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EVALUATION OF ANTI INFLAMMATORY EFFECTS OF BLUEBERRY (VACCINIUM) FRUIT EXTRACT IN WISTAR RATS : AN EXPERIMENTAL STUDY

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ABSTRACT

Background: Inflammation is a very common problem for physician consultation worldwide. Cyclooxygenase (COX) is involved in the development of inflammation and non-steroidal anti-inflammatory drugs (NSAIDs) represent one of the most common classes of medications used. Most of the NSAIDs are carboxylic acid and so cause severe side effects such as gastrointestinal ulceration, perforation, obstruction, and bleeding. In the recent years, the use of traditional medicine information on plant has again received considerable interest. One of the herbs, Blueberries, contains antioxidants which neutralize free radicals linked to the development of cancer, cardiovascular disease, and other age-related conditions. These berries contain anthocyanins, other polyphenols and various phytochemicals which are very good antioxidants. Blueberry contains polyphenols which attenuate inflammatory responses probably by reducing oxidative stress. The present study was undertaken to find a tasty and safe way to combat inflammations. **Objectives** of this study were to observe the effect of Blueberry on the volume of edema in a rat-paw-model and to compare this effect with that of Ibuprofen. **Result:** Vaccinium (Blueberry Fruit Extract) at the dose of 300mg/kg body weight was found to be prophylactic efficient on suppressing carrageenan induced acute inflammation in albino rats. Anti-inflammatory ability of Blueberry was found significant and comparable to Ibuprofen.

KEYWORDS: Anti-inflammatory effect, digital plethysmograph, Ibuprofen, Vaccinium sp., Rat-paw-edema model.

INTRODUCTION

Next to pain, inflammation is a very common problem for physician consultation worldwide. Cyclooxygenase (COX) is a prostaglandin endoperoxide synthase enzyme involved in the metabolism of arachidonic acid (AA) and synthesis of potent proinflammatory prostaglandins (PGE2, PGF2a).[1] This enzyme is an important target for the design and development of anti-inflammatory agents. Secondly, the free radicals especially, the reactive oxygen species (ROS) create oxidative stress in the cells leading to inflammatory conditions. Besides their defensive effects these excessively produced ROS deregulate the cellular functions causing cellular and tissue damage, which in turn augments the state of inflammation.[2]

Non steroidal anti-inflammatory drugs (NSAIDs) represent one of the most common classes of medications used worldwide with an estimated usage of >30million per day for inflammation and related disorders.[3] Most of the NSAIDs are carboxylic acid. This class includes salicylate derivatives (aspirin), carboxylic and heterocyclic acid derivatives (indomethacin), propionic acid derivatives (ibuprofen, ketoprofen, flurbiprofen) and phenyl acetic acid derivatives. Unfortunately, besides the excellent anti-inflammatory potential of the NSAIDs, the severe side effects such as gastrointestinal (GI) ulceration, perforation, obstruction, and bleeding has limited the therapeutic usage of NSAIDs.

According to World Health Organization (WHO), about three-quarters of the world population depends on traditional medicines (mainly herbs) for their healthcare. Ayurveda and Chinese medicinal systems are the most acceptable traditional system which has a considerable amount of research on pharmacognocy, chemistry, pharmacology and clinical therapeutics.[4] It is evident that several plants have been used in traditional ayurvedic medicine for treatment and management of distinct inflammatory disorders and wound healing activities.[5]

In the recent years, the use of traditional medicine information on plant has again received considerable interest.

Vaccinium has many species, among which cyanococcus is the commonest.[6] Blueberries contain antioxidants, which neutralize free radicals linked to the development of cancer, cardiovascular disease, and other age-related conditions. These little powerhouses provide tasty ways of staying healthy.

Medically important chemical present in blueberries are anthocyanins and polyphenols. Related with blueberries, many studies have been conducted and it was found that these polyphenols and anthocyanins are having very good antioxidant activity. Blueberry polyphenols attenuate inflammatory responses probably by reducing oxidative stress.[7]

There is a well-known saying that "a blueberry closes the doctor's door", which tells us a lot about the value of this berry.

The present study has been undertaken to evaluate the anti inflammatory effect of blueberry (Vaccinium cyanococcus) and to find a tasty way to manage pain and inflammation. This study will might give a future prospect of its use in the patients not willing to consume NSAIDS and pediatric patients.

OBJECTIVES

1. To observe the effect of Blueberry on the volume of edema induced by Carrageenan in Albino Rat.
2. To compare this effect of Vaccinium (Blueberry) with that of Ibuprofen.

MATERIALS

Experimental Animal: Wistar rats.

