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ANALGESIC, ANTI-INFLAMMATORY AND ANTI-OXIDANT ACTIVITY OF INDIAN MEDICINAL PLANT ON RODENTS

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ABSTRACT
Inflammation is the response to injury of cells and body tissues through different factors such as infections, chemicals, thermal and mechanical injuries. Most of the anti inflammatory drugs presently available are potential inhibitors of cyclooxygenase (COX) pathway of arachidonic acid metabolism which produces prostaglandins. Prostaglandins are the mediators of inflammation. Inflammation is accompanied by redness, swelling, pain (analgesia). Inhibition of prostaglandins synthesis is essential to treat inflammation. Hence, for treating inflammatory diseases, analgesic and anti-inflammatory agents are required. Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most widely used medications due to their efficacy for a wide range of pain and inflammatory conditions. But, the long-term administration of NSAIDs may induce gastro-intestinal ulcers, bleeding and renal disorders due to their non-selective inhibition of both constitutive (COX-1) and inducible (COX-2) isoforms of the cyclooxygenase enzymes. Therefore, there is a need to develop new anti-inflammatory and analgesic drugs with potent activity, less side effects are being searched all over the world as alternatives to NSAIDs and opiates. Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects. Cauliflower is a vegetable that belongs to the Cabbage family, which is also the Brassicaceae family or Cruciferae. Its scientific name is *Brassica oleracea var. botrytis*. The aim of this study was to carry out the phytochemical and pharmacological evaluation of *BRASSICA OLERACEA* as potential anti-inflammatory and analgesic agent on rats and mice and in-vitro evaluation of antioxidant property. To investigate the phyto-constituents present in *Brassica oleracea* (stem, leaves and flowers).To perform acute toxicity studies on *Brassica oleracea*. To investigate the analgesic effect of *Brassica oleracea*on mice using:

1) Acetic acid induced abdominal writhing in mice.
2) Eddy's hot plate plate method.
3) To investigate the anti-inflammatory effect of *Brassica oleracea* on rats using.
4) Carageenan induced paw edema.

The experimental findings in the study demonstrated the peripheral analgesic, anti-inflammatory and antioxidant activity of *BRASSICA OLERACEA (Cauliflower florets)*. Mainly EECF (200 and 400 mg/kg) was found to be highly effective. The results suggested that the mechanism of action of EECF seems to be similar to NSAID's rather than to steroidal drugs. The study justified and supported scientifically the ethno-pharmacological use of the plant as an anti-inflammatory agent to treat pain and inflammation.

KEY WORDS: Analgesic, Anti-Inflammatory, Anti-Oxidant, Medicinal Plant, Rodents, Screening, Evaluation.

INTRODUCTION
Cauliflower is one of several vegetables in the species Brassica oleracea, in the family Brassicaceae. It is an annual plant that reproduces by seed. Typically, only the head (the white curd) of aborted floral meristems is eaten, while the stalk and surrounding thick, green leaves are used in vegetable broth or discarded. Its name is from Latin caulis (cabbage) and flower, an acknowledgment of its unusual place among a family of food plants which normally produces only leafy greens for eating. *Brassica oleracea*also includes cabbage, Brussels sprouts, kale,

broccoli, and collard greens, though they are of different cultivar groups.

Inflammation is the response to injury of cells and body tissues through different factors such as infections, chemicals,thermal and mechanical injuries(Oyedapo*et al*., 2008). Most of the anti inflammatory drugs presently available are potential inhibitors of cyclooxygenase (COX) pathway of arachidonic acid metabolism which produces prostaglandins. Prostaglandins arethe mediators of inflammation. Inflammation is accompanied byredness, swelling, pain (analgesia). Inhibition of prostaglandins synthesis is essential to treat inflammation. Hence, for treating inflammatory diseases, analgesic and anti-inflammatory agentsare required (Anilkumar, 2010). Nonsteroidal anti-inflammatorydrugs (NSAIDs) are among the most widely used medicationsdue to their efficacy for a wide range of pain and inflammatoryconditions (IMS Health, 2005). But, the long-term administrationof NSAIDs may induce gastro-intestinal ulcers, bleeding and renaldisorders due to their nonselective inhibition of both constitutive(COX-1) and inducible (COX-2) isoforms of the cyclooxygenaseenzymes (Robert, 1976; Peskar, 1977; Tapiero*et al*., 2002).Therefore, there is a need to develop new anti-inflammatory andanalgesic drugs with potent activity, less side effects are beingsearched all over the world as alternatives to NSAIDs and opiates(Dharmasiri*et al*., 2003; Kumara, 2001). Medicinal plants arebelieved to be an important source of new chemical substanceswith potential therapeutic effects.

