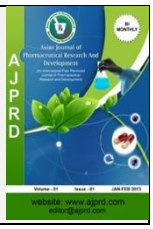
Available online on 15.08.2020 at http://ajprd.com

Asian Journal of Pharmaceutical Research and Development

Open Access to Pharmaceutical and Medical Research



Open Access Research Article

Assess the Anti-Inflammatory and Analgesic Activity of Leaves of *Raphanus Sativus* – An *In Vivo* Design

Devi Manisha, Karnati Naga Rashi, Kapilavai Harini, Valluri Swarna, Narender Boggula, Vasudha Bakshi, Mohammed Sayeed*

School of Pharmacy, Anurag University, Venkatapur, Ghatkesar, Telangana, India.

A B S T R A C T

Background: Natural products found from plants have been conveying a vital role among human being since ancient times. An enormous number of scientific reports evidenced of using the medicinal plants as natural remedies. Nowadays, the medicinal plant is using as an alternative medicine to synthetic drugs. The use of medicinal plants singly or in combination in treating different ailments has been practiced by traditional medical healers for a very long time. People of Mesoamerica have utilized plants and products from plants in curing and relieving ailments for centuries.

Aim: The present aim of the study is to assess the anti-inflammatory and analgesic activity of Ethanolic Extract of leaves of *Raphanus sativus* (EERS) on animal models and therefore to determine the scientific basis for its use in traditional medicine in the management of anti-inflammatory condition.

Methods: Anti-inflammatory activity was evaluated using carrageenan and histamine-induced paw edema methods. Analgesic activity of Ethanolic Extract of leaves of *Raphanus sativus* (EERS) was estimated against a hot plate, acetic acid induced writhing and formalin tests.

Results: The extracts showed significant anti-inflammatory and analgesic activities with a dose-dependent manner. Anti-inflammatory activity of olive tree extract at 250 and 500mg/kg doses was more important compared to the used standard drugs (p<0.05), in both carrageenan and histamine-induced paw edema tests. In analgesic assays, results showed that 500mg/kg dose of olive tree extract has a significant analgesic effect through both peripheral and central mechanisms.

Conclusion: This study provided evidence on the traditionally claimed uses of the plant in pain and inflammatory diseases, and *Raphanus sativus* could be potential source for development of new analgesic and anti-inflammatory drugs.

Key words: *Raphanus sativus*, anti-inflammatory activity, Carrageenan-induced rat paw oedema, Hot plate test, writhing test.

A R T I C L E I N F O: Received 2020; Review Completed 2020; Accepted 2020; Available online 2020

Cite this article as:

Devi M, Karnati N R, Kapilavai H, Valluri S, Narender B, Vasudha B, Mohammed S, Assess the Anti-Inflammatory and Analgesic Activity of Leaves of Raphanus Sativus – An In Vivo Design, Asian Journal of Pharmaceutical Research and Development. 2020; 8(5):37-44. **DOI:** http://dx.doi.org/10.22270/ajprd.v8i5.840

***Address for Correspondence:**

Mohammed Sayeed,Assoc. Professor,Department Of Pharmacology,School Of Pharmacy, Anurag University,Venkatapur, Ghatkesar, Medchal, Hyderabad, Telangana, INDIA-500088.

INTRODUCTION

Plants are one of the important sources of medicine. The application of plants as medicine dates back to prehistoric period. Several indigenous drugs used in modern medicine have figured in ancient manuscripts such as Rigveda, The Bible, and Quran. Over six thousand years ago, the ancient Chinese were the first to use the natural vegetation as medicine[1]. In India, the Ayurvedic system of medicine has been in use for over three thousand years. Hippocrates, the 'Father of Medicine' was the first to give a scientific explanation of diseases. Indian system's of medicine includes Ayurveda, Siddha, Unani, Tibetan and Naturopathy. Herbal therapy provides rational means for the treatment of many internal diseases which are considered to be obstinate and incurable in other systems of medicine. It aims at both the prevention and cure of diseases[2].

Man has been using herbs and plant products for combating diseases since times immemorial. The Indian subcontinent is enriched by a variety of flora-both aromatic and medicinal plants. This is used in a wide diversity of climatic conditions in India ranging from deserts to swamp lands. Numerous types of herbs have been well recognized and catalogued by botanists from the high ranges of the Himalayan tract up to the sea shore Kanyakumari. This extensive flora has been greatly utilized as a source of many drugs in the Indian traditional system of medicine[3]. In India, the earliest mention of the use of medicinal plants was found in the Rigveda which was written between 4500-1600 BCE. A detailed account of the world's first symposium on medicinal plants is given the first chapter of Brihat Samhita and since 1600 BCE the amount of literature on the subject is boundless. The traditional system of medicine is so engrained in our culture that, even now, 75% of the Indian population depend on this indigenous system for relief. With such a huge section of an ever-increasing population relying on herbal remedies, it is imperative that the plant products which have been in use for such a long time be scientifically supported for their efficacy[4,5].