Chemicals and Test Material: Inflammatory agent Carrageenan was bought online from eBay.com (Batch: 150413, Seaweed Fertilizer Co, Bhavnagar, Gujrat). Test plant material Vaccinium (blueberry dry fruits) was bought online from Snapdeal.com (Invoice Number: S047D7/16-17/534, HW Wellness, Solutions PVT LTD, Hinjewadi, Pune). Standard anti inflammatory agent ibuprofen suspension was purchased from a local medical store.

Instruments: Digital Plethysmograph (PLM 01 Plus Model), Orchid.

METHODOLOGY

It was an experimental study which was conducted in Dept of Pharmacology, JNMC, Sawangi, Wardha and completed in 3 Months.

For this project, as a total 30 Wistar Rat of both sexes was used. Healthy rats with body weight 100 -150 gm were selected while special precaution was taken for not taking pregnant rats and rats with abrasive wounds in paws. After selecting the animals, all 30 animals were acclimatized in the environment (25 ± 3°C), with light/dark control each 12 hours (7 a.m. to 7 p.m.) and were placed in cages up to 6 rats & were provided with proper meal & water ad libitum. They were kept without any food 12 hours before the experiments, but water ad libitum.

Preparation

1 % suspension of Carrageenan was prepared in NS. Extract of blueberries were prepared with the help of soxhlet apparatus. Before using soxhlet apparatus blueberries were finely grounded in a grinder and semisolid paste of it was prepared.

All the 30 animals were divided into 5 equal groups (n=6). Group I (Control) was given 1 ml distilled water orally. Group II was given ibuprofen 10 mg/kg orally.[8] Group IIIa, IIIb, and IIIc were given aqueous blueberry fruit extract (orally) in doses of 100 mg/kg, 200 mg/kg and 300 mg/kg respectively. After 30 minutes of administration of standard anti-inflammatory drug ibuprofen and test plant extract of blueberries, 0.1 ml 1 % Carrageenan was injected intradermally[9] in left paw of all the 30 animals.

Volume of edema in all five groups was observed by digital plethysmography at 1hour, 3 hours and 5 hours. Reductions in edema by NS, ibuprofen and mentioned increasing doses of blueberry extract were calculated and depicted in tables. Then percentage of reduction in edema was calculated with the formula:

% Reduction in Edema =

$$\frac{(\text{Mean edema in Control Group} - \text{Mean Edema in drug Treated Group}) * 100}{\text{Mean edema in Control Group}}$$

All the observations were analyzed statistically unpaired student test, one Way ANOVA test. Multiple Comparison was done by Tukey Test. Software used in the analysis were SPSS 17.0 version & EPI-INFO 6.0 version.

OBSERVATIONS

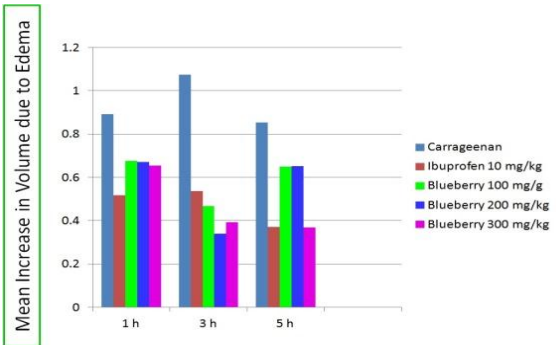

Fig. 1: Mean Edema after 1, 3 and 5 h of Carrageenan administration in Group I, II, IIIa, IIIb and IIIc.

Table 1: Means and Standard Deviation (SD) Edema in the rat-paw observed at 1, 3 and 5 hours of Carrageenan administration.

Groups (n=6)	Material & Dose	Mean & SD	After 1 h	After 3 h	After 5 h
Group I Control	Carrageenan 1 ml of 1% solution	Mean SD	0.89166 .16857	1.075 .51795	0.85333 0.33404
Group II Standard Drug	Ibuprofen 10 mg/kg	Mean SD	0.51833 0.04355	0.53666 ± 0.25672	0.37 0.34415
Group IIIa	Blueberry 100 mg/kg	Mean SD	0.675 0.318292	0.46667 ± 0.29642	0.64833 0.48201
Group IIIb	Blueberry 200 mg/kg	Mean SD	0.67 0.20552	0.34 ± 0.29786	0.65167 0.36842
Group IIIc	Blueberry 300 mg/kg	Mean SD	0.65333 0.270456	0.39167 0.1477	0.36833 0.14289

RESULT

Table 2: Percentage of reduction in edema by Ibuprofen and Vaccinium in rat-paw-model.