Plants and plant extracts have been used since the dawnof civilization by mankind. The uses of ethnobotanicalpreparations for various reasons justified or not, are still continuedby various cultures all over the world. Considering structural andbiological diversity of terrestrial plants, they offer a uniquerenewable resource for the discovery of potential new drugs andmodern medicine has developed a rational strategy for drugdiscovery which involves the study of plants and plant materialsbased on their ethnobotanicalusage (Cordell *et al*., 1991). Naturalproducts are sources of active compounds that may be useful in thedevelopment of new and potent drugs. Cauliflower is a vegetable that belongs to the Cabbage family, which is also the Brassicaceae family or Cruciferae. Its scientific name is *Brassica oleracea var. botrytis* (http://www.biodatabase. de/Cauliflower). The plants in this family all share a common feature: their four-petaled flowers resemble to a Greek cross and are often refereed to as crucifers or cruciferous vegetables. Its scientific name originates from the classical Latin word for Wild Cabbage (Quattrocchi, 349). The name cauliflower comes from the Latin words *caulis*, meaning-stalk, and *floris*, meaning -flower. As suggested by its name, cauliflower is actually a flower. The editable part of the plant is the head of underdeveloped, tender flower stems and buds.

One cup of boiled cauliflower is an excellent source of vitamin C (91.5% of the DV), folate (13.6% of the DV), and dietary fiber (13.4% of the DV). That same amount of cauliflower also serves as a very good source of vitamin B5, vitamin B6, manganese and omega-3 fatty acids. Consumption of cauliflower is known to reduce the risk of a number of cancers, such as lung, colon, breast, ovarian and bladder cancer. However till now no pharmacological studies about the analgesic and anti-inflammatory activity of *Brassicaoleracea (var.botrytis)* have been reported. The objective of the study was to investigate theanalgesic and anti-inflammatory activities of extracts of *Cauliflower*in different animal models.

Analgesia and Inflammation

The accepted definition of pain is ‘an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage. Analgesia is the alleviation or the absence of pain.

Pain is a multidimensional experience that is essential for the maintenance and preservation of an individual. It warns of the danger of bodily harm and alerts to trauma and injury. Pain is a specific enteroceptive sensation; it can be perceived as arising from a particular portion of the body, its temporal properties can be detailed, it can be differentiated qualitatively (for example, as stinging, pricking, burning, throbbing, dull or aching), and it involves dedicated subsets of peripheral and centralneurons. The experience of pain has a distinctly unpleasant character, that is, an affective or motivational aspect that can be distinguished from its discriminative sensory aspects and from the long-term emotional experience of ‘suffering’. The unpleasantness ranges in intensity from the discomfort of a cold room, fatigued muscles or colonic tension to the excruciating agony of a severe burn, toothache, gallstone or migraine. Under normal circumstances, primary afferent pain fibres activate particular central pathways that engage protective mechanisms at several functional levels: autonomic, homeostatic, motoric, behavioural and mnemonic. However, injury or disease can alter the balance of this system and result in persistent, pathological pain. Analgesic substances, such as aspirin and morphine, that interact with the transmitters and modulators of the pain system are helpful for many people with pain, but there is a great need for the development of better methods for the alleviation and control of both acute(immediate) and chronic(long-term, pathological) pain.[1]

