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RESEARCH ARTICLE

EVALUATION OF ANALGESIC ACTIVITY OF CRUDE EXTRACT OF BLUEBERRY (VACCINIUM) FRUIT IN WISTAR RATS

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ABSTRACT

Background: Pain is a very common complaint for a medical shop visit worldwide. Prostaglandin, a Cyclooxygenase (COX) pathway intermediate product is involved in the development of inflammation and pain. Non-steroidal anti-inflammatory drugs (NSAIDs) represent one of the most common classes of medications used with or without prescription. Most of the NSAIDs are liable to cause severe side effects such as gastrointestinal ulceration, perforation, obstruction, and bleeding because of its carboxylic acid moiety. Nowadays, the use of traditional medicine information on plant has got considerable interest. Among many medicinal herbs, Blueberry is very important as it 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 aim of this study was to evaluate the potential effects of Crude extract of Blueberry fruits (Vaccinium) on nociception and to get a tasty and safe way to fight with pain.
Objectives of this study was to observe the effect of Blueberry on the response time by licking of rat-paw and to compare this effect with that of Aspirin.
Result: Blueberry Fruit Extract at the dose of 300mg/kg body weight was found prophylactically efficient on suppressing pain and increasing pain threshold induced temperature in albino rats. Analgesic ability of Blueberry was found significant and comparable to Aspirin at this dose.

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INTRODUCTION

Pain is probably the most common cause for physician consultation worldwide. Cyclooxygenase, an enzyme and prostaglandin endoperoxide synthase enzyme involved in the metabolism of arachidonic acid (AA) and synthesis of potent proinflammatory prostaglandins (PGE2, PGF2a). The free radicals especially, the reactive oxygen species (ROS) create oxidative stress in the cells leading to inflammatory conditions and so pain.. Besides their defensive effects these excessively produced ROS derange the cellular functions causing cellular and tissue damage, which in turn augments the state of pain and inflammation (Vittorio Limongelli *et al*., 2010). Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage. In medical diagnosis, pain is regarded as a symptom of any unpleasant underlying condition. Pain motivates the individual to withdraw from damaging situations, to protect a damaged body part while it heals, and to avoid similar experiences in the future. Most of the NSAIDs are carboxylic acid and Aspirin is a very commonly used drug of this class. This class also 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. Systems of Ayurvedic and Chinese medicine are the most acceptable traditional system which has a considerable amount of research on pharmacognocy, chemistry, pharmacology and clinical therapeutics (Gacche *et al*., 2011). 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 (Subarnas and Wagner, 2000). In the recent years, the use of traditional medicine information on plant has again received considerable interest. Previous studies suggest that proanthocyanidin of Vaccinium species might have analgesic and anti-inflammatory activity. These activities are might be due to the inhibition of prostaglandin biosynthesis, because the proanthocyanidin fraction had an inhibitory effect

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on cyclooxygenase, but not on 5-lypoxygenase enzymes (Lisa J. Rowland and Freddi A. Hammerschlag, 2005). Vaccinium has many species, among which cyanococcus is the commonest. 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, flavonoids 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 and decreases pain sensation probably by reducing oxidative stress (Kalt *et al*., 2001). 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.

Herbal approaches usually have been pursued because of the perception that many of these therapies are gentler and cause fewer side effects than pharmaceuticals. Among herbs, blueberries are among the edible fruits that are recognized best for their potential health benefits. However, this perception must be tested in each case. It is important to know and remember that herbs contain active chemicals that may have side effects or interactions with foods and other drugs. For the management of pain, a constant research based on natural pharmacophores and its interaction with targets, has led to search of many potential therapeutic agents[6]. The present study has been undertaken to evaluate the analgesic ability of blueberry (Vaccinium) 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 response time with prophylactic Blueberry extract to pain stimulus induced by heat in Albino Rat.
2. To compare this effect of Blueberry (Vaccinium) with that of Aspirin.

MATERIALS AND METHODS

Experimental Animal: Wistar rats

Chemicals and Test Material: Distilled water was prepared in department of Biochemistry, Jawaharlal Nehru medical College, Sawangi, Wardha. Standard analgesic drug, Aspirin, was purchased from a local medical shop. Test plant material blueberry (Vaccinium) dry fruits) was bought online from Snapdeal.com (Invoice Number: S047D7/16-17/534, HW Wellness, Solutions PVT LTD, Hinjewadi, Pune).

Instruments: Hot Plate Analgesiometer.