Raphanus sativus L. belongs to the family Brassicaceae (Cruciferae, an older name). It is known as Radish in English, Daikon in Japanese, and "Laifu" or "Luobo" in Chinese. With its high adaptive ability, high yield, and abundant nutritional value, *Raphanus sativus* L. has long been grown as a food crop worldwide, especially in China, Japan, Korea, and Southeast Asia. The leaf, seed, and root of *Raphanus sativus* L. are claimed to have various medicinal uses[6]. The major active compounds in Raphani Semen are alkaloids, glucosinolates, brassinosteroids, and flavonoids. Fatty acids are its main nutritional contents. Raphani Semen has been demonstrated to have beneficial effects on hypertension, obesity, diabetes mellitus, constipation, and cough. So far, there is no report about the adverse/toxic effects of this herb on humans. In Unani, Greeko-Arab, and Indian folk medicine, radish is used as a household remedy for the treatment of many diseases such as jaundice, gallstone, liver diseases, rectal prolapse, indigestion, and other gastric pains. In general, radish contains carbohydrates, sugars, dietary fibers, protein, and even some fat and fluoride[7,8].

Radish (*Raphanus sativus* L.) is the one of the most important root crops belonging to the family Cruciferae. It is grown both in tropical and temperate regions of the world and is probably a native of Europe and Asia. Radish is grown for its edible young, tender and fusiform roots which are eaten raw as salad or cooked as vegetable along with leaves and also stuffed in paranthas. Besides this, its immature pods usually called 'mougree' are either eaten raw or cooked as vegetable, alone or mixed with other vegetables. It is a good source of vitamin-C (ascorbic acid) containing 34-40mg per 100g of edible portion and supplies a variety of minerals. Trace elements in radish include aluminium, barium, lithium, manganese, silicon, titanium, fluorine and iodine (up to 18µg/100g). Roots are also rich in carbohydrate and protein. Radish is useful in the treatment of liver, gall bladder troubles, urinary complaints and piles etc. In Homeopathy, it is used for neurological disorders, headache, sleeplessness and chronic diarrhoea[9-11].

Based upon the climatic conditions, radish has been divided into two groups - 'Asiatic' or 'Tropical' and 'European' or 'Temperate' types. The Asiatic type produce roots and seeds both under tropical and temperate climate. European types produce roots under both the climatic conditions but its seed can only be produced in hills or temperate climate. In India, radish is grown in one and other parts of the country throughout year, occupying an area of 1,77,000 hectares and producing 25,40,000 MT roots. Keeping in view the increasing Indian population and decreasing cultivated crop area, there is a need to enhance the production as well as productivity to meet out the vegetable requirement of the country. Production of any crop can be increased by supplying quality inputs and seed is the most important input in any crop production programme. Without healthy and good quality seed, all expenditure incurred on other inputs go waste. Good quality seed is also one of the most important criteria to increase productivity[12-14].

Figure: 1 *Raphanus sativus*

Figure: 2 *Raphanus sativus* leaves

Broadly speaking, radishes can be categorized into four main types according to the seasons they are grown in and a variety of shapes lengths, colours, and sizes, such as red, pink, white, gray-black or yellow radishes, with round or elongated roots that can grow longer than a parsnip[15]. Algesia (pain) is an ill-defined, unpleasant sensory and emotional experience associated with actual or potential tissue damage, which varies from person to person and in

the same person, from time to time. Unrelieved acute pain can cause chronic pain and long standing pain can cause anatomical and even genetic changes in the nervous system. Pain is warning signal, primarily protective in nature, but causes discomfort and suffering. Excessive pain may produce other effects such as sinking sensation, apprehension, sweating, nausea, palpitation, and rise or fall in BP, tachypnoea[16,17].

The present aim of the study is to screen the anti-inflammatory and analgesic activity of Ethanolic Extract of leaves of *Raphanus sativus* (EERS) in Wistar rats.

MATERIALS AND METHODS

Ethical approval

This experiment was approved by the Institutional Animal Ethical Committee (IAEC) (I/IAEC/AGI/008/2019 WR ♂), School of Pharmacy, Anurag University, Venkatapur, Ghatkesar, Telangana.

Study area

The investigation was conducted at Pharmacology Laboratory, Department of Pharmacology, School of Pharmacy, Anurag University, Venkatapur, Ghatkesar, Telangana.

Plant material

The freshly *Raphanus sativus* (radish) leaves were collected from local vegetable market, Koti, Hyderabad, Telangana.

Animals

For studying the *in-vivo* activities, male adult Wistar rats (120-180g) and Swiss albino mice (20-25g) of both sexes were obtained from the animal house. They were housed in polypropylene cages with free access to food and water. The animals were maintained under controlled conditions of temperature (22 ± 2 ^{0}C) with a 12 h light-dark cycle. The animals were used after an acclimatization period of 7 d in the laboratory environment. Housing conditions and *in-vivo* experiments were approved according to the guidelines. The experiment protocol was approved by an Institutional Animal Ethical Committee (IAEC) and care of the animals was taken as per guidance of the Committee for the Process of Control and Supervision of Experiments on Animals (CPCSEA).