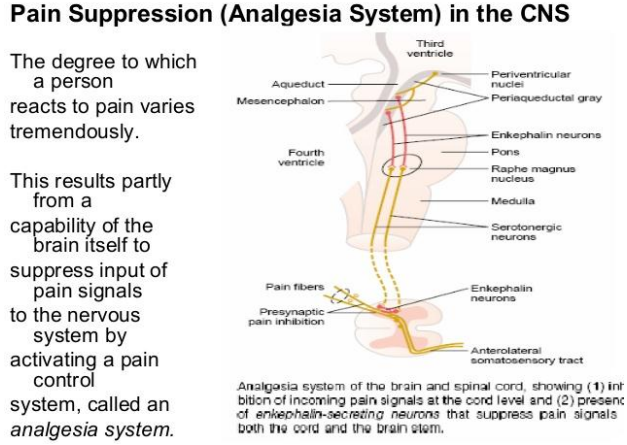

Fig No. 1 Pain Suppression (Analgesia System) in the CNS

Analgesics

Analgesics refer to a group of drugs used to temporarily relieve pain. They are sometimes known as painkillers. They block pain signals by changing how the brain interprets the signals and slowing down the central nervous system. Combining analgesics with alcohol, prescription or illegal drugs can create dangerous and unpredictable effects. Even low doses can impair driving ability. There are two main types of analgesics: non-narcotic and narcotic.

Plant Introduction

Fig No. 2 Cauliflower

Classification

Scientific name: *Brassica oleracea*var. botrytis L.
Common names: Cauliflower
Family name: Brassicaceae (Cruciferae)

AIMS AND OBJECTIVES

Aims

The aim of this study is to carry out the phytochemical and pharmacological evaluation of *BRASSICA OLERACEA* as potential anti-inflammatory and analgesic agent on rats and mice and in-vitro evaluation of antioxidant property.

Objectives

1. To investigate the phytoconstituents present in *Brassica oleracea* (stem, leaves and flowers).
2. To perform acute toxicity studies on *Brassica oleracea.*
3. To investigate the analgesic effect of *Brassica oleracea*on mice using:
   1) Acetic acid induced abdominal writhing in mice
   2) Eddy's hot plate plate method
4. To investigate the anti-inflammatory effect of *Brassica oleracea*on rats using:
   -Carageenan induced paw edema

Plan of Study

The work was carried out in phases as mentioned below:

1) Collection of plant and preparation of extract
2) Investigation of bioactive phytochemicals
3) Acute toxicity studies
4) Analgesic activity using acetic acid induced abdominal writhing in mice,Eddy's hot plate method
5) Anti-inflammatory activity using carragenaan induced paw edema method

MATERIALS AND METHODS

Collection of plant and identification

Cauliflower (Brassica oleracea var.botrytis) buds used for investigation was purchased from a local vegetable market in Hyderabad, Telangana, India. The plant can be identified authenticated by department of botany research office (Botanist) University College of Science, Osmania University, Hyderabad.

Preparation of Extract

The chopped vegetable material was divided into three portions (500 g each) to dry by different drying processes. One portion was air-dried (ambient conditions, 10 days), another portion was sun-dried (7 days) and a third portion was oven-dried at 40^{o}C (3 days). All samples were ground and the material that passed through 80-mesh was used for extraction purposes.

Ethanolic extract of cauliflower (250gm) was loosely packed in the thimble of Soxhlet apparatus and extracted with methanol as a solvent. The extract so obtained was thick and syrupy and was evaporated to dryness under vaccum and dried in vaccum desiccator.

Acute oral toxicity study

Healthy female Swiss albino mice were subjected to acute oral toxicity studies as per OECD guideline- 425. The animals were fasted overnight and divided into 3 groups with 5 animals in each group. Extracts (EECF and AECF) were administered orally at one dose level of 2000 mg/kg body weight. The mice were observed continuously for behavioural and autonomic profiles for 2 hrs and for any signs of toxicity or mortality up to 48 hr.[9]

Analgesic activity
The acetic acid (chemical) and hot plate (thermal) analgesic test methods were used.

Acetic acid induced abdominal writhing in mice
Swiss albino mice were treated according to the method described by Colier *et al*, 1968. Mice were pre-treated orally with EECF, AECF and acetylsalicylic acid, 60 min before administration of acetic acid solution at a dose of 10 ml/kg (0.6%, I.P.). The number of abdominal constrictions (full extension of both hind paws) was cumulatively counted over a period of 15 min.

