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Evaluation of Antidiarrhoeal, Antinociceptive and Anti-Inflammatory activity of *Centella asiatica* in rats

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Abstract
Pain is a warning sensation, defensive in nature but causes uneasiness. Inflammation is accompanied by pain, stiffness and swelling or oedema of the affected area. Diarrhoea is too frequent passage of watery faeces. The present study investigated the potency of the ethanolic extract of *Centella asiatica* in the above described disorders in the dose dependent manner. The animals were divided into four groups for each test with 6 rats in each group and tagged properly. Castor oil induced diarrhoea model was used for the study of antidiarrhoeal effect of the drug (p<0.05). The basal reaction time of the rats increased by the extract in hot plate test for antinociception. The ethanolic extract of the *Centella asiatica* was administered to the rats at doses of 200mg/kg and 400mg/kg for the antidiarrhoeal and antinociceptive activity (p<0.05) significantly reduced the watery faeces and pain in the rats when compared with the control group. The extract at the doses of 100mg/kg and 200mg/kg significantly reduced the inflammation in carragenan induced inflammation in rats (p<0.001) when compared with the control group. The results significantly depicted the antidiarrhoeal, antinociceptive and anti-inflammatory activity of the *Centella asiatica* extract but in the dose dependent manner, which supports its use as a remedy in these disorders.

Keywords: *Centella asiatica*, antidiarrheal, antinociceptive, anti-inflammatory, carragenan

Introduction

Pain

The word pain is used in various contexts. Most often it is connected to emotions of grief, sorrow and suffering. The word "pain" comes from the Latin word "Poena" meaning a fine, a penalty. "Pain as the fifth vital sign" has been introduced by the American Pain Society in 1996 [1]. According to the International Association for the Study of Pain (IASP) it is defined as "Pain is an unpleasant sensory and emotional experience arising from actual or potential tissue damage [2]." It is a sensation that can range from mild discomfort localized to agony. Pain has both physical and emotional components. The physical part of the pain results from nerve stimulation. The pain may be contained in a discrete area, such as in an injury, or may be more diffuse, as in disorders such as fibromyalgia. Common side effects of opioid administration include sedation, dizziness, nausea, vomiting, constipation, physical dependence, tolerance, and respiratory depression. Physical dependence and addiction are clinical concerns that can prevent correct prescribing and, consequently, inadequate pain management. Less common side effects may include delayed gastric emptying, hyperalgesia, immunological and hormonal dysfunction, muscle rigidity and myoclonus. The most common side effects of opioid use are constipation (which has a very high incidence) and nausea [3].

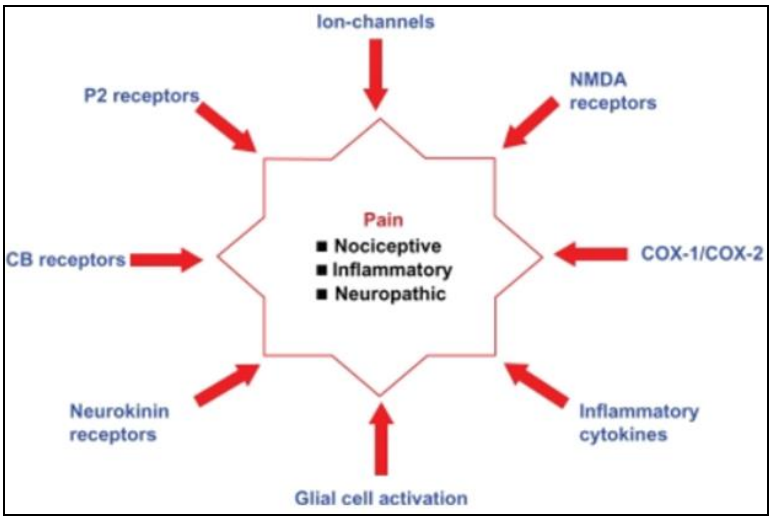

Fig 1: Summary of pain pathophysiology and some pain targets. Abbreviations: CB, cannabinoid; COX, cyclooxygenase; NMDA, N-Methyl-D-aspartate13

