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EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF ETHANOLIC AND ETHYLACETOACETATE EXTRACTS OF SWEET CHERRY

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ABSTRACT

Inflammation is a process that is accompanied by the local liberation of chemical mediators like histamine, 5-hydroxy tryptamine, bradykinin and eicosanoids. Eicosanoids are extremely potent compounds involved in most types of inflammation and are formed in almost tissue in the body, thus, inhibition of their biosynthesis is the mainstay of anti-inflammatory therapy for example corticosteroids inhibit the formation of arachidonic acid (AA), the starting compound of eicosanoids. Normally AA is further metabolized by the enzyme cyclooxygenase (COX) to a group of compounds called prostaglandins (PG_S). It has been shown that PGE_2 and PGI_2 cause erythema and increase local blood flow and PGE_2 and $PG_{2\alpha}$ cause intense local pain when given i. m, s. c and PGE_1 causes itching and finally PGE_2 is associated with production of fever. Inhibition of COX by NSAIDS prevents formation of PG_S and brings about its therapeutic actions. However, two things must be kept in mind while dealing with NSAIDS: a). In general, these drugs only offer symptomatic relief without affecting the underlying disease and appropriate drugs need to be given simultaneously to correct the pathological state e. g appropriate antibiotic in a febrile patent and (b) by virtue of its COX inhibiting property the use of these drugs also leads to the manifestation of certain common adverse effects, which may actually limit the use of an NSAID e. g aspirin and gastric ulceration. The objective of the present work was to evaluate the anti-inflammatory activity of various extracts like ethanolic and ethylacetoacetate extracts of sweet cherry by ***in vivo*** method. Anti-inflammatory activity of both extracts (EEC and EAAEC) was evaluated by carrageen an induced paw edema method. The activity was studied at a 200 mg/kg b. w. p. o. and their responses were measured at **30, 60, 120 and 180 min.** The present experimental data displayed that both extracts exhibited mild to good anti-inflammatory activity. Graded dose response was also observed. Both the extracts exhibited highest activity **at 120 min**. When compared with standard drug diclofenac sodium (10 mg/kg i. p) the percent protection of EAAEC was found to be **68.9%** and EEC was found to be **68.5%.**

KEYWORDS: Inflammation, prostaglandins, eicosanoids, carrageen and percent protection etc.

INTRODUCTION

Wonderfully delicious, cherry fruit is packed with full of health-benefiting nutrients and unique antioxidants. Cherries are native to Eastern Europe and Asia Minor regions. Botanically, the fruit is a “drupe” (stone fruit), belonging to the broad Rosaceae family of small tree fruits in the genus, Prunus. Some of the common “drupe” family fruits are plums, peaches, apricots etc. Although several species of cherries exist, two popular cultivars are wild or sweet-cherry, and sour or tart-cherry. While sweet cherries belong to the species of Prunus avium, tart variety belongs to that of Prunus cerasus. Cherries are drupe fruits with a central “stony-hard” seed surrounded by fleshy edible pulp measuring about 2 cm in diameter. Externally they covered by bright "shiny" red or purple, thin peel. The West Indian cherry, known as acerola (Malpighia emarginata) is native to West Indian islands and grown in Mexico, Texas regions in North America. Acerola belongs to tropical fruit-bearing shrub or small tree in the family Malpighiaceae and contains 2-3 tiny seeds. Acerola contains exceptionally high levels of vitamin-C and vitamin-A than North American and European cherries. Cherries are one of the very low calorie fruits. Nonetheless, they are rich source of phytonutrients, vitamins, and minerals. Both sweet as well as tart cherries are packed with numerous health benefiting compounds that are essential for wellness. Cherries are pigment rich fruits. These pigments, in fact, are polyphenolic flavonoid compounds known as anthocyanin glycosides. Anthocyanins are red, purple or blue pigments found in many fruits and vegetables, especially concentrated in their skin, known to have powerful anti-oxidant properties.

Fig: Cherry fruits.

Scientific studies have shown that anthocyanins in the cherries are found to act like anti-inflammatory drugs by blocking the actions of enzymes cyclooxygenase-1 and 2. Thus, consumption of cherries may offer potential health effects against chronic painful episodes such as gout arthritis, fibromyalgia (painful muscle condition) and sports injuries. Research studies also suggest that anti-oxidant compounds in tart cherries can help the human body to fight against cancers, aging and neurological diseases, and pre-diabetes condition. Cherries compose of melatonin anti-oxidant. Melatonin can cross the blood-brain barrier easily and has soothing effects on the brain neurons, calming down nervous system irritability. It, thus, can help relieve neurosis, insomnia and headache problems.[1-2]

Cherry Taxonomy[3]

Cherries are members of the Rosaceae family, subfamily Prunoideae. They occupy the Cerasus subgenus within Prunus, being fairly distinct from their stone fruit relatives plums, apricots, peaches, and almonds. Prunus avium L. is the Sweet Cherry, and Prunus cerasus L. the Sour, Pie, or Tart Cherry.