Fig No. 3 Mice

The mice were divided into eight groups of 6 mice each.
Group 1: - Vehicle control (2% Tween 80).
Group 2: - Standard (Acetylsalicylic acid 100 mg/kg p.o.).
Group 3, 4 and 5: - EECF (200 and 400 mg/kg, p.o.), respectively.
Group 6, 7 and 8: - AECF (200 and 400 mg/kg, p.o.), respectively.

The percent inhibition of writhing was calculated as follows:

% Inhibition = (VC-VT/VC) * 100

Where, VT, number of writhes in drug treated mice.
VC, number of writhes in control group of mice.

Hot plate method in mice
Swiss albino mice were treated according to the method describedby Eddy and Leimback, 1953. Mice were screened by placingthem on hot plate (UGO Basile, Italy. Model No. DS-37) maintained at 55+- 1 °C and the reaction time were recorded in seconds. The timefor paw licking or jumping on the hot plate was considered as areaction time. The responses were recorded before and after 30, 60, 90, 120, 150 and 180 min after the administration of EECF, AECF and Pentazocine. A cut-off time of 15s was used to avoid injury to the animals.[10]

Fig No. 4 Mice during Analgesic, Anti-Inflammatory And Anti-Oxidant activity.

The mice were divided into eight groups of 6 mice each.
Group 1: - Vehicle control (2% Tween 80).
Group 2: - Standard (Pentazocine 5 mg/kg s.c.).
Group 3, 4 and 5: - EECF (200 and 400 mg/kg, p.o.), respectively.
Group 6, 7 and 8: - AECF (200 and 400 mg/kg, p.o.), respectively.

Anti-inflammatory activity
Carrageenan induced rat paw edema
Female Wistar rats were treated according to the method described by Winter *et al*, 1962[11]

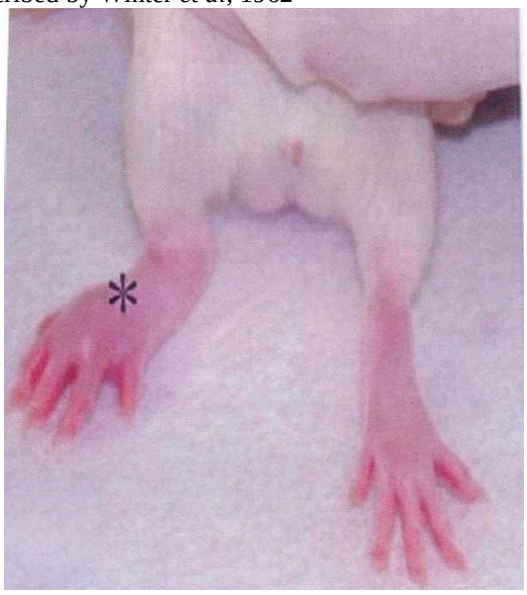

Fig No. 5 Mice during Analgesic, Anti-Inflammatory activity

Female Wistar rats were divided into eight groups of 6 rats each.

Group 1: - Vehicle control (2% Tween 80).
Group 2: - Standard (Diclofenac 10 mg/kg p.o.).
Group 3, 4 and 5: - EECF (200 and 400 mg/kg, p.o.), respectively.
Group 6, 7 and 8: - AECF (200 and 400 mg/kg, p.o.), respectively.

Inflammation was produced by injecting 0.1ml of 1% lambda carrageenan (Sigma Chemical Co., USA) in sterile normal saline intothe sub plantar region of the right hind paw of the rat. Rats were pre-treated orally with EECF, AECF and Diclofenac 1h before the carrageenan injection. The paw volume was measured from 0-6h, at an hourly interval using pleythsmometer (UgoBasile, Italy, Model No. 7140). The mean changes in injected paw volume with respect to initial paw volume were calculated. Percentage inhibition of paw volume between treated and control group was calculated by the following formula,

% Inhibition = (1-VT / VC *100)

Where, VT and VC are the mean increase in paw volume in treated and control groups, respectively.

