthy analgesic and antipyretic capabilities especially at the dose of 200 mg/kg that are almost comparable with standard drug (aspirin). Although the finding of present investigation highlighted the medicinal importance of the plant but there is dire need to carry out the fractional extraction of crude extract with different solvents based on polarity to identify the active constituents with exact analgesic and antipyretic mechanistic action of *Raphanus caudatus* as well as clinical trials are required in future for medicinal use of the plant in humans.

INTRODUCTION

Raphanis sativus var. *caudatus*, commonly known as rat-tailed radish is an important healthy food crop belongs to Brassicaceae family. Member of this family are enriched in isothiocyanates, polyphenols and vitamins and considered to be chemopreventive and health promoting agents (Sangthong *et al*., 2014).

R. sativus commonly called radish have been known since long for prevention and treatment of various diseases. All the parts of plant have been reported to possess different pharmacological activities (Gutiérrez and Perez, 2004). The fresh extract of leaves possesses diuretic and purgative properties, roots are utilized for urinary ailments, gastric problems, hemorrhoids and different gastric illnesses. The seeds of this herb have been reported for expectorant, purgative, digestive, diuretic, and carminative effects (Magesh *et al*., 2016).

Pain is basic factor that influences individuals worldwide consistently. Pain can emerge from damage or regular events like sprain or acidity (Pinky *et al*., 2015). Pain is an inclination activated in the sensory system.

Over the counter medications such as ibuprofen or paracetamol have unfavorable symptoms like gastric ulceration and hepatotoxicity. Thus there is dire need to identify new medicines of enhanced efficacy and minimum side effects (Sultana *et al*., 2014).

The physiological action for analgesics was proposed by a few scientists that they restrain prostaglandin combination in the central nervous system to interfere with nociceptive receptors in the cerebrum; others have suggested that they trigger specifically on nociceptive neural pathways to develop analgesic effect. This class of medications additionally has antipyretic action (Shimada *et al*., 1994).

Fever is a condition of raised core temperature, which is usually, part of the defensive mechanism of living beings. The agents used to decrease the body temperature are called as antipyretic agents. People have been using such compounds for more than two centuries. Antiquated Assyrian, Egyptian, and Greek doctors all evidently worked for assessing medicinal plants and their antipyretic properties (Plaisance and Mackowiak, 2000).

Radish has been reported for analgesic potential (Mikaili *et al*., 2010). Historically, it was used for treating the pain of migraine (Wu *et al*., 2014). A study evaluated that the crude extract of *Raphanus sativus* leaves showed actions to relieve spasm in a dose dependent manner.

* Corresponding author: afshanpharma@gmail.com
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They have significantly produced evidence for its activity against constipation and relieving spasm for having analgesic activity (Gilani and Ghayur, 2004).

Raphanus was found to be rich in saturated fatty acids and eicosenoic acids, thus, supposed to have some correlation towards analgesic and antipyretic activity (Mandal *et al*., 2002).

Hence the present study was undertaken to evaluate the analgesic and antipyretic actions of *R. sativus* L. var *caudatus*. The study was carried out first time on this variety of radish so it will add new information regarding pharmacological potential of plant.

MATERIALS AND METHODS

The study was conducted at Department of Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences in the months of September to November 2016.

Collection and extraction of plant material

The pods of *Raphanus sativus* L. were purchased from local market of Karachi identified from Herbarium, Department of Botany, University of Karachi. The plant material was air dried and subjected to grinding to make powder. The powdered plant material of *R. sativus* L. var. *caudatus* was subjected to extraction with ethanol using Soxhlet apparatus. Then the extract was subjected to rotary evaporator (Buchi) to obtain semi solid mass and then air dried.

Animal selection

Before the experiments the animals were placed in separate cages for 7 days. For evaluation of antipyretic and analgesic activity, total of 60 albino mice (25±2g) of either sex were used. Standard sized cages made up of iron were employed for animals housing at Animal House, Department of Pharmacology, University of Karachi. The animals were housed at at 25±1°C on 12/12 h light and dark cycle. All animals were allowed food and water *ad libitum*. University Board of Advanced Studies and Research approved this study with reference # 02419/Pharm.