Inflammation

Inflammation is a normal response of the body to protect the tissues against infections, injuries or diseases. The inflammatory response begins with the production and release of chemicals by infected, injured or diseased tissue cells. These agents cause redness, swelling, pain, heat and loss of function. The inflamed tissues generate additional signals that recruit leukocytes to the site of inflammation. Leukocytes destroy any infectious or harmful agent and remove cellular debris from damaged tissues [14]. This inflammatory response generally promotes healing but can, if left unchecked, become harmful. The inflammatory response can be acute or chronic. Acute inflammation usually lasts only a few days. If a wound heats up, becomes red, hurts and swells, we recognize that the inflammation is working. In this case, inflammation is a beneficial process,

It serves to immobilize the area of the lesion while the rest of the immune system is mobilized to heal. Inflammation is the first line of defense against injury or infection. The treatment of acute inflammation, which includes administration of aspirin and other non-steroidal anti-inflammatory agents, provides relief of pain and fever to patients. In contrast, chronic inflammation lasts for weeks, months or even indefinitely and causes tissue damage.

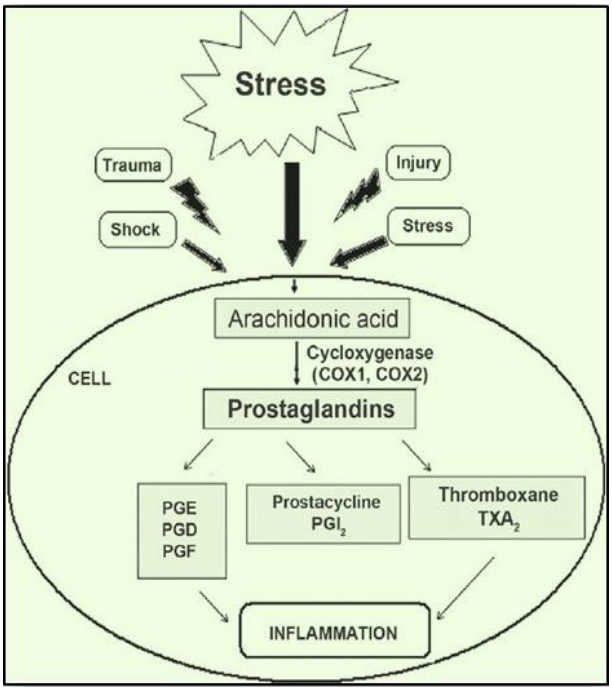

Fig 2: Process of inflammation by various causes

Chronic Inflammation

In chronic inflammation, inflammation becomes the problem rather than the solution to infection, injury or disease [16]. Tissues with chronic inflammation continues to generate signals that attract blood leukocytes. When leukocytes migrate from blood to tissue, they amplify the inflammatory response. This chronic inflammatory response can degrade healthy tissue during attempted repair and poorly directed healing [4].