In vitro* antioxidant activity of *Cauliflower (brassica oleracea)

DPPH free radical scavenging assay

The 1, 1-diphenyl -2-picylhydrazyl (DPPH) - free radical scavenging activity was determined using the method described by Blois M. The antioxidant activity of the EECF, AECF and ascorbic acid were assessed on the basis of the radical scavenging effect of the stable DPPH free radical. 10-100 µl of each extract or standard was added to 2 ml of DPPH in methanol (0.33%) in a test tube. After incubation at 37ºC for 30 minutes the absorbance of each solution was determined at 517 nm using spectrophotometer.[12] The corresponding blank reading were also taken and the remaining DPPH was calculated by using the following formula,

DPPH radical scavenging activity (%)=
[Abs (control) – Abs (standard) /Abs(Control)] × 100.
Where, Abs (control): Absorbance of DPPH radical + methanol.
Abs (standard): Absorbance of DPPH radical + extract/standard.
IC50 value calculated denotes the concentration of the sample required to scavenge 50% of DPPH radical.

Hydrogen peroxide radical scavenging assay

The ability of the EECF, AECF and ascorbic acid to scavenge hydrogen peroxide was determined according to the method of Ruch*et al*. A solution of hydrogen peroxide (2 mmol/l) was prepared in phosphate buffer (pH 7.4). Extracts (10–100 µg /ml) were added to hydrogen peroxide solution (0.6 ml). Absorbance of hydrogen peroxide at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. For each concentration, a separate blank sample was used for background subtraction.[13] The percentage scavenging activity of hydrogen peroxide by EECF,AECF and ascorbic acid was calculated using the following formula,
% scavenging activity [H2O2] =
[Abs (control) – Abs (standard) / Abs (control)]× 100.
Where, Abs (control): Absorbance of the control and methanol.
Abs (standard): Absorbance of the extract/standard.
IC50 value calculated denotes the concentration of the sample required to scavenge 50% of hydrogen peroxide radical.

Hydroxyl radical scavenging assay

The scavenging capacity for hydroxyl radical was measured according to the method of S. Ganapaty *et al*.[14] The assay was performed by adding 0.1 ml of 1mM EDTA, 0.01 ml of 10 mM FeCl3,0.1 ml of 10 mM H2O2, 0.36 ml of 10 Mm deoxyribose, 1.0 ml of different dilutions of the EECF, AECF and ascorbic acid (10 –100 µg/ml) dissolved in distilled water, 0.33 ml of phosphate buffer(50 mM, pH 7.4) and 0.1 ml of ascorbic acid in sequence. The mixture was then incubated at 37 °C for 1 h. A 1.0 ml portion of the incubated mixture was mixed with 1.0 ml of 10% TCA and 1.0 ml of0.5% TBA (in 0.025M NaOH containing 0.025% butylated hydroxyl anisole to develop the pink chromogen measured at 532 nm.The hydroxyl radical scavenging activity of the extracts is reported as % inhibition of deoxyribose degradation and is calculated as,
OH- scavenged (%) = [Abs (control) – Abs (standard) / Abs (control)] × 100.

Where, Abs (control): Absorbance of the control reaction
Abs (standard): Absorbance of the extract/standard.

IC50 value calculated denotes the concentration of the sample required to scavenge 50%of hydroxyl radical.

Total phenolic content

The total soluble phenolic in the EECF, AECF and Gallic acid was determined with using Folin-Ciocalteau reagent. 1 ml of extract solution containing 1 g extract in a volumetric flask wasdiluted with 46 ml of distilled water. 1 ml of Folin-Ciocalteau reagentwas added and the content of the flask mixed thoroughly. 3 min later3 ml of 2% sodium carbonate was added and the mixture was allowed to stand for 2h with intermittent shaking. The absorbance of the blue color that developed was read at 760 nm. Gallic acid was used as a standard. The concentration of total phenolic compounds in the extracts and Gallic acid was determined as µg of gallic acidequivalent using an equation obtained from the standard gallic acid.[15]

Statistical Analysis

The experimental results were expressed as the mean +- standard error of mean (SEM).Data was assessed by the method of analysis of ANOVA. P value of <=0.05 and <=0.01 were considered as statistically significant.

RESULTS

Phytochemical screening

Acute oral toxicity study: Administration 2000 mg/kg, p.o. of all the three extracts i.e. PECP, MECP and AECP did not produce any behavioral abnormalities and mortality. Therefore three doses (100,200 and 400 mg/kg b.w) were selected for pharmacological studies.