	Percentage of reduction in edema		
Ibuprofen 10 mg/kg	41.9	50	56.64
Blueberry 100 mg/kg	24.3	56.6	24.02
Blueberry 200 mg/kg	25	68.37	23.63
Blueberry 300 mg/kg	26.72	63.6	56.83

Table 3: One Way ANOVA Test indicating Significance of Difference.

		Sum of Squares	Df	Mean Square	F	p-value
1 hour	Between Groups	.427	4	.107	2.163	.103,NS
	Within Groups	1.235	25	.049		
	Total	1.662	29			
3 hour	Between Groups	2.107	4	.527	4.946	.004,S
	Within Groups	2.663	25	.107		
	Total	4.770	29			
5 hour	Between Groups	1.040	4	.260	2.103	.111,S
	Within Groups	3.093	25	.124		
	Total	4.133	29			

Table 1 shows that reduction in amount of edema produced by 100 mg/kg blueberry was very less than that of 10 mg/kg ibuprofen.

When dose of blueberry was increased to 200 mg/kg, reduction in edema was found more than Ibuprofen at 3 hours. 300 mg/kg blueberry was found more effective at 3 hours and equally effective after 5 hours, compared to ibuprofen.

One Way ANOVA Test shows statistically significant reduction in rat-paw-edema (P= 0.004 at 3 hours with 200 mg/kg blueberry and P=0.111 at 3 and even at 5 hours with 300 mg/kg blueberry).

DISCUSSION

Carrageenan produces biphasic inflammatory responses. First phase is due to the release of histamine and other kinins while second phase is due to prostaglandins, PGE2.[1] Anthocyanins content of Blueberry have inhibitory effect on cyclooxygenase -2 leading to decrease in PGs synthesis. This is the most probable mechanism of inhibition of acute inflammation. Antioxidants activities of blueberries are effective in controlling in sub acute and chronic inflammation.

Experiments of Neuza Mariko and colleagues explain presence some chemical like Cyanidin-3-Glucoside responsible for COX-2 inhibition and its role in chronic inflammation.

Blueberry fruit like most berries, is rich in flavonoids, tannins and phenolic acids. Many studies have indicated that the blueberry has several beneficial health properties associated with the presence of such bioactive compounds, especially anthocyanins.

This fruit, like most berries, is rich in flavonoids, tannins and phenolic acids. Many studies have indicated that the blueberry has several beneficial health properties associated with the presence of such bioactive compounds, especially anthocyanins.[10]

Reduction in amount of edema produced by blueberry was found significantly effective at all the given doses compared to control group. It was noted that minimum percentage in reduction of edema occurred with 100 mg/kg blueberry at 1 hour and it was found 24.3 %. This 24.3 % reduction indicates a good control over inflammatory reaction. But this anti-edema was very less compared to 10 mg/kg ibuprofen.

When dose of blueberry was increased to 200 mg/kg, reduction in edema was found more than Ibuprofen at 3 hours and less than it after 5 hours. This decrease in anti-edema effect was probably due to faster excretion of blueberry. Compared to 10 mg/kg ibuprofen, 300 mg/kg, blueberry was found more effective at 3 hours and equally effective after 5 hours, compared to ibuprofen.

Difference in the percentage reduction of edema depends on the amount of anthocyanins and polyphenols present in blueberry. Mazza and colleagues (2002) studied the various pharmacokinetic aspects of blueberry anthocyanins and found that its peak plasma level is achieved from 2 – 3 hours depending on the dose of oral blueberry.[11]

Sonia R. Pereira and colleagues have demonstrated, in an in vitro intestinal cell model, the higher anti-inflammatory activity of cyanidin-3-glucoside in comparison with 5-aminosalicylic acid (5-ASA), a well-established anti-inflammatory drug in IBD.[12]

The important difference in anti-inflammatory actions of NSAIDS and blueberry is due to its components and mechanism. The major component of NSAIDS is carboxylic acid while major components of blueberries are anthocyanins and ploy phenols. Anti-inflammatory action of NSAIDS is due inhibition of prostaglandin but blueberry decreases inflammatory cytokines and scavenges free radicals. This leads to significant down regulation of total ROS, resulting in anti-inflammatory action by increasing 5-HT levels without increasing NE levels.

CONCLUSION

Aquous extrat of blueberry fruit (Vaccinium) was found prophylactically as effective as standard drug ibuprofen, at the dose of 300 mg/kg after 3 and 5 hours of administration of carrageenan. Therefore, it can be concluded that a pleasant herbal approach using blueberries might be a useful addition for its anti-inflammatory action.

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