Diarrhoea

Diarrhoea is one of the leading causes of illness and death among children in developing countries, where an estimated 1.3 billion episodes and 4 million deaths occur each year in children under five. Worldwide, these children live an average of 3.3 episodes per year, but in some areas, the average exceeds nine episodes per year. When episodes are frequent, young children can spend more than 15% of their days with diarrhea. About 80% of diarrhea deaths occur in the first two years of life. The leading cause of death from acute diarrhea is dehydration, which results from the loss of fluids and electrolytes in stool diarrhea. Dysentery and malnutrition are other important causes of death [17]. Diarrhoea is a major cause of malnutrition. In fact, patients eat less during diarrhea and their ability to absorb nutrients is reduced. In addition, nutrient requirements increase due to infection. Each episode of diarrhea contributes to malnutrition; as the episodes continue, their impact on growth increases [5]. Diarrhoeal diseases also represent an economic burden for developing countries. In many countries, over one-third of children's hospital beds are occupied by patients with diarrhea. These patients are often treated with expensive intravenous fluids and ineffective drugs. Although diarrheal diseases are generally less harmful for adults than for children, they can also affect a country's economy by reducing the health of its workforce. [18] Fortunately, there are simple and effective treatments that can significantly reduce the number of deaths from diarrhea, make hospitalization unnecessary in most cases, and prevent the adverse effects of diarrhea on nutritional status. Practical preventive measures can be taken to significantly reduce the incidence and severity of diarrhoea episodes. This unit provides information on the epidemiology and etiology of diarrhoea, which is essential for understanding the principles of treatment and prevention. Treatment and prevention of diarrhoea are considered in subsequent units. The synthetic drugs available for the treatment of the these diseases are not optimal and are not able to fulfill the desired criteria for treating the diseases. There is an immense need to switch over to the herbal medications that offers maximum healing with the minimum amount of the side effects. *Centella asiatica (C.A)* is at the interface between traditional medicine and modern medicine, with a scientific orientation. *Centella asiatica* Urban is a genus of the family Apiaceae (Umbelliferae), which contains 20 different species. In addition to this name, which is generally used in scientific work, the synonym Hydrocotyle asiatica is the most frequently found designation. *Centella asiatica* is the most widespread species of Centella. It is found in Southeast Asia, Sri Lanka, parts of China, the Western Isles of the South Sea, Madagascar, India, South Africa, the southeastern United States, United States, Mexico, Venezuela and Colombia, as well as in the eastern regions of South America. *Centella asiatica* is a perennial climbing plant that blooms between August and September. Its flowers are light purple. The gray to greenish green plant has an odor reminiscent of tobacco leaves and a slightly bitter taste. The leaves have long petioles that are in the form of a rosette from a common base (node) and rosettes of individual leaves (nodes) are connected by means of stolons or thin air corridors. The leaves are thin and smooth, with patterned nerves, without hair or with some hair, and measure about 2 to 5 cm in diameter. The edge of the leaf is crenate or slightly lobed. The petioles are between 5 and 15 cm long, are thin and hairless or have only a few scattered hairs [12]. *Centella asiatica* has been reported to contain a large number of compounds belonging to different types of

chemicals classes. Triterpene saponosides are the main class of chemicals found in this plant. The main ones are Asiatic acid, madecassic acid (6-hydroxy- Asiatic acid), asiaticoside, madecassoside and mad Asiatic acid, betulinic acid, Thankunic acid and isothankunic acid. In addition, there are other triterpenes such as brahmic acid, centellin, centellicin, asiaticin, bayogenin, terminolic acid, 3β, 6β,3-O- [α-L-arabinofuranosyl] 2α, 3β, 6β, 23-α-tetrahydroxy-12-en-28-oic acid, saponins of centella AD, ursolic acid, pomolic acid, 3-epimaslinic acid, 23 -O acetone madecassoside and 23-O-acetylasiaticoside centella saponins AD, ursolic acid, pomolic acid, 3- epimaslinic [11]. *Centella asiatica* is one of the essential plants to revitalize the nerves and cells of the brain. *Centella asiatica* has justified its use as a panacea for treating a wide variety of health problems since time immemorial. Countless clinical and experimental studies have demonstrated their important role in improving memory and improving cognition, promoting brain repair, antiepileptic control, anxiety, wound healing and anti-stress activities. Clinical trials have been shown to be effective in supporting the treatment of autism and mental retardation [10]. The present study focus on the anti-inflammatory, anti-diarrhoreal and anti-nociceptive activity of the *Centella asiatica*.

Material and Methods

Plant Collection and Authentication

Dry leaves of *Centella asiatica* was purchased from the market. The plant leaves was identified by botanist at M.J.P. Rohilkhand University. A voucher specimen of the plant material was deposited at department of plant science, M.J.P Rohilkhand University, Bareilly. All parts of the plants were thoroughly washed with water and dried in a shade at room temperature for 7 days; after that they were dried in an oven at 40° C for the next 2 days to facilitate grinding.

Preparation of Extract

The fresh leaves of *Centella asiatica* were washed thoroughly under running tap water followed by sterile distilled water and dried under shade. They were grounded into coarse powder by mechanical support (pulverizer). The dried leaf powder sample (500g) was extracted in Soxhlet apparatus at 60-70oC for 6 hours continuously in 70% ethanol. The extracted material was evaporated into dryness under reduced pressure at 40-50oC in vacuum rotary evaporator and stored in sterilized air tight container at 4oC for further use [19].

Selection of Test Animals

Male rats six in each group having weight (150-200g) was used for the study. The animals were housed at temperature 22±2%, relative humidity 60±10% and 12h light and dark cycle. All the study was performed in Department of pharmacy M.J.P Rohilkhand University, Bareilly as per the guidelines of the Institutional Animal Ethical Committee (IAEC) and Committee for purpose of control and supervision of experiments on animals (CPCSEA).