Table 1: Phytochemical screening of Brassica oleracea (cauliflower).

Plant constituents	Present(+)/Absent(-)
Alkaloids	+ve
Steroids	+ve
Flavanoids	+ve
Tannins	+ve
Saponins	+ve
Triterpinoids	+ve
Carbohydrates	+ve
Phenols	+ve
Glycosides	+ve
Proteins	+ve
Resins	+ve

Analgesic activity

Acetic acid induced abdominal writhing in mice: EECF (400 and 200 mg/kg) significantly ($p<0.001$ and $p<0.05$, respectively) reduced the number of wriths induced by 0.6% acetic acid at the dose of 10 ml/kg. While AECP (200 and 400 mg/kg) showed non-significant results. The number of wriths in the acetic acid vehicle control group was found to be 68±1.5. Acetylsalicylic acid (100 mg/kg) appears to be more effective in reducing the number of wriths, it significantly ($p<0.001$) reduced the number of wriths with 64.71 % inhibition. (Table 2).

Table 2: Effect of *Cauliflower florets* in acetic acid-induced abdominal writhing in mice.

Treatment	Dose (mg/kg p.o)	No of writhing	Percentage inhibition
Vehicle control	-	68±1.5	-
Acetyl salicylic acid	100	24±2.1***	64.71
EECF	200	56±2.2*	17.65
EECF	400	44±3.3***	35.29
AECF	200	65±2.5	4.41
AECF	400	65±3.0	4.41

Values are expressed as mean ± SEM for six animals and analysed by One way ANOVA followed by Dunnett's test, *$p<0.05$, ***$p<0.001$ when compared to vehicle control.

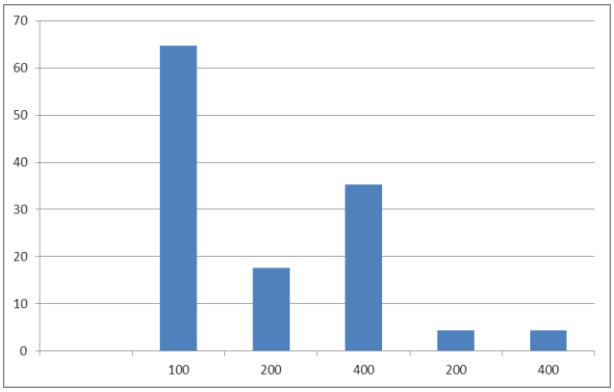

Fig No. 6 Graph 1

Where,
X axis denotes-Dose (mg/kg)
Y axis denotes-percentage (%) inhibition

Hot plate method in mice: All the extracts (EECF and AECF) did not exhibit analgesic activity in hot plate model at all the doses tested. On the other hand, Pentazocine (5mg/kg, subcutaneously) showed significantly ($P<0.001$) increased pain threshold in mice when compared to the vehicle control group at 60and 90 min.

Anti-inflammatory activity

Carrageenan induced rat paw edema: There was a gradual increase in paw edema volume of rats in the carrageenan control group. In the test groups, the EECF (200 and 400 mg/kg) showed a significant ($p<0.001$) reduction in paw volume in a dose dependent manner at 3rd and 5th h. The inhibitory effect of the EECF at 400mg/kg was recorded 40.92 % at 3rd h and 51.47 % at 5th h. However, On treatment with AECF there was no significant inhibition at all the doses when compared to carrageenan control group. Diclofenac (10 mg/kg) caused significant ($P<0.001$) inhibition of increase in paw edema at 3rd and 5th h. The inhibitory effect of the diclofenac at 10 mg/kg was recorded 46.84 % at 3rd hand 53.99 % at 5th h (Table 3).

Table 3: Effect of *Cauliflower florets*on carrageenan induced rat paw edema.