Methodology

This experimental study which was conducted in Dept of Pharmacology, JNMC, Sawangi, Wardha and completed in 3 Months. The hot plate test was used to measure latency response, according to method previously described by Eddy and Leimbach, with minor modifications. All animals were selected beforehand, based on their reactivity in the model. Rats that exhibited a pre-treatment reaction time greater than 12 seconds were not used in subsequent tests (Maria Rosana Ramirez *et al*., 2010). For this project, as a total 18 Wistar Rat of both sexes was used. Healthy rats with body weight 100-150 gm were used while special precaution was taken for not taking pregnant rats and rats with abrasive wounds in paws. After selecting the animals, they 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. For the study of antinociceptive activity, rats were divided into three equal groups (n=6): Group I, Group II and Group III. Group I was served as the negative control group and pretreated with distilled water (DW) 0.5 ml orally while Group II was served as positive control and pretreated with the standard analgesic drug Aspirin 200 mg/kg orally (Ghosh 6th edition). Group III was served as the test group and given Blueberry fruit extract 100, 200 and 300 mg/kg on day 1, 2 and 3 respectively, hence named Group III D1, Group III D2 and Group III D3.

Preparation

Dispersible Aspirin tablets were dissolved in distilled water. Extract of blueberry was 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. After 30 min of administration of Distilled water, Aspirin and Blueberry fruit extract. All animals were individually exposed to heat by using Eddy's hot plate analgesiometer apparatus. Heat was used as the pain stimulus. This instrument was maintained at 55±0.50 degree centigrade. The reaction (response) was noted for all the animals. Response time was considered as the duration of time spent by rats from keeping them on the hot plate to start of licking of the footpad or jumping. The cut off time used were 30 seconds to avoid thermal injury. The observations were taken at 0, 30, 60, 90 and 120 and 150 minutes after treatments with DW, Aspirin and Blueberry extract. Response time for each drug and dose were depicted in tables and the analgesic activity of Blueberry extract was compared with that of Aspirin (positive control) and DW (negative control). Differences were statistically analyzed by One Way ANOVA test. Multiple Comparison was done by Tukey Test. Software used in the analysis were SPSS 17.0 version and EPI-INFO 6.0 version.

OBSERVATION AND RESULTS

For all the response time, Mean and Standard Deviation (SD) was calculated and depicted in table and bar chat. Table 1 showed no significant difference between negative control and effect of blueberry 100 mg/kg. But at the dose of 200 and 300 mg/kg, Blueberry induced statistically significant increase in response time compared to Negative Control. Aspirin was found clearly better analgesic than blueberry 100 and 200 mg/kg. While there was no significant difference between analgesic ability of Aspirin 200 mg/kg and Blueberry 300 mg/kg. One Way ANOVA result showed means for all the time-interval, for Group I (DW), Group II (Aspirin 200 mg/kg), Group III D1 (Blueberry 100 mg/kg), Group III D2