Anti-diarrhoeal activity

Castor oil induced diarrhoea

This experiment was carried out according to the method described by Awouters *et al*. The experimental mice were fasted for 18 hours. Four groups of mice were taken for this experiment. Group I was treated as a control (saline solution at 2 ml / kg body weight orally), group II received the standard drug (loperamide 5 mg / kg) and group III- IV received a ethanolic extract (200 and 400 mg / kg i.p). Then, 1 hour later, castor oil (0.4 ml / rat) was administered orally. The rats were individually housed in cages lined with white transfer paper. The papers were changed every hour. The total number of dry and wet faeces excreted was counted every hour for 4 hours and compared to the control group. The total number of diarrheal faeces in the control group was considered 100% [7].

Anti-nociceptive activity

Hot plate method

The antinociceptive activity of the extract was measured by the hot plate method. Rats were treated in groups of four with vehicle (normal saline), diclofenac sodium (10 mg / kg, i.p) and *Centella asiatica* (200 and 400 mg / kg, p.o). The rats were placed on a heating plate maintained at 55 ± 0.5 ° C. The reaction time was taken when the interval between the moment the animal reached the heating plate and the moment when the animal licked his feet or jumps out. A cutting time of + 20 s was followed to avoid any thermal damage to the legs. The reaction time was recorded before and after +0, +30 and +60 min after administration of the standard and test drug repectively [8].

Carrageenan Induced Paw Edema in Mice

The effect of oral administration of 100 and 200 mg / kg *Centella asiatica* extract, 10 mg / kg diclofenac Na or vehicle (saline, 10 ml / kg) on posterior paw edema induced by a subplantar injection of 1 ml of carrageenan (1% w / v) was evaluated according to the method described by Winter *et al*. (1962). In summary, 0.1 ml of 1% w / v carrageenan was injected into the subplantar tissue of the left hind paw of each rat. The swelling of the injected foot with carrageenan was measured at 1, 2, 3 h using the plethysmometer [19]. The animals were treated with the test extract 1 hour before carrageenan injection. The measurement was carried out immediately before and 4 hours after the injection of carrageenan. The amount of inhibition of the drugs tested was observed relative to the control vehicle (100%) [9].

Result

Anti- diarrhoeal effect

In the castor oil- induced diarrhoea experiments, the ethanolic extract of C.A produced significant anti-diarroheal effect in the rats, as shown in table no.1. at doses of 200mg/kg and 400mg/kg, the ethanolic extract produced very significant inhibition of ($p<0.05$) defecation when compared with control group. The total no. of wet faeces produced on administration of castor oil decreased with 7.24±0.44 in 200mg/kg of C.A extract and 6.21±0.62 in 400mg/kg of C.A extract respectively. The standard drug loperamide further decrease total no. of diarrhoeal facese 5.45±0.62 at dose of 5mg/kg.

Table 1: Effect of ethanolic extract of *Centella asiatica* on castor oil induced diarrhoea in rats

Groups	treatment	dose and routes	total no. of feaces	total no. of diarrhoeal faeces
I	castor oil+ saline	2ml/kg, p.o	17.2±0.72	14.52±0.62
II	castor oil+ loperamide	5mg/kg, i.p	9.45±0.47	5.45±0.62
III	castor oil+ extract	200mg/kg, p.o	17.00±0.89	7.24±0.44
IV	castor oil+ extract	400mg/kg, p.o	13.46±0.67	6.21±0.62

The values are expressed as mean±SEM, p<0.05 when compared with the control group