Change in Paw Volume(ml)				
Treatment	**Dose(mg/kg),p.o**	**1h**	**3h**	**5h**
Vehicle(cearrageenan)	-	1.43±0.24	2.56±0.10	2.78±0.07
Diclofenac	10	1.10±0.11{23.14}	1.36±0.04***{46.84}	1.28±0.04***{53.99}
EECF	200	1.22±0.05{15.23}	1.82±0.09***{28.82}	1.62±0.07***{41.50}
EECF	400	1.12±0.06{21.63}	1.51±0.04***{40.92}	1.35±0.05***{51.47}
AECF	200	1.38±0.09{3.04}	2.56±0.11{0.13}	2.68±0.12{3.48}
AECF	400	1.40±0.04{2.44}	2.53±0.12{1.24}	2.68±0.13{4.2}

Values are expressed as mean ± SEM for six animals and analysed by Two way ANOVA followed by Bonferroni post-hoc test, *p<0.05, **p<0.01 and***p<0.001 when compared to carrageenan control. The figures in parenthesis indicates the percentage inhibition

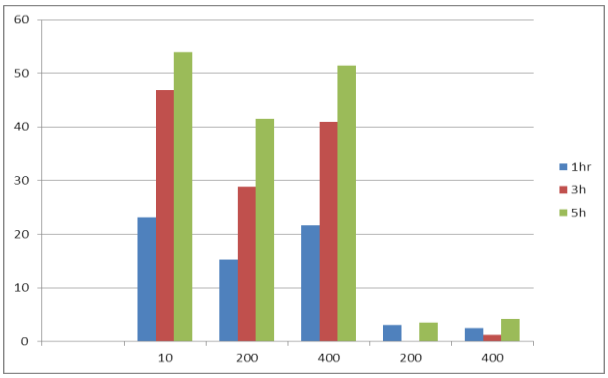

Fig No. 7 Graph 2

Where,
X axis denotes-dose (mg/kg)
Y axis denotes-percentage (%) inhibitionie; change in paw volume (ml) noted at an interval of 1 hr.

In vitro* antioxidant activity of *Cauliflower florets

EECF and AECF showed promising scavenging effect in DPPH free radical assay, hydrogen peroxide assay and hydroxyl radical assay. IC50 value calculated indicates the concentration required to inhibit the radicals by 50 percent. EECF was found to be more active in inhibiting radicals than AECF. Although the scavenging abilities of the extracts were significantly lower than ascorbic acid used as reference standard.

Table 4: *In vitro* antioxidant activity of *cauliflower florets.*

Antioxidant activity	DPPH assay. IC50µg/ml	Hydrogen peroxide. IC50µg/ml	Hydroxyl radical.IC50µg/ml	TPC µg/100 µg
EECF	62	77	67	78
AECF	94	95	75	66
Ascorbic acid	58	65	52	-
Gallic acid	-		-	96

DISCUSSION

Pain and inflammation are associated with pathophysiology of various diseases like arthritis, cancer and vascular diseases. A number of natural products are used in various traditional medicinal systems to relief symptoms of pain and inflammation.[16] *BRASSICA OLERACEA(cauliflower)*is reported to contain chemical constituents which may exert analgesic and anti-inflammatory effect; however till now there has been no investigation supporting the pharmacological properties of this plant. Acute oral toxicity study performed at thedose of 2000 mg/kg revealed the non-toxic nature of all the two extracts i.e. EECF and AECF.