Botanical Description

Plant: Sweet Cherry. Vigorous tree with strong apical control with an erect-pyramidal canopy shape, capable of reaching 50 ft. In cultivation, sweet cherries are maintained 12-15 ft in height. Leaves are relatively large (largest of cultivated Prunus), elliptic with mildly serrate margins, acute tips, petioled, and strongly veined.

Sour Cherry. Medium sized tree with a rounder, more spreading habit than the erect sweet cherry. Kept <15 ft in cultivation. Leaves elliptic with acute tips, mildly serrate margins, smaller than sweet cherry, with long petioles.

Flowers: Sweet Cherry. White, with long pedicels, borne in racemose clusters of 2-5 flowers on short spurs with multiple buds at tips; the distal bud is vegetative and continues spur growth. Spurs are long-lived, producing for 10-12 years. Ovary position. is perigynous with a distinct hypanthium, characteristic of stone fruits. Sour Cherry. Individual flowers are the same as for sweet cherry. Sour cherry inflorescence buds usually produce 2-4 flowers, with long pedicels, as in sweet cherry. However, many are borne laterally on 1-yr wood, not exclusively on spurs as in sweets. Spurs are shorter-lived on sour than sweet, gradually declining in productivity over 3-5 years. Sour cherries are the latest blooming of the stone fruits.

Pollination: Sweet Cherry. Pollination is absolutely essential for production, since sweet cherries are self-incompatible and need a high degree of fruit set (25-50%) for a commercial crop. In addition to self-incompatibility, there is a high degree of cross-incompatibility. Pollinizers are set every third tree in every third row, or a ratio of 8-9:1. Honey bees are the main pollinator.

Sour Cherry: Sour cherries are self-fertile, and require no pollinizers.

Sweet Cherry: A drupe; ½" to 1 1/4″, round or heart-shaped, glabrous, with long pedicel attached. The pit is generally smooth, and encloses a single seed. The skin color is generally deep red or purple (often referred to as "black"), yellow, or rarely white. Yellow fruit often have a red cheek. The flesh color varies from white to dark red. Fruit is borne on short spurs that arise from older wood. Sweet cherries require only about 2-3 months for fruit development. Thinning is unnecessary.

Pharmacological Actions[4]

Cherries are a nutritional powerhouse fruit with so many incredible health benefits. One cup of raw cherries has 87 calories, 22 grams of carbohydrates, 1 gram of protein and 3 grams of fiber. Enjoy them now while they are at their peak because their season is way too short. Read on for some of the great health benefits of eating cherries.

Ten Great Health Benefits of Eating Cherries

1. Cherries, known as a "super-fruit", are packed with antioxidants called anthocyanins which aid in the reduction of heart disease and cancer.
2. Cherries are one of the few food sources that contain melatonin, an antioxidant that helps regulate heart rhythms and the bodies sleep cycles.
3. Cherries are an excellent source of beta carotene (vitamin A). In fact they contain 19 times more beta carotene than blueberries and strawberries.
4. Cherries are rich in vitamins C, E, potassium, magnesium, iron, folate and fiber.
5. Cherries are referred to as "brain food", aiding in brain health and in the prevention of memory loss.
6. Because cherries contain anthocyanins, they can reduce inflammation and symptoms of arthritis and gout.
7. Eating cherries reduces the risk of diabetes.
8. Cherries are a good source of fiber which is important for digestive health.
9. Cherries are a great snack or dessert choice important for weight-maintenance.

10. Because of their powerful anti-inflammatory benefits, cherries are said to reduce pain and joint soreness for runners and athletes after workouts.

MATERIALS AND METHOD

Drugs and chemicals: The standard drug diclofinac purchased from Local Retail Pharmacy Shop and solvents and other chemicals used for the extraction and phytochemical screening were provided by Institutional Store and were of LR and AR grade.