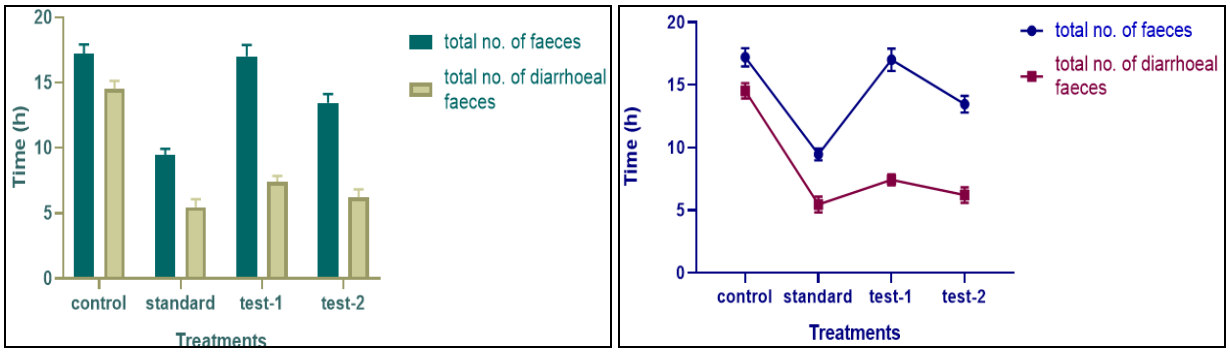

Fig 3: Effect of ethanolic extract of *Centella asiatica* on castor oil induced diarrhoea in rats

Hot plate method

C.A administered at doses of 200mg/kg and 400mg/kg significantly elevated the mean basal reaction time as compared to central group. The highest nociceptive inhibition was shown by C.A extract at a dose of 400 mg/kg at 60 minute. C.A extract at dose of 200 mg/kg produced significant nociceptive effect at 60 minute. When compared to central the maximum nociception inhibition by standard drug diclofenac Na was observed at 45 minute.

The table number 2 indicates the nociception inhibition of C.A extract at various doses respectively.

Table 2: Effect of ethanolic extract of *Centella asiatica* on hot plate test

Groups	treatment	dose and routes	0 minute	30 minute	60 minute
I	saline water	10mg/kg, p.o	7.76±0.78	7.45±0.52	6.12±1.24
II	diclofenac sodium	10mg/kg, i.p	9.91±1.96	14.56±1.40	17.45±1.74
III	extract-1	200mg/kg, p.o	7.82±0.82	9.45±1.40	9.42±1.28
IV	extract-2	400mg/kg, p.o	7.56±0.64	12.45±1.64	13.45±1.21

The values are expressed as mean±SEM, p<0.05 when compared with the control group

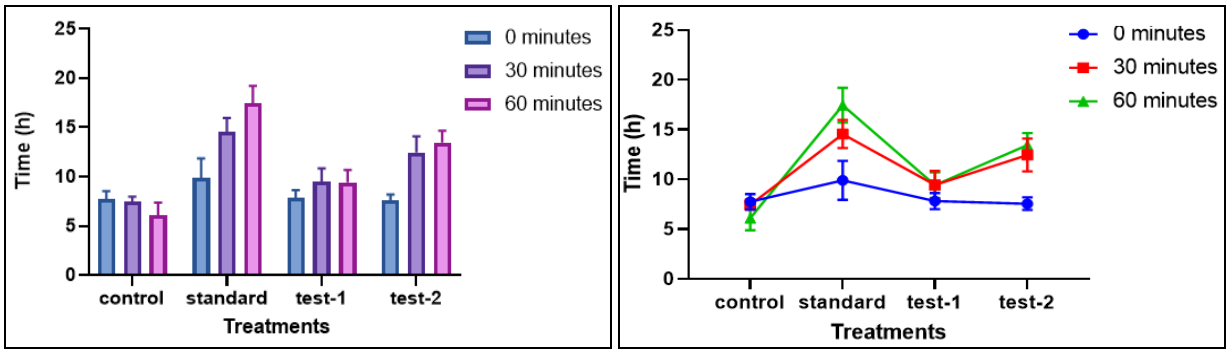

Fig 3: Effect of ethanolic extract of *Centella asiatica* on hot plate test

Carrageenan induced rat paw edema method

The C.A extract administered at doses of 100mg/kg and 200mg/kg significantly showed anti-inflammatory activity. The result of inflammation of inhibition when compared with central proved anti-inflammatory activity of C.A (p<0.0001) a dose of 200mg/kg of C.A extract maximum inhibition of inflammatory was observed. The standard drug diclofenac Na showed inflammation inhibition maximum when compared respectively.