Protocol of the experimental study

The protocol of the current study was designed on the basis of pilot study. Mice were divided into five groups (I, II, III, IV and V) of six animals in each group. Group I and II were kept as control and standard groups, administered with normal saline and aspirin (100 mg/kg), respectively. Group III, IV and V were set as treated groups, administered orally with ethanolic extract of *R. caudatus* in doses of 50 mg/kg, 100 mg/kg and 200 mg/kg, respectively (Ahmed *et al*., 2015).

The animals were subjected to following different tests to evaluate analgesic and antipyretic activities.

Analgesic activity

The anti-nociceptive activities of ethanolic extract of *R. caudatus* were carried out in albino mice by using acetic acid-induced writhing and tail flick methods, respectively.

For acetic acid-induced writhing test the anti-nociceptive activity of the ethanolic extract of *R. caudatus* was evaluated in five groups of adult albino mice (Koster, 1959). Each group contained six animals. Group I and II were kept on normal saline and aspirin (100 mg/kg), respectively. The mice of III, IV, and V groups (six animals each) were orally given the tested extract in three doses; 50, 100 and 200 mg/kg, respectively. Writhing was induced 30 min later by intraperitoneal injection of 0.6% v/v acetic acid solutionat a dose of 10 ml/kg. After the injection of acetic acid, numbers of writhes (abdominal contractions) in all animals were calculated immediately for 10 min. Analgesic activity interm of percent inhibition of writhing was calculated as follows (Ahmad *et al*., 2015).

$$\% \text{ Inhibition} = \frac{A - B}{A} \times 100$$

Where A is average number of writhing of control per group, and B is average number of writhing of test per group.

For tail flick test in mice Luiz *et al*. (1988) method was used to determine analgesic activity as the reaction time or latency period (flick the tail out of the hot water). The reaction time was measured by immersing the distal part of tail (3.5cm) into hot water bath maintained temperature at 51±0.5ºC (Luiz *et al*., 1988). This reaction time considered as zero reading. The reaction time in seconds also determined at a time interval of 30, 60, 90, 120, 150 min. All the experiments were carried out in triplicates. The data obtained were analyzed statistically to determine the level of significance.

Antipyretic activity

On the testing day food was withdrawn and animals were habituated to test condition for 2 h. Temperature was noted by utilizing calibrated thermometers in to the rectum of animals. The baseline temperature was set as 39.2 ºC to 39.8 ºC for any animal to be employed in to the test. After taking the rectal temperature at 0 h, the animals were injected subcutaneously with 20% w/v aqueous suspension of brewer's yeast (1 ml / kg) (Vaz *et al*., 1996) and the animals with elevated temperatures up to 0.5ºC preferred for the study. The standard group was given Asprin and test groups were given plant extracts of different stated doses at 0 h time. The rectal temperatures of all mice were recorded at 1 h interval up to 05 hs after yeast injection.

Statistical analysis

All the results were represented as mean ± SEM and analyzed by applying SPSS- 20 using one-way analysis of variance (ANOVA) followed by post hoc Tukey HSD. The significant cut –off value was considered at $p < 0.05$.

RESULTS

Writhing induced test

Ethanolic extract of *R. caudatus* showed notable peripheral anti-nociceptive activity in acetic acid-induced writhing test in mice when compared with standard drug (aspirin). The results of analgesic activity of *R. caudatus* are tabulated in Table I. By increasing the dose (200 mg/kg), the antinociceptive efficacy was observed to be enhanced. It is evident that the means of increasing treatments were apparently lowered.

Tail flick test

In case of heat induced pain model, ethanolic extract of *R. caudatus* exhibited significant increase ($p < 0.05$) in reaction time when compared with control in mice showing central analgesic effect. The highest anti-nociceptive activity was observed at 150 minute after the treatment. The extract showed significant anti-nociceptive activity at the dose of 200 mg/kg and remarkable ($p < 0.05$) analgesic effect was observed as compared to other groups (Fig. 1).