Chemicals

Carrageenan; histamine; indomethacin; diclofenac sodium (DFS); acetylsalicylic acid (ASA); tramadol hydrochloride (Tramadol Hcl); formalin; acetic acid were purchased from Sigma-Aldrich.

Processing and extraction[18]

Leaves of *Raphanus sativus* was under the shade air dried for 4 weeks and mechanically pounded into fine particles using electronic grinder. About 500g of the pounded plant materials were weighed and extracted by soxhlation for 72 h in absolute ethanol. The extract was then filtered, evaporated to dryness, and stored in capped bottles inside the refrigerator at 4 ^{0}C until required.

Experimental Design:

In-vivo anti-inflammatory activity

Carrageenan-induced rat paw oedema[19]:

Ethanolic extract of leaves of *Raphanus sativus* (EERS) anti-inflammatory activity was evaluated using carrageenan-induced paw oedema in rats. Male Wistar rats were divided into 8 groups of five animals each. (1) Control group (10ml/kg of 0.9% NaCl solution); (2) and (3) groups received reference drugs (10mg/kg of indomethacin and diclofenac sodium); (4), (5), (6), (7) and groups (8) were orally administered EERS in 50, 100, 250, 500 and 1000mg/kg doses, respectively. Animals were pre-treated with drug and EERS 60 min before injection of carrageenan. Inflammation of the hind paw was induced by injecting 0.1ml of 0.5% carrageenan suspension into the sub-plantar surface of the right hind paw. Measures of the paw circumference were determined at 3, 4, 5 and 6 h (after edematogenic agent injection) intervals later (St) using the method of Bamgbose and Noamesi. The difference between St (3, 4, 5 and 6 h) and S0 was taken as the oedema size. The inhibition percentage of the inflammatory reaction was determined for each animal by comparison with controls and calculated by the following equation:

$$\% \text{ Inhibition} = \frac{(St-S0)control-(St-S0)treated}{(St-S0)control} \text{ X } 100$$

Histamine-induced rat paw oedema[20]:

Eight groups of rats (five rats each) were used for this test. Group (1) served as a control group (10 ml/kg of 0.9% NaCl solution), animals in groups (2) and (3) were orally treated with indomethacin and diclofenac sodium (10mg/kg), while rats in groups (4), (5), (6), (7) and (8) received the EERS at doses of 50, 100, 250, 500 and 1000mg/kg, respectively. Animals were treated by drug controls and EERS 1h before histamine injection (0.1 ml of a 1% solution in 0.9% NaCl solution) into the plantar region of the right-hand paw. Paw size was measured before injection of histamine and at 3, 4, 5 and 6 h, after injection. The average increase in paw size of each group was determined. The percentage inhibition was obtained using this formula:

$$\% \text{ Inhibition} = \frac{(St-S0)control-(St-S0)treated}{(St-S0)control} \text{ X } 100$$

Where,

St = the paw size for each group after histamine treatment and

S0 = paw size for each group before histamine injection.

In vivo analgesic activity

Hot plate test[21,22]:

Twenty-five Swiss albino mice (20-25g) were divided into 5 groups of five mice per group. Group (1) received control solution (0.9% NaCl solution), group (2) received tramadol hydrochloride (10mg/kg), while groups (3), (4) and (5) received 100, 250 and 500mg/kg of EERS, respectively. 1h after the orally administration, mice were placed onto a hot plate (55 ± 2 ^{0}C), and the reaction time for licking of paw

or jumping for the control and treated mice was recorded (in seconds). A cut-off time of 15 s was used to avoid damage to the paw. The percentage increase in reaction time was determined thus:

$$\% \text{ Increase in reaction time} = \frac{Tt - T0}{T0} \text{ X } 100$$

Writhing test[23,24]:

Overnight fasted mice were divided into five groups of five each. Groups (1) and (2) received control solution (0.9% NaCl solution) and acetylsalicylic acid (10mg/kg), while groups (3), (4) and (5) received 100, 250 and 500mg/kg of EERS, respectively. 1h after, the animals were intraperitoneally injected with acetic acid (0.6%, v/v in 0.9% NaCl solution) [30], the analgesic activity was quantified by counting the total number of writhes over a period of 25 min after a latency period of 5 min. The percentage of analgesic activity was calculated as follows:

$$\% \text{ Inhibition} = \frac{\text{No.of writhes (control)} - \text{No.of writhes (test)}}{\text{No.of writhes (control)}} \text{ X } 100$$

Formalin licking test[25-27]:

Formalin licking test was carried out using male mice under same experimental conditions of acetate writhing test. 1 h after orally EERS administration, 20µl of 1 % formalin solution (in 0.9% NaCl solution) was injected subcutaneously into the plantar surface of the right hind paw of each mouse. Licking the injected paw time was measured over 30 min divided into two phases. The early phase was observed during the first 5 min and the late phase was recorded in 15-30 min. These phases represented neurogenic and inflammatory pain responses, respectively.