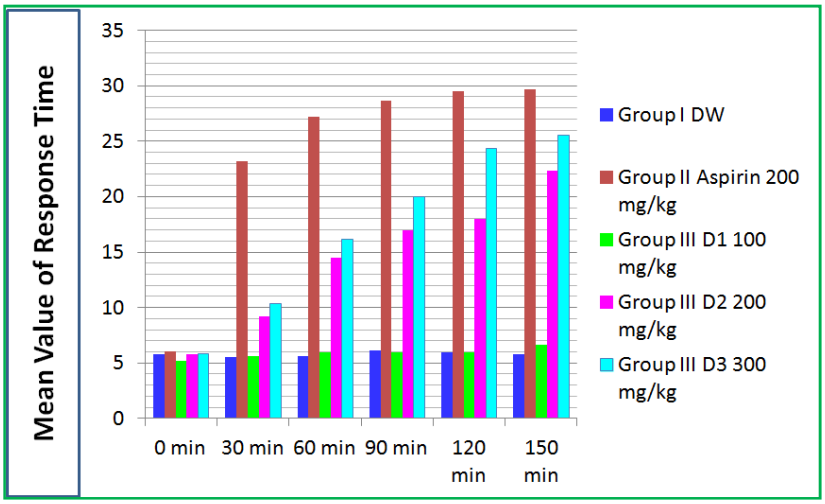

Fig.1. Bar-Chart showing Mean value of response time on Hot Plate

Table 1. Means and Standard Deviation (SD) response time for all groups

Groups	Material & Dose (Oral)	Mean & Standard Deviation (SD)	0 min	30 min	60 min	90 min	120 min	150 min
Group I Negative Control	DW	Mean	5.8	5.5	5.6	6.1	6	5.8
	0.5 ml	SD	1.57	1.05	1.37	0.8	1.1	1.16
Group II Positive Control	Aspirin	Mean	6.06	23.16	27.16	28.66	29.5	29.66
	200 mg/kg	SD	1.30	2.3	1.72	1.2	0.83	1.16
Group III D1	Blueberry	Mean	5.16	5.66	6	6	6	6.66
	100 mg/kg	SD	1.16	0.51	1.2	0.63	1.09	0.81
Group III D2	Blueberry	Mean	5.83	9.16	14.5	17	18	22.33
	200 mg/kg	SD	0.75	0.75	1.76	1.54	1.09	2.25
Group III D3	Blueberry	Mean	5.83	10.33	16.16	20	24.33	25.5
	300 mg/kg	SD	1.16	1.5	2.4	2.1	2.6	2.07
P Value	Group I vs Group III D1, p = 0.19		Group II vs Group III D1, p = 0.007					
	Group I vs Group III D2, p = 0.0049		Group II vs Group III D2, p = 0.02					
	Group I vs Group III D3, p = 0.005		Group II vs Group III D3, p = 0.18					

(Blueberry 200 mg/kg) and Group III D3 (Blueberry 300 mg/kg) as 5.8, 24.03, 5.91, 14.47 and 16.97 in seconds respectively. The f- ratio value was found 10.01739 and $p<0.05$, which was statistically significant. When analgesic effect of blueberry extract 100 mg/kg was compared to negative control, the difference was found insignificant. But this plant extract was found statistically very much significant analgesic when given in dose of 200 mg/kg and 300 mg/kg. Blueberry extracts 300 mg/kg found comparable to positive control Aspirin.

DISCUSSION

Pain is acute as well as chronic symptom of many physiological, psychological and pathological conditions. It may be associated with prostaglandins, PGE2. 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 pain. Antioxidants activities of blueberries are effective in controlling in sub acute and chronic inflammation and inflammatory pain. 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 (Masuelli *et al*., 2012). Preliminary studies indicate that flavonoids may affect anti-inflammatory mechanisms via their ability to inhibit reactive oxygen or nitrogencompounds (Wang *et al*., 1999). Flavonoids have also been proposed to inhibit the pro-inflammatory activity of enzymes involved in free radical production, such as cyclooxygenase, lipoxygenase or inducible nitric oxide synthase, and to modify intracellular signaling pathways in immune cells, or in brain cells after a stroke. Increase in response time indicates the increased threshold for pain. This is due to central and peripheral control over the production of nociceptive substances. According to previous studies, analgesic and anti-inflammatory activity also responded to proanthocyanidin content of berries. In the present study it was noted that minimum increase in response time occurred when Blueberry was given 100 mg/kg. Blueberry in higher dose 200 mg/kg was found effective in increasing response time in the experimental rats, compared to negative control. In another study Aglycone cyanidin showed better anti-inflammatory activity than aspirin when used an in vitro model that monitors the ability to inhibit cyclooxygenase conversion of arachidonic acid to prostaglandins (Mazza *et al*., 2002).

But in this study antinociceptive effect of blueberry 200 mg/kg was again very less compared to 200 mg/kg aspirin. When dose of blueberry was increased to 300 mg/kg, analgesic ability was found comparable to aspirin after 150 min administration. Such finding indicated that blueberry was found less potent analgesic and with delayed onset of action. Aspirin's action was obvious after 30 min while analgesic action Blueberry was not noticeable up to 60 min. This may be due to its faster excretion at low dose. At the same time peripheral mechanism may also be the reason for late effectiveness. In this model, Vaccinium inhibited the licking response of rats more effectively after 120 min, suggesting this

compound exerts its antinociceptive effects connected with peripheral mechanisms. Difference in the amount of increase in response time on hot plate 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. 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. The important difference in analgesic 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 and nociceptive 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. And less inflammation causes less pain.

Conclusion

Analgesic effect of Blueberry (Vaccinium) was not comparable to aspirin at lower dose like 100 and 200 mg/kg but at higher dose 300mg/kg this fruit extract was found significantly analgesic compared to negative control and almost equally effective to aspirin. That means blueberry is effective analgesic with low potency and slow onset of action. Therefore, it can be concluded that a pleasant herbal approach using blueberries might be a useful addition for its analgesic action.

Recommendations

Study of Analgesic effect of Blueberry is required in the clinical set up so that we may have an herbal analgesic preparation for the patient suffering from mild pain and having inclination for the herbal medication. This would be specially helping to pediatric patient because of its sweet taste.

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