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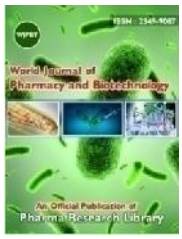

Research Article **Open Access**

Phytochemical screening and Evaluation of Analgesic activity of various extracts of beet root (*Beta vulgaris*)

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A B S T R A C T

Analgesia, loss of sensation of pain that results from an interruption in the nervous system pathway between sense organ and brain. Different forms of sensation (e.g., touch, temperature, and pain) stimulating an area of skin travels to the spinal cord by different nerve fibres in the same nerve bundle. Therefore, any injury or disease affecting the nerve would abolish all forms of sensation in the area supplied by it. When sensory nerves reach the spinal cord, however, their fibres separate and follow different courses to the brain. Thus, it is possible for certain forms of sensation to be lost, while others are preserved, in diseases that affect only certain areas of the spinal cord. Because pain and temperature sensations often travel the same path, both may be lost together. Diseases of the spinal cord that may cause analgesia without loss of the sensation of touch are tabes dorsalis, syringomyelia, and tumours of the cord. The term is also used for pain relief induced by the action of such medications as aspirin, codeine, and morphine. An analgesic or painkiller is any member of the group of drugs used to achieve analgesia, relief from pain. Analgesic drugs act in various ways on the peripheral and central nervous systems. They are distinct from anaesthetics, which temporarily affect, and in some instances completely eliminate, sensation. Analgesics include paracetamol, the non-steroidal anti-inflammatory drugs (NSAIDs) such as the salicylates, and opioid drugs such as morphine and oxycodone. The main aim and objective of my present research work was the preliminary phytochemical screening of various extracts of beet root (***Beta vulgaris,*** EEBT, MEBT and CEBT) and evaluation of analgesic activity. The analgesic activity of various extracts **EEBT**, **MEBT and CEBT of Beta vulgaris** were evaluated by Tail-immersion method. The present experimental data displayed that all the extracts (**EEBT** and **MEBT and CEBT) of Beta vulgaris executed very** good analgesic activity at 200 mg/kg body weight. The highest analgesic activity was observed at 60 min for all the extracts (200 mg/kg). The percentage protection of EEBT and MEBT and CEBT and standard drug Pentazocine for analgesic activity were found to be **84.12%, 81.26%, 83.54%, 45.94% etc.**

Keywords: Analgesia, anaesthetics, NSAIDs, oxycodone, Tail-immersion method

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1. Introduction

Beta vulgaris (beet) is a plant in the Amaranthaceae family (which is now included in Betoideae subfamily) [1-5]. It has numerous cultivated varieties, the best known of which is the root vegetable known as the beetroot or garden beet. Other cultivated varieties include the leaf vegetable chard; the sugar beet, used to produce table sugar; and mangelwurzel, which is a fodder crop. Three subspecies are typically recognised. All cultivated varieties fall into the subspecies Beta vulgaris subsp. vulgaris. Beta vulgaris subsp. maritima, commonly known as the sea beet, is the wild ancestor of these and is found throughout the Mediterranean, the Atlantic coast of Europe, the Near East, and India. A second wild subspecies, Beta vulgaris subsp. adanensis, occurs from Greece to Syria.The roots are most commonly deep red-purple in color, but less common varieties include golden yellow and red-and-white striped roots [6]. Beta vulgaris is an herbaceous biennial or, rarely, perennial plant with leafy stems growing to 1–2 m tall. The leaves are heart-shaped, 5–20 cm long on wild plants (often much larger in cultivated plants). The flowers are produced in dense spikes; each flower is very small, 3–5 mm diameter, green or tinged reddish, with five petals; they are wind pollinated. The fruit is a cluster of hard nutlets.

Figure 1: Yellow-stemmed chard (with purple-leaved kale).