The percentage of inhibition was obtained by the following formula:

$$\%\text{Inhibition} = \frac{\text{Reaction time (control)} - \text{reaction time (treated)}}{\text{Reaction time (control)}} \text{ X } 100$$

Statistical analysis

Statistical analyzes were performed using GraphPad Prism software version 6.00 (GraphPad Inc., San Diego, California). Data were analyzed by analysis of variance (ANOVA Analysis of Variance) followed by posthoc Dunnet test if the sample distribution follows a normal distribution or by the Kruskal-Wallis if the sample distribution does not follow the normal law. Values between groups were considered statistically significant for at $P<0.05$.

RESULTS

Anti-inflammatory activity

Results plotted in Tables 1 and 2 illustrate the dose-dependent EERS's effect on paw oedema formation after induction by carrageenan (Table 1) and histamine (Table 2). The subplantar injection of carrageenan-induced a progressive local oedema reaching its peak at the 3rd h (Table 1). The orally administration of the EERS showed a dose-dependent reduction in carrageenan-induced paw oedema from the 3rd to the 6th h. The highest EERS's inhibition activity (80 %) was recorded after 4th h at 500mg/kg dose, compared to reference drugs indomethacin (53.24%) and diclofenac (69.34%) ($p<0.05$). Besides, no significant difference ($p<0.05$) was observed when the treatment dose, rise to 1g/kg (compared to 500mg/kg dose).

Table 1: Effect of EERS on carrageenan-induced rat paw oedema in rats

	Inhibition (%)			
Dose (mg/kg)	**3 h**	**4 h**	**5 h**	**6 h**
50	^{a}0.27±0.13	^{a}10.00±2.20	^{a}20.21±2.21	^{a}0.45±0.05
100	^{b}44.45±3.45	^{b}60.00±2.33	^{b}64.24±2.22	^{b}16.67±1.00
250	^{b}50.27±3.61	^{b}75.00±2.76	^{c}73.40±1.56	^{c}37.50±1.11
500	^{c}66.76±3.69	^{c}80.00±2.11	^{c}78.73±1.72	^{d}50.00±4.27
1000	^{c}67.81±5.06	^{c}81.97±2.63	^{c}78.79±1.18	^{d}50.00±3.00
Indomethacin*	^{d}19.05±2.20	^{b}53.24±2.54	^{d}60.99±2.33	^{e}8.34±1.22
Diclofenac sodium*	^{b}47.11±2.22	^{b}69.34±3.25	^{d}63.12±1.65	^{e}3.34±0.18

$^{a-e}$Values in the same column with different superscripts are significantly different ($p<0.05$) [mean ± SD, n= 6]. *Reference drugs (indomethacin 10mg/kg and diclofenac sodium 10mg/kg).

Table 2: Effect of EERS on histamine-induced rat paw oedema in rats

	Inhibition (%)			
Dose (mg/kg)	**3 h**	**4 h**	**5 h**	**6 h**
50	^{a}0.13±0.03	^{a}8.90±0.55	^{a}18.96±0.59	^{a}0.26±0.04
100	^{b}25.00±2.43	^{b}52.00±1.54	^{b}60.50±1.35	^{b}12.00±1.67
250	^{c}51.00±2.39	^{c}70.90±1.67	^{c}69.00±2.22	^{c}34.00±1.50
500	^{d}62.70±0.84	^{d}76.00±2.03	^{d}76.30±0.70	^{d}45.50±1.34
1000	^{d}63.98±0.57	^{d}78.22±1.18	^{d}77.10±0.86	^{d}47.00±2.37
Indomethacin*	^{e}15.50±0.50	^{b}51.00±0.59	^{b}56.80±1.04	^{a}3.21±0.79
Diclofenac sodium*	^{f}43.90±1.01	^{e}64.96±1.66	^{b}58.60±1.09	^{a}2.60±0.10

$^{a-f}$Values in the same column with different superscripts are significantly different ($p<0.05$) [mean ± SD, n= 6]. *Reference drugs (indomethacin 10mg/kg and diclofenac sodium 10mg/kg).

Similarly, the paw oedema induced by histamine was reduced after EERS administration (Table 2). High inhibition activity was observed for the 500 and 1000mg/kg doses (76.00 and 78.22%), but with no significant difference (p<0.05). Thus, EERS's administration has a significant anti-inflammatory effect compared to the used reference drugs, even at low doses like 250mg/kg.

Analgesic activity

Graphs of Figures 3, 4 and 5 show results related to the EERS's analgesic activity assessed by means of the hot plate, acetic acid-induced abdominal writhing and formalin *in-vivo* tests.