There were no toxic reactions or mortality found with these extracts. The peripheral analgesic effect may be mediated through inhibition of cyclooxygenase and/or lipooxygenases, while central analgesic action may be mediated through inhibition of central pain receptors.[17] Therefore peripheral (acetic acid induced writhing) and central (hot plate test) models were selected to observe the analgesic effect of *Cauliflower*. Acetic acid induced writhing test is a simple, reliable and affords rapid evaluation of analgesic drugs.[18] The intraperitoneal administration of agents that irritate serous membranes provokes a stereotypical behavior in mice which is characterized by abdominal contractions, movements of the body as a whole, twisting of dorso-abdominal muscles, and a reduction in motor activity and coordination.[19] The abdominal constrictions induced in mice results from an acute inflammatory reaction with production of prostaglandins E2 and F2 in the peritoneal fluid.[20] EECF (400 mg/kg) significantly (p<0.001) inhibited the number of wriths with 35.29 % inhibition. EECF (200mg/kg) also significantly (p<0.05) inhibited the number of wriths compared to vehicle control group. Acetyl salicylic acid (100 mg/kg) showed maximum activity with 64.71 % inhibition. It has been reported that NSAID's prevent prostaglandin production, thus sensitization of pain receptors by prostaglandin at the inflammatory site is inhibited.[21] The mechanism of peripheral analgesic action of EECF, likewise other NSAID's, could probably be due to the blockade of the effect or due to the release of endogenous substances that excite pain nerve endings. The hot plate model has been found to be suitable for the evaluation of centrally acting analgesics.[5] Hence, the hot plate test was performed to check if *Cauliflower* would have any central analgesic effect. There were no significant results obtained in these test with *Cauliflower* on all the extracts tested. On the other hand Pentazocine (5 mg/kg, s.c.) Showed a significant result by elevating the pain threshold. Hence it can be assumed that *Cauliflower* has no effect on central nervous system. Carrageenan induced rat paw edema is a suitable model for evaluating anti-inflammatory drugs.[22] Carrageenan has been widely used as an inflammagen capable of inducing experimental inflammation for screening of compounds possessing anti-inflammatory effect.[23] The development of edema induced by carrageenan is a biphasic event; the early phase (90-180 min) of the inflammation is due to release of histamine, serotonin and similar substances. The later phase (270-360 min) is associated with the activation of kinin- like substances and the release of

prostaglandins, proteases and lysosome.[24] Statistical analysis revealed that EECF (200 and 400 mg/kg) significantly ($p<0.001$) inhibited the development of paw edema induced by carrageenan from 3h onwards. Therefore it may be assumed that EECF is associated with inhibition of later phase regulated by prostaglandins, proteases and lysosome. Moreover, Diclofenac (10 mg/kg) exhibited an enhanced effect of inhibiting the paw edema than EECF with 53.99 %inhibition at 5 h. (Table 3).

Oxidation is essential in many organisms for the production of energy to fuel biological processes. However the uncontrolled production of oxygen derived free radicals is involved in the onset of many diseases such as rheumatoid arthritis, atherosclerosis and also cancer.[23] DPPH radical has been widely used as a model system to investigate the scavenging activities of extracts of plants. DPPH radical is scavenged by antioxidants through the donation of a hydrogen atom, forming the reduced DPPH-H.[25] Hydrogen peroxide itself is not very reactive, but it can sometimes be toxic to cell because it may give rise to hydroxyl radical in the cells. Thus, the removal of hydrogen peroxide is very important for antioxidant defense in cell or food systems.[21] Hydroxyl radical is the principal contributor for tissue injury.[26] The results for DPPH, hydrogenperoxide, and hydroxyl radical scavenging activity exhibited by the extracts were comparable with the standard compound, ascorbic acid. The IC50 value of EECF showed higher antioxidant activity.while less activity for AECP. The phenolic compounds contribute directly to the antioxidative action and play an important role in stabilizing lipid peroxidation.[27] The phenolic contents varied significantly among all the extracts.

Phytochemical analysis of the *BRASSICA OLERACEA (cauliflower)* has mainly demonstrated the presence of flavonoids, steroids, phenols, tannins and saponins. Steroids, alkaloids and terpenoids have been reported to have analgesic and anti-inflammatory activity. Flavonoids and phenolic compounds have multiple biological effects such as antioxidant activity.[28] Flavonoids have also been reported to have anti-inflammatory effect.[29] Steroids can decrease inflammation and reduce the activity of the immune system, while triterpenoids impairs histamine release from mast cells and exerts anti-inflammatory effects.[30] Therefore peripheral analgesic and anti-inflammatory activity of *Cauliflower*might be attributed to phytoconstituents present in the extract like flavonoids, steroids, tannins, phenols and saponins.

CONCLUSION

The experimental findings in the study demonstrated the peripheral analgesic, anti-inflammatory and antioxidant activity of *BRASSICA OLERACEA (Cauliflower florets)*. Mainly EECF (200 and 400 mg/kg) was found to be highly effective. The results suggested that the mechanism of action of EECF seems to be similar to NSAID's rather than to steroidal drugs. The study justified and supported scientifically the ethno pharmacological use of the plant as an anti-inflammatory agent to treat pain and inflammation.

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