Figure 2: Beet root plant

The taxonomy of the various wild and cultivated races of beets has a long and complicated history. Mansfeld's Encyclopedia of Agricultural and Horticultural Crops following Letschert's 1993 treatment of Beta, section Beta recognizes the following taxa [7] for cultivated varieties, which are grown for their taproots, leaves, or swollen midribs: B. v. ssp. vulgaris convar. cicla (leaf beet or chard) - The leaf beet group has a long history dating to the second millennium BC. The first cultivated forms were believed to have been domesticated in the Mediterranean, but were introduced to the Middle East, India, and finally China by 850 AD. These were used as medicinal plants in Ancient Greece and Medieval Europe. Their popularity declined in Europe following the introduction of spinach. B. v. ssp. v. convar. cicla. var. cicla (spinach beet) - This variety is widely cultivated for its leaves, which are usually cooked like spinach. It can be found in many grocery stores around the world [8]

Nutrition

Beets are low in calories (about 45 kcal per 100 g) and have zero cholesterol and a minute amount of fat. Nutrition comes from the beets' vitamins, minerals, and unique plant-derived anti-oxidants. A phytochemical compound, glycine betaine, is found in the root. Betaine lowers the chance of coronary heart disease (CHD), stroke, and peripheral vascular diseases. Beets in raw form are high in folates. Folates are essential in the synthesis of DNA within cells. Vitamin-C is found in small amounts.The root provides B-complex vitamins including niacin (B-3), pantothenic acid (B-5), and pyridoxine (B-6), and minerals such as iron, manganese, copper, magnesium, and potassium, lowers the heart rate and regulates metabolism in the cells. Beet greens contain vitamin C, carotenoids, flavonoid anti-oxidants, and vitamin-A [9-10].

Possible health benefits of consuming beetroot [10]

Consuming fruits and vegetables of all kinds has long been associated with a reduced risk of many lifestyle-related health conditions. Many studies have suggested that increasing consumption of plant foods like beetroot decreases the risk of obesity and overall mortality, diabetes, heart disease and promotes a healthy complexion and hair, increased energy, overall lower weight. Heart health and blood pressure: A 2008 study published in Hypertension examined the effects of ingesting 500 mls of beetroot juice in healthy volunteers and found that blood pressure was significantly lowered after ingestion. Researchers hypothesized this was likely due to the high nitrate levels contained in beet juice and that the high nitrate vegetables could prove to be a low cost and effective way to treat cardiovascular conditions and blood pressure. Another study conducted in 2010 found similar results that drinking beetroot juice lowered blood pressure considerably on a dose-dependent basis.

Dementia:

Researchers at Wake Forest University have found that drinking juice from beetroot can improve oxygenation to

the brain, slowing the progression of dementia in older adults. According to Daniel Kim-Shapiro, director of Wake Forest's Translational Science Center, blood flow to certain areas of the brain decrease with age and leads to a decline in cognition and possible dementia. Consuming beetroot juice as part of a high nitrate diet can improve the blood flow and oxygenation to these areas that are lacking.

Diabetes:

Beets contain an antioxidant known as alpha-lipoic acid, which has been shown to lower glucose levels, increase insulin sensitivity and prevent oxidative stress-induced changes in patients with diabetes. Studies on alpha-lipoic acid have also shown decreases in peripheral neuropathy and/or autonomic neuropathy in diabetics. However, a meta-analysis suggests that the benefits of alpha-lipoic acid for symptomatic peripheral neuropathy may be restricted to intravenous consumption of the acid; the authors conclude that "it is unclear if the significant improvements seen after 3-5 weeks of oral administration at a dosage of >600 mg/day are clinically relevant."6

Digestion and regularity:

Because of its high fiber content, beetroot helps to prevent constipation and promote regularity for a healthy digestive tract.

Inflammation:

Choline is a very important and versatile nutrient in beetroot that helps with sleep, muscle movement, learning and memory. Choline also helps to maintain the structure of cellular membranes, aids in the transmission of nerve impulses, assists in the absorption of fat and reduces chronic inflammation.