Considering the results of anti-inflammatory tests, analgesic tests were performed at three EERS's doses (100, 250 and 500mg/kg). EERS administration has increased the insensibility to pain without loss of consciousness (analgesic activity) in mice placed onto the hot plate (55 ± 2 ^{0}C). The observed EERS's analgesic effect was dose-dependent, reaching its maximum (36.77%) at 500mg/kg dose (Figure 3). This effect was, however, compared to that observed (35.84%) for tramadol hydrochloride (no significant difference at p<0.05).

On the writhing response in mice, EERS induced a potent dose-dependent anti-nociceptive activity at all used doses (Figure 4). This activity was up to 68% for 500mg/kg dose, which was similar to that shown by the reference drug significantly (no differences at p<0.05).

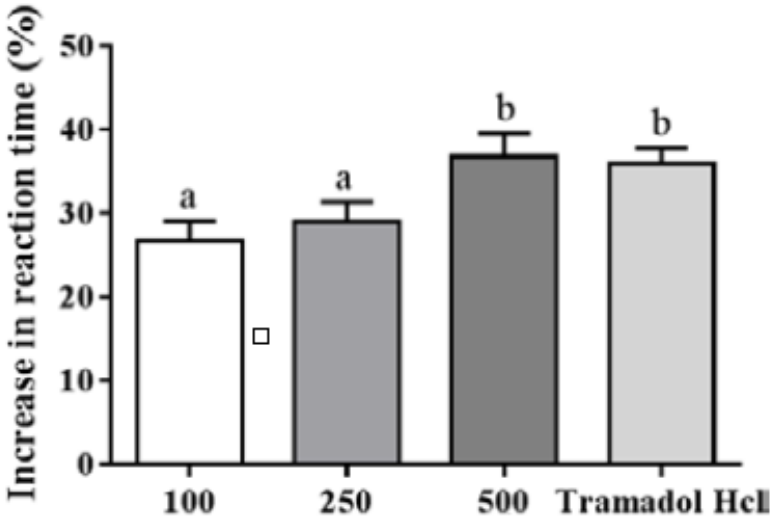


Figure 3: Effect of EERS on hot plate-induced pain in mice. Data is expressed as mean ± SD [n= 5]. Different letters indicate significant differences (p<0.05).

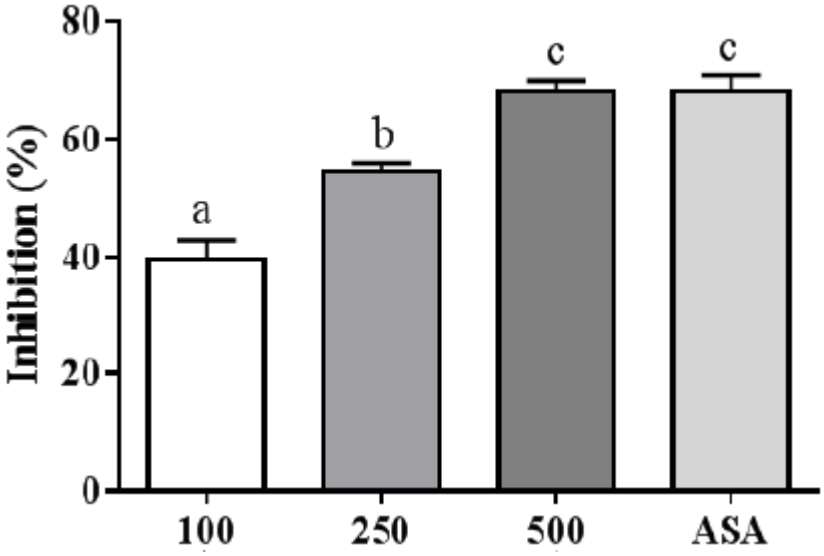


Figure 4: EERS's analgesic activity in mice treated with acetic acid. Data are expressed as mean ± SD [n= 5]. Different letters (a-c) indicate significant differences (p<0.05).

Two phases showed in Figure 5 represented neurogenic and inflammatory pain responses, respectively. The subcutaneous injection of formalin solution into the plantar surface of the right hind paw of mice produced an analgesic

response of licking of the treated paw. EERS showed dose-dependent effect in both early and late phases. The EERS's analgesic activity was incomparable to that of reference drug (diclofenac sodium) during the early phase (corresponding to the neurologic pain) (Figure 5).
.

However, this effect was significantly higher in mice treated with 500mg/kg dose (84.70%) compared to diclofenac sodium (75.20 %) (p<0.05) in the late phase (corresponding to the inflammatory pain).