Yeast-induced pyrexia

In the present investigation, ethanolic extract of *R. caudaus* produced significant ($p < 0.05$) reduction in body temperature of yeast induced mice at different tested doses. it is noted that the means of increasing treatments were apparently lowered. To test each treatment to others, post hoc Tukey HSD was used. It was observed by post hoc analysis that by increasing the dose, anti-pyretic effects were potentiated (Table I).

DISCUSSION

Non-steroidal anti-inflammatory drugs (NSAIDs) are among very popular medication especially for analgesic and anti-inflammatory properties. In spite of these benefits, NSAIDs are also responsible for serious adverse effectsincluding gastrointestinal and cardiovascular problems (Sostres *et al*., 2010).Thus current studies were focused to identify plant derived pharmacologically active compounds to overcome such type of problems.

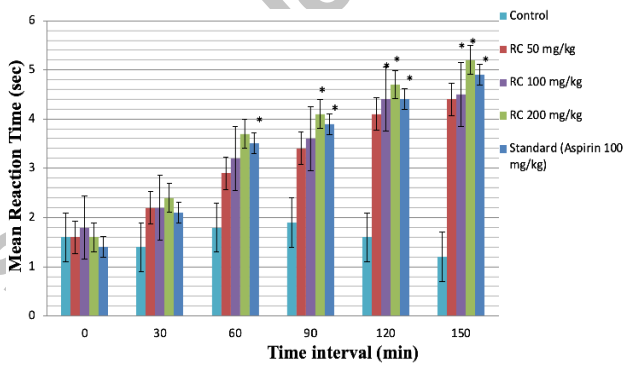

Fig. 1. Analgesic effect of ethanolic extract of *Raphanus caudatus* and aspirin in tail flick test in mice.
RC, *Raphanus caudatus;* n=6; One-Way ANOVA followed by post hoc Tukey HSD; *p < 0.05 significant when compared to control.

Table I. Analgesic effect and antipyretic activity of ethanolic extracts of *Raphanus caudatus* (RC) and aspirin on acetic acid induced writhing test and yeast-induced pyrexia, respectively in mice.

	Control (n=6)	RC 50 mg/kg (n=6)	RC 100 mg/kg (n=6)	RC 200 mg/kg (n=6)	Aspirin 100 mg/kg (n=6)
No. of writhing (Mean±SEM)	20.50±1.22	16.16±0.47	11.22±0.27*	7.83±0.29*	8.35±0.44*
% inhibition	0.00	21.17	45.26*	61.80*	59.26*
Rectal temperature (ºC) (Mean±SEM)					
After yeast injection at 0h	39.43±0.04	39.76±0.09	39.22±0.14	39.06±0.03	39.17±0.09
After drug administration					
1h	39.22±0.23	39.59±0.14	39.06±0.29	38.73±0.19	38.77±0.14
2h	39.15±0.23	37.61±0.05	38.75±0.15	38.56±0.22*	38.59±0.05*
3h	39.30±0.21	37.33±0.12	38.30±0.04*	37.82±0.08*	38.26±0.12*
4h	39.23±0.10	37.06±0.20	38.02±0.28*	37.66±0.33*	38.07±0.20*
5h	39.07±0.14	39.90±0.28	37.92±0.24*	37.42±0.27*	37.77±0.28*

RC, *Raphanus caudatus;* n=6; One-Way ANOVA followed by post hoc Tukey HSD; *p < 0.05 significant when compared to control.

The present study indicated *R. caudatus* as analgesic and antipyretic agent for the first time so it has added new information regarding this pharmacological potential of plant.