Exercise and athletic performance:

Beetroot juice supplementation has been shown to improve muscle oxygenation during exercise, suggesting that increased dietary nitrate intake has the potential to enhance exercise tolerance during long-term endurance exercise. Quality of life for those with cardiovascular, respiratory, or metabolic diseases, who find the activities of daily living physically difficult because of lack of oxygenation, could be improved. Beetroot juice improved performance by 2.8% (11 seconds) in a 4-km bicycle time trial and by 2.7% (45 seconds) in 16.1-km time trial.

2. Materials and Methods

Drugs and chemicals:

The standard drugs pentazocin (Analgesic) was purchased from Local Retail Pharmacy Shop and solvents and other chemicals used for the extraction and phytochemical screening used from Institutional Store and were of 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/12/2015**.

Methodology for extraction [11]:

Weigh 20 g of beet root paste (root can be mashed or grined 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 beet root **(EEBT) of *Beta vulgaris*.** Same procedure was followed for the preparation of methanolic and chloroform extracts **(MEBT, CEBT)** of ***Beta vulgaris.***

Phytochemical scereening [12-14]:

Preliminary Phytochemical screening of EEBT, MEEBT and CEBT had shown the presence of various bioactive compounds such as carbohydrates, amino acids and peptides, phytosterols, carotenoids, and polyphenols etc. Evaluation pharmacological activity

Evaluation of Pharmacological activity

Acute oral toxicity studies [15]: In the present study the acute oral toxicity of the **EEBT, MEEBT and CEBT** were performed by acute toxic class method. In this method the toxicity of the extract was planned to test using step wise procedure, each step using three Wister rats. The rats were fasted prior to dosing (food but not water should be withheld) for three to four hrs. Following the period of fasting the animals were weighed and the extract was administered orally at a dose of 2000 mg/Kg b. w. Animals were observed individually after dosing at least once during the first 30 min; periodically the surveillance was carried out for the first 24 hrs with special attention given during the first 4 hrs and daily thereafter, for a total of 14 days

Evaluation of Analgesic activity by Tail-Immersion method [16]: The analgesic activity of various extracts such as **EEBT, MEEBT and CEBT** were evaluated by tail-immersion method. Wister rat (n=6)** of either male or female sex was chosen by random sampling technique and used for the evaluation of analgesic activity. The standard drug **PNTZ** (Pentazocine) was administered at the dose of 20 mg/kg (i. p.) for comparison. The test extracts were administered at 200 mg/kg by the oral route. The rats were hold in position by a proper restrainer with the tail extending out and the tail (up to 6 cm) was taken and dipped in a beaker. In the beaker the temperature of water should be maintained at 56 ± 4^0C. The time in second taken by the rats to withdraw their tail completely out of water. These were the reaction time. The observation was carried out at 0, 15, 30, 60 and120 min after the administration of extracts [16]. A cut off point of 15 sec was observed to avoid the tail damage. The percentage analgesic activity was easily calculated by the following formula:

PAA = [(B-A)/B] X 100

B is the reaction time (in sec) before treatment.

A is the reaction time (in sec) after treatment.

PAA is the percentage analgesic activity.

3. Results and Discussion

Phytochemical scereening:

Preliminary phytochemical screening of EEBT, MEEBT and CEBT had shown the presence of various bioactive compounds such as carbohydrates, amino acids and peptides, phytosterols, carotenoids, and polyphenols etc. Evaluation pharmacological activity

Acute oral toxicity study:

Acute oral toxicity studies were performed according to the OECD guideline 423 method. This method has been designed to evaluate the substance at the fixed doses and provide information both for hazard assessment and substance to be ranked for hazard classification purposes. The extract was administered initially at a dose of 2000 mg/kg b. w and 1% CMC (p .o) and observed 14 days mortality due to acute toxicity. Careful observation were made at least thrice a day for the effect on CNS, ANS, motor activity, salivation and other general signs of toxicity were also observed and recorded. Since no sign of toxicity observed at 2000 mg/kg b. w. to the group of animals, the LD_{50} value of the **extract** expected to exceed 2000 mg/kg b. w. and represented as class 5 (2000 mg/kg < LD50 < 2500 mg/kg). From the toxicity studies the data revealed that all the synthesized compounds proved to be non toxic at tested dose levels and well tolerated by the experimental animals as there LD_{50} cut of values > 2000 mg/kg b. w.