Table 3: effect of ethanolic extract of *Centella asiatica* on carragenan induced rat paw edema method

Groups	treatment	dose and routes	1 hr.	2 hr.	3 hr.	4 hr.
I	saline water	10mg/kg, p.o	44.46±1.40	92±3.40	131±2.41	131.72±2.41
II	diclofenac sodium	10mg/kg, i.p	15.23±0.96	39±2.14	42.84±2.72	48.90±2.31
III	extract-1	200mg/kg, p.o	35.44±0.74	73±1.92	112.42±2.42	111.74±0.94
IV	extract-2	400mg/kg, p.o	29.30±0.74	52.14±1.92	71.32±0.97	85.29±1.30

The values are expressed as mean±SEM, p<0.001 when compared with the control group

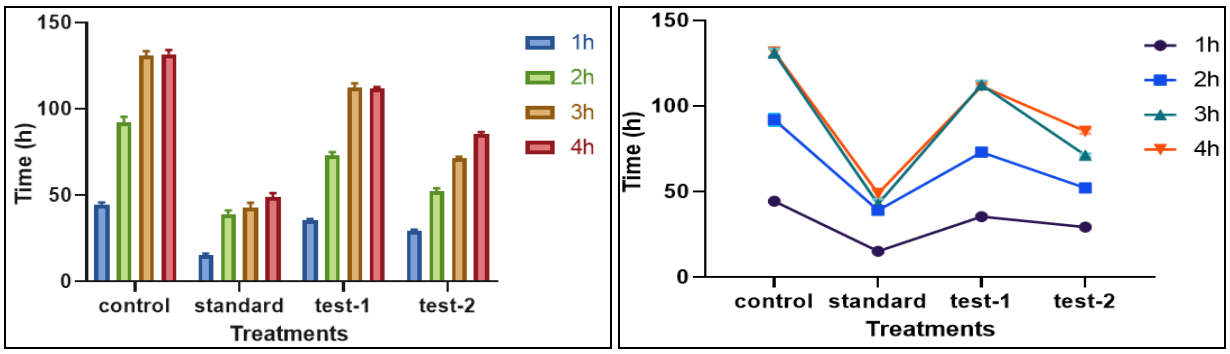

Fig 4: Effect of ethanolic extract of *Centella asiatica* on carragenan induced rat paw edema method

Discussion

A variety of medicinal remedies are available for the treatment of the various diseases prevailing in the present scenario. *Centella asiatica* possesses a numerous amount of active chemical constituents such as alkaloids, triterpenoids, volatile oils, saponins and trace amount of other chemicals. In the present study castor oil induced diarrhoea is significantly improved by the administration of *Centella asiatica* extract at doses of 200mg/kg and 400mg/kg ($p<0.05$), when compared with the other groups respectively. The mechanism available for the anti-diarrhoeal effect of the drug include inhibition of Na^+k^+ATPase activity [20], thus reducing normal fluid absorption, activation of adenyl cyclase or mucosal cAMP-mediated active secretion [21]. Castor oil administered for inducing diarrhoea produces diarrhoea due to the presence of its most active constituent ricinoleic acid through a hyper secretory response [22, 23]. The ethanolic extract of the drug *Centella asiatica* is also investigated for the anti-nociceptive activity of the drug. The Hot plate method significantly proved the potency of the extract in pain at doses of the 200mg/kg and 400mg/kg ($p<0.05$) when compared with the control group and standard drug for the study was Diclofenac Na at a dose of 10mg/kg i.p.

The mechanism that lies for the effect could be inhibition of the synthesis of the arachidonic acid metabolites and basal reaction time was elevated of the rats investigated for the study. In the carragenan induced rat paw edema method injection of carragenan produces a typical biphasic edema with the production of the inflammatory mediators such as bradykinin, prostaglandins, nitric oxide (NO) and cytokines. In our study, inflammation was significantly declined by the treatment with *Centella asiatica* extract at doses 100mg/kg and 200mg/kg ($p<0.001$). The mechanism behind the response may be through the L-arginine-NO pathway, since *Centella asiatica* significantly inhibits the NO production [24].

Conclusion

In conclusion, the results of the present study clearly depict the anti-diarrhoeal, anti-nociceptive and anti-inflammatory activity of the ethanolic extract of the centella asiatica at the various doses. The results of the study are significant and hence the drug can be used as an herbal remedy for the treatment of the diarrhoea, pain and inflammation respectively.

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