Brassicaceae is a plant family of great significance. The plants of this family have been reported for variety of activities. *R. sativus* L. originally belongs to Asia and Europe (Gutiérrez and Perez, 2004). *R. sativus* L. var. *caudatus*are actually pods of *R. sativus*. The vernacular name in Pakistan is "Mungra" and rat-tailed radish in English. This edible part of radish is available in Pakistan between the months of November and March and is cooked in the form of delicious dishes (Khare, 2007).

Already different plants have been reported to possess analgesic and antipyretic activities (Almeida *et al*., 2001; Khattak *et al*., 1985).The results of present study showed significant antipyretic and analgesic potential of *R. caudatus*.

To evaluate central and peripheral analgesic activity, two tests were utilized i.e. acetic acid induced writhing test and tail flick test, respectively. The ethanolic extract of *R. caudatus* exhibited notable activities. The current study is supported by an earlier study that indicated radish seeds in prevention of pain after hemorrhoid surgery (WU *et al*., 2010). Similarly methanolic extract of aerial parts of *Raphanus sativus* var. *hortensis* indicated analgesic potential (Sharmin and Mohammed, 2014).

The acetic acid and tail flick latency time are responsible to release prostaglandins and other endogenous substances from the nerve endings and abdominal constrictions (writhes) are produced due to interaction of prostagalndin to pain receptors (Bentley *et al*., 1983). Thus analgesic activity in the present writhing test might be attributed to *R. caudatus* involvement in prostaglandin pathway.

Regarding antipyretic activity evaluation, ethanol extract of *R. caudatus* showed noteworthy activity in Yeast induced pyrexia model and it is comparable with standard antipyretic drug (Asprin). Nevertheless the extract showed antpyretic activity in dose dependant manner. Prostaglandins have been known since long for their role in the pyrexia (Milton, 1982; Sugita *et al*., 2016; Engström *et al*., 2012). So it is obvious that inhibition in prostaglandin pathway could lead to antipyretic potential. Flavonoids are plant derived compounds (also present in *R. caudatus*) reported to inhibit prostaglandins (Ferrandiz and Alcaraz, 1991; Baumann *et al*., 1980). Thus the occurrence of flavonoids in *R. caudatus* could be linked to its antipyretic activity in yeast induced pyrexia method. With regard to antipyretic potential of radish limited research data is available but previously different plants showed antipyretic effects (Ikram *et al*., 1987; Awaad *et al*., 2011; Muhammad *et al*., 2012; Ajaz *et al*., 2019; Vinod and Malgi, 2019)

Isothiocyanates, sulphur containing compounds, have been reported in the plants of the Brassicaceae family (Bednarek, 2012). These compounds are present in these plants as glucose inolatesprecursors. GC–MS analysis of *R. caudatus* in aprevious study had revealed the presence of two isothiocyanates compounds named sulforaphane and sulforaphene (Pocasap *et al*., 2013). On the other hand, phenolic compounds have also been reported in *R. caudatus* (Beevi *et al*., 2012; Takaya *et al*., 2003). Thus the presence of these constituents may be responsible for observed analgesic and anti-pyretic activity of *R. caudatus* in the present study.

Thus the present study identified *R. caudatus* as analgesic and antipyretic agent. The results recommend use of this plant in pain and pyrexia. Moreover, the study is limited because of animals thus it should be replicated on large number of animals and also in humans to develop this plant as useful medicines in such ailments.

CONCLUSION

Conclusively, the present study reportedin vivo analgesic and anti-pyretic effects of ethanolic extract of *Raphanus sativus* L. var. *caudatus*. In future, there is dire need to carry out the fractional extraction ofethanolicextract with different solvents based on polarity to identify the active constituents with exact analgesic and antipyretic mechanistic action of *R. caudatus*.

This study discovered the analgesic and antipyretic pharmacological potential of *R. caudatus* that can be beneficial in pains and pyrexia conditions. This study will help the researcher to uncover the pharmacological potential and possible mechanism of action. Thus, a new theory of pharmacological actions of this radish variety may be arrived at.

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Statement of conflict of interest

The authors have declared no conflict of interest.

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