Experimental animals: White male albino Wister rats weighing about 200-250 g were used. They were obtained from the animal house of C.L. Baid Metha College of Pharmacy, Chennai. They were kept under observation for about 7 days before the onset of the experiment to exclude any intercurrent infection, had free access to normal diet and water. The animals were housed in plastic well aerated cages at normal atmospheric temperature (25±5 °C) and normal 12- hour light/dark cycle under hygienic conditions. The experimental protocol was approved by Institutional Animal Ethics Committee (IAEC) of CPCSEA: IAEC/XXIX/03/2016.

Apparatus: Round bottom flask, water condenser, heating mantle, motor and pestle**.**

Methodology:[5] Weigh 20 g of sweet cherry fruits paste (ripen can be mashed to prepare a paste) into a 250 ml round-bottomed flask. Add 50 ml of ethanol and 60 ml of dichloromethane. Heat the mixture under reflux for 5 min on stem-bath with frequent shaking. Filter the mixture under suction and transfer the filtrate to a separating funnel. Wash this mixture containing bioactive compounds with three portions of 150 ml each with sodium chloride solution. Dry the organic layer over anhydrous magnesium sulfate. Filter and evaporate most of the solvent in vacuum without heating and obtained ethanolic extract of sweet cherry (EEC) of *Prunus avium*. Same procedure was followed for the preparation of ethylacetoacetate extract of sweet cherry (EAAEC) of *Prunus avium.*

Phytochemical screening:[6-8] Preliminary Phytochemical screening of ethanolic and ethyl acetoacetate extracts of sweet cherry of Prunus avium had shown the presence of various bioactive compounds such as carbohydrates, amino acids and peptides, phytosterols, carotenoids, and polyphenols etc.

Evaluation of Acute Oral Toxicity[9]

In the present study acute oral toxicity of the EEC and EAAEC were performed by the acute toxic class method. No sign of toxicity and mortality were observed at 200 mg/kg b. w of the animals and the LD_{50} value expected to exceed at 2500 mg/kg b. w and represented as class 5 (200 mg/kg < LD_{50} < 2500 mg/kg). From the toxicity studies the data revealed that both extracts proved to be non toxic at tested dose levels and well tolerated by the experimental animals as their LD_{50} cut of values > 2500 mg/kg b. w.

Evaluation Anti-inflammatory activity by Carrageen an Induced Paw Edema Method in Rats[10]

Anti-inflammatory activity was performed by carrageenan induced paw oedema method in rats. Diclofenac sodium (10 mg/kg I. p) was administered as standard drug for comparison. The extracts were administered at one dose level (200 mg/kg) by orally then 30 minutes prior to the administration of 0.1ml/kg body weight of carrageenan used in saline (1%w/v) into the lateral malleolus of sub-planter region of the rats left their hind paw. The paw volumes were measured using the mercury displacement technique with the help of a plethysmonograph immediately before and 30 minutes, 1, 2 and 3 hour after carrageenan injection. The percentage inhibition of paw edema was calculated by using the following formula:

Percentage protection = [(control-test)/control] ×100

RESULTS AND DISCUSSION

Anti-inflammatory activity of both extracts (EEC and EAAEC) was evaluated by carrageen an induced paw edema method. The activity was studied at a 200 mg/kg b. w. p. o. and their responses were measured at 30, 60, 120 and 180 min. The present experimental data shown in Table 1, displayed that both extracts exhibited mild to good anti-inflammatory activity. Graded dose response was also observed. Both the extracts exhibited highest activity at 120 min. When compared with standard drug diclofenac sodium (10 mg/kg i. p) the percent protection of EAAEC was found to be 68.9% and EEC was found to be 68.5%.

Table 1: for the Evaluation of Anti-inflammatory activity.

Groups	30 min		60 min		120 min		180 min	
	MEAN±SEM	%	MEAN ±SEM	%	MEAN ±SEM	%	MEAN ±SEM	%
Control	0.70 ±0.08	-	0.72±0.07	-	0.74±0.34	-	0.72±0.04	-
Diclofenac	0.42 ± 0.06	40.00	0.30±0.05	58.33	0.22±0.23	70.27	0.35±0.23	51.38
EEC	0.40± 0.049	38.42	0.288±0.48	57.10	0.22±0.21	68.5	0.34±0.22.9	52.20
EAAEC	0.41± 0.05	39.42	0.299±0.49	57.11	0.22±0.22	68.9	0.34±0.22	52.16

Significant differences with respect to control was evaluated by (ANOVA), Dunnet's t test * P<0.05. **P<0.01, NS (Non significant) % (Percentage reduction of edema).

CONCLUSION

In vivo anti-inflammatory activity displayed that both extracts (EEC and EAAEC) exhibited a good anti inflammatory activity and had percentage protection was found to be 68.5%, 68.9% and 70.27%.

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