Table 1: for the dose selection by acute toxicity class method (OECD) guide lines 423 of EEBT, MEBT & CEBT

Sl. No.	Treatment group	Dose mg/kg	Sign of toxicity	Onset of toxicity	Duration
1.	EEBT	200	No	No	14 days
2.	MEBT	200	No	No	14 days
3.	CEBT	200	No	No	14 days

Table 2: For the analgesic activity of various extracts of watermelon

Extracts	Dose (mg/ kg)	0 min	15 min		30 min		60 min		120 min	
		MEAN + SEM	MEAN ± SEM	%	MEAN ± SEM	%	MEAN ± SEM	%	MEAN ± SEM	%
EEBT	200	6.30ï0.23	6.34ï0.25*	37.00	9.53ï0.2*	49.83	12.97ï0.27*	84.12	10.02ï0.22*	67.91
MEBT	200	5.87ï0.21	6.46ï0.23*	39.71	9.73ï0.52*	50.10	12.19ï0.27*	81.26	8.20ï0.28*	61.21
CEBT	200	5.79ï0.24	6.2ï0.20*	26.62	9.01ï0.22*	42.50	12.05ï0.25*	83.54	8.81ï0.33*	59.08
PNTZ	20	5.90ï0.22	6.28ï0.22	24.76	8.67ï0.33	36.94	10.51ï0.22	45.94	8.51ï0.25	60.91

Each value is mean pain reaction time (in sec) + SEM using 6 animals in each group. Significant differences with respect to 0 min reaction time was evaluated by (ANOVA), Dunnet's t test *P< 0.05, **P<0.01, NS (Non significant), % (Percentage Analgesic activity).

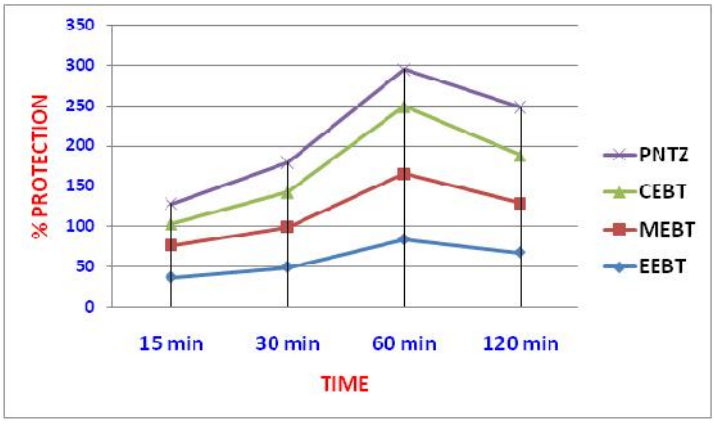

Figure 1: Analgesic activity of the various extracts of Beta vulgaris at dose of 200 mg/kg body weight

Evaluation of Analgesic Activity: The analgesic activity of various extracts (**EEBT, MEBT & CEBT**) of ***Beta vulgaris*** were evaluated by using the tail-immersion method. The activity was studied at 200 mg/kg b. w. (p. o) and effect was measured at the time interval of 15, 30, 60 and 120 min. **All the extracts had shown** significant analgesic activity and also the graded dose response was observed. The highest analgesic activity was observed at 60 min for all the extracts (200 mg/kg). When compared with standard drug (Pentazocine 20 mg/kg, i. p) all the extracts