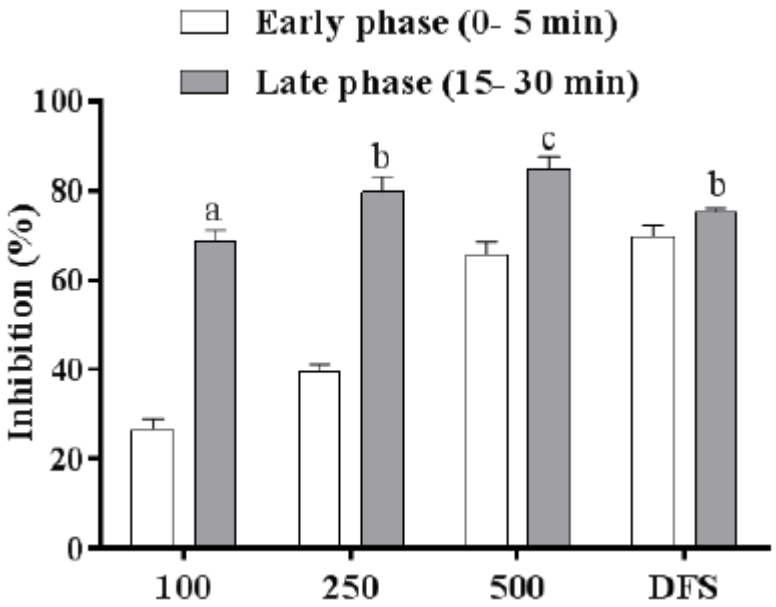


Figure 5: Effects of EERS on the formalin-induced licking response in mice. Data is expressed as mean ± SD [n= 5]. Different letters (a-c) indicate significant differences (p<0.05).

DISCUSSION

Leaves of medicinal plants are common ingredients of many folk and herbal medicines, and leaf extracts of a number of medicinal plants have been reported to possess pharmacological activity, including anti-inflammatory activity. The present study reveals that the dried leaf extract of *Raphanus sativus* possesses significant anti-inflammatory and analgesic activities in experimental animals. The carrageenan-induced paw oedema is frequently used as an experimental model for acute inflammation studying. The inflammatory reaction carrageenan-induced (in rats) is a biphasic response, (i) oedema formation involving the production of inflammatory mediators such as histamine, serotonin, and kinins; (ii) the biosynthesis of prostaglandin and other autacoids release and attributed to the induction of cyclooxygenase (COX-2) in the tissue. Actually, the results of this study suggest that the EERS could antagonize the action and/or inhibit the production of the circulating inflammation mediators. On injection, histamine acts as an inflammatory mediator, which increases vascular permeability[28-30].

Results herein presented show the anti-edematogenic effect of orally administered EERS in rats injected by histamine. This could be, in fact, attributed to the anti-inflammatory activity of studying extract through an anti-histamine mechanism. The rich phenolic *Raphanus sativus* tree extracted may act by inhibiting the release and/or histamine action, which can explain its inhibitory activity on oedema development. Thus, observed EERS's anti-inflammatory effects could be related to its phenolic composition. Actually, EERS is a rich phenolic extract. In general, *Raphanus sativus* phenolic compounds have been known to inhibit both COX-1/2 inflammatory enzymes (in a dose-dependent manner) more efficiently than ibuprofen drug[31,32].

Results from the current study reveal the effectiveness of natural EERS as oedema inhibitor compared to reference drugs (indomethacin and diclofenac sodium). These classical non-steroidal anti-inflammatory drugs (NSAIDs) mainly inhibit COXs. However, they have side effects such as irritation of the gastric mucosa, caused by the inhibition of prostaglandin biosynthesis, which has a protective role in the gastrointestinal tract. Many NSAIDs are also acids that may cause additional harm in the gastrointestinal tract. Thus, the use of natural anti-inflammatory agents is one of the proposed solutions to overcome problems caused by side effects of NSAIDs.

Hot plate test was selected to investigate the central analgesic effect of EERS, which is known to elevate the pain threshold of mice towards heat. It also indicates narcotic involvement with opioid receptor and measures the complex response to a non-inflammatory acute nociceptive input. The high percentage inhibition (36.77%) shown by EERS in this test suggests that EERS is a centrally acting analgesic. Acetic acid-induced writhing response is useful for the evaluation of peripherally acting analgesics. Acetic acid stimulates the tissue to produce several inflammatory mediators such as histamine, serotonin, cytokines, and eicosanoids with an increase in peritoneal fluid levels of these mediators. In this sense, EERS inhibited mice abdominal writhes, suggesting that EERS's anti-nociceptive

activity could be related to the reduction of inflammatory mediator's liberation and/or to direct blockage of receptors resulting in peripheral analgesic response.

The formalin test is a valid model of pain and analgesic research for clinical pain compared to tests using phasic thermal or mechanical stimulation. It has two distinctive phases, reflecting different types of pain. The early phase (0 to 5 min) reflects centrally mediated pain, which was a result of direct stimulation of nociceptors and believed to be a non-inflammatory pain. The late phase (15 to 30 min) persistent period caused by local tissue inflammation. Experimental results demonstrated that substance P and bradykinin participate in the early phase, while histamine, serotonin, prostaglandins, nitric oxide, and bradykinin are believed to be involved in the late phase of the formalin test response.

Our results show that EERS has an inhibitory effect on the analgesic response of both early and late phases of the formalin test. Moreover, significant pain relief activity observed in the late phase (compared to the early phase) indicates the peripherally acting protective effect of EERS, which was correlated with anti-inflammatory tests results. In that way, EERS attenuate pain response better than diclofenac sodium (NSAID), commonly used as a reference due to its anti-inflammatory and analgesic effects.

This drug has the ability to reduce inflammation, swelling and pain by inhibiting either the release of arachidonic acid or the prostaglandin synthesis. This fact corroborates with published data about, *in-vivo*, phenolic compounds anti-nociceptive effects, mainly attributed to flavonoids.

CONCLUSION

In most of the developing nation's natural sources in particular the phytomedicine is the sole source within the financial and physical reach of the needy people. Furthermore, people had gained faith felt satisfied and happy that most of the therapeutically effective chemical molecules have come from plants. Natural medicine flourished by sharing knowledge both locally as well as across nations. Hence the natural products must continue to hold their quality and significant efforts have to be made to trap the real potential of natural source of medicine.

Results of pharmacological tests performed in the present study suggest that EERS with high polyphenols content is safe and presented potential anti-inflammatory and analgesic activities, which are comparable with the reference drugs. Considering high consumer demand due to the beneficial health effects, radish plant can be beneficially used as a natural food supplement to contend inflammation and pain in the case of inflammatory diseases.

The result of the present study indicates that ethanolic extract of *Raphanus sativus* leaves possess significant analgesic and anti-inflammatory activity on both acute and chronic inflammation. Further detailed investigation is underway to determine the exact phytoconstituents, which are responsible for the anti-inflammatory and analgesic activity. Further studies involving the purification of the chemical constituents of the plant and the investigations in the biochemical pathways may result in the development of a potent anti-inflammatory agent with low toxicity and better therapeutic index. This knowledge about the medicinal plants usage can also be extended to other fields like field of pharmacology. A large scale isolation and further spectral techniques are required to isolate and identify a particular compound responsible for anti-inflammatory and analgesic activity.

Declarations

Acknowledgement

The authors wish to thank the management of School of Pharmacy, Anurag University, Venkatapur, Ghatkesar, Telangana, India for providing necessary equipment for research, praiseworthy inspiration, constant encouragement, facilities and support.

Author contributions

All authors contributed to data collection, drafting or revising the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Competing interest statement

All authors declare that there is no conflict of interests regarding publication of this paper.

Additional information

No additional information is available for this paper.

Financial support and Sponsorship

None.

REFERENCES

1. Ferrucci L, Corsi A, Lauretani F, Bandinelli S, Bartali B, Taub DD et. al. The origins of the age-related proinflammatory state. Blood. 2005; 105:2294-2299.
2. Narender Boggula, Ananda Kumar Chettupalli, Swetha Reddy Naram reddy, Vasudha Bakshi. Anti-diabetic effect of *Alstonia scholaris linn* bark in Alloxan induced diabetic rats. Journal of Global Trends in Pharmaceutical Sciences. 2017; 8(1):3590-3598.
3. Emmanuel S, Ignacimuthu S, Perumalsamy R and Amalraj T. Anti-inflammatory activity of *Solanum trilobatum*. Fitoterapia. 2006; 77:611-612.
4. Ch. Krishna Mohan, V. Soundarya, R. Vasanth Kumar, L. Kiran Kumar, K. Vamshi Sharathnath, B. Narender. *In-vitro* anti inflammatory activity of dried leaves of *Borassus flabellifer*. Indo American Journal of Pharmaceutical Sciences. 2016; 3(8):809-813.
5. Fito M, Cladellas M, De la Torre R, Marti J, Munoz D, Schroder H et. al. Anti-inflammatory effect of virgin olive oil in stable coronary disease patients: a randomized, crossover, controlled trial. Eur J Clin Nutr. 2008; 62:570-574.
6. Singh P, Singh J. Medicinal and therapeutic utilities of *Raphanus sativus*. International journal of plant, animal and environmental sciences. 2013; 3(2):103-105.
7. Rosa Martha Perez Gutierrez, Rosalinda Lule Perez. *Raphanus sativus* (Radish): Their Chemistry and Biology. The Scientific World Journal. 2004; 4:811-837.

8. Guisti MM, Ghanadan H, Wroslstad RE. Elucidation of the structure and conformation of red radish (*Raphanus sativus*) anthocyanins using one-and two dimensional nuclear magnetic resonance techniques. J Agric Food Chem. 1998; 46:4858-4863.
9. Shyamala, G, Singh PN. An analysis of chemical constituents of *Raphanus sativus*. Proc. Natl. Acad. Sci. India Sect. B. 1987; 57:157-159.
10. Ahmad F, Hasan I, Chishti D K, Ahmad H. Antibacterial Activity of *Raphanus sativus* Linn. Seed Extract. Global Journal of Medical Research. 2012; 12(11):25-34.
11. Sreelekshmi G, Pratibha K. A safe body through moolaka-*Raphanus sativus* linn. International Ayurvedic Medical Journal. 2015; 3(10):3091-3096.
12. Teklic T, Hancock J T, Engler M, Paradikovic N, Cesar V, Lepedus H, Stolfa I, Beslo D. Antioxidative responses in radish (*Raphanus sativus* l.) Plants stressed by copper and lead in nutrient solution and soil. Acta Biologica Cracoviensia Series Botanica. 2008; 50(2):79-86.
13. Alqasoumi S, AL-Yahya M, AL-Howiriny T, Rafatullah S. Gastroprotective effect of radish *Raphanus sativus* L. on experimental gastric ulcer models in rats. Farmacia. 2008; 56(2):204-214.
14. Badar A, Jan M. Effect of crude extract of *Raphanus sativus* roots on isolated trachea of albino rat. Pak j physiol. 2012; 8(1):23-26.
15. Shukla S, Chatterji S, Yadav DK, Watal G. Antimicrobial efficacy of *Raphanus sativus* root juice. International Journal of Pharmacy and Pharmaceutical Sciences. 2011; 3(5):89-92.
16. Rahman I, Biswas SK, Kirkham PA. Regulation of inflammation and redox signaling by dietary polyphenols. Biochem Pharmacol. 2006; 72:1439-1452.
17. Yaso Deepika Mamidisetti, Nikhila Yammada, Harihara Kumar Siddamsetty, Vasudha Bakshi, Narender Boggula. Phytochemical and analgesic, anti-inflammatory screening of methanolic extract of *Ficus religiosa* fruits – An *in vivo* design. The Pharma Innovation. 2018; 7(6):69-74.
18. N.K. Manaswini, S Nazneen, Garige Baba Shankar Rao, Narender Boggula, Vasudha Bakshi, Manda Ram Mohan Evaluation of *Ocimum tenuiflorum* and *Syzygium aromaticum* phenolic ethereal oils for *In-vitro* anti-inflammatory and anti-bacterial activities. Journal of Drug Delivery & Therapeutics. 2019; 9(2):93-96.
19. Bamgbose SOA, Noamesi BK. Studies on cryptolepine. Inhibition of carrageenan-induced edema by cryptolepine. Planta Med. 1981; 42:392-396.
20. Farshid A, Tamaddonfard E, Morvaridi A. Effects of histidine and dexamethasone on the local inflammation induced by histamine in rats. Vet Res Forum. 2011; 2:31-36.
21. Sutar NG, Pal SC. Evaluation of analgesic activity of leaf extracts of *pergularia daemia* [forsk] in experimental animals. Int J Pharm Pharm Sci. 2014; 6:137-139.
22. Omisore NOA, Adewunmi CO, Iwalewa EO, Ngadjui BT, Watchueng J, Abegaz BM, et. al. Antiniociceptive and anti-inflammatory effects of *Dorstenia barteri* (Moraceae) leaf and twig extracts in mice. J Ethnopharmacol. 2004; 95:7-12.
23. Dahiya RS, Kaur P, Kashyap P, Katoch N, Gupta S. Pharmacological evaluation of hydroalcohol and chloroform extracts of nyctanthes arbour-tristis l. for antioxidant, anti-inflammatory and analgesic activity. Int J Pharm Pharm Sci. 2014; 6:460-465.
24. Koster R, Anderson M, De Beer E. Acetic acid for analgesic screening. Fed Proc. 1959; 18:412.
25. Mahgoub AA. Grapefruit juice potentiates the anti-inflammatory effects of diclofenac on the carrageenan-induced Rat's Paw oedema. Pharmacol Res. 2002; 45:1-4.
26. Hunskaar S, Hole K. The formalin test in mice: dissociation between inflammatory and non-inflammatory pain. Pain. 1987; 30:103-114.
27. Raman R, Parthiban S, Karthikeyan S, Muthuraman MS, Sivasubramanian A. Antimicrobial and anti-inflammatory studies on *sargassum wightii* extracts. Int J Pharm Pharm Sci. 2014; 6:611-614.
28. Ribeiro IA, Rocha J, Sepodes B, Mota-Filipe H, Ribeiro MH. Effect of naringin enzymatic hydrolysis towards naringenin on the anti-inflammatory activity of both compounds. J Mol Catal B: Enzym. 2008; 52:8-13.
29. Vineger R, Truax JF, Selph JL. Quantitative studies of the pathway to acute carrageenan inflammation. Fed Proc. 1976; 35:2447-2456.
30. Di Rosa M, Giroud JP, Willoughby DA. Studies of the mediators of the acute inflammatory response induced in rats in different sites by carrageenan and turpentine. J Pathol. 1971; 104:15-29.
31. Vasudevan M, Gunman KK, Parle M. Antinociceptive and anti-inflammatory effects of *Thespesia populnea* bark extract. J Ethnopharmacol. 2007; 109:264-270.
32. Linardi A, Costa SKP, DeSilva GR, Antunes E. Involvement of kinins, mast cells, and sensory neurons in the plasma exudation and paw edema induced by staphylococcal entrotoxin B in the mouse. Eur J Pharmacol. 2002; 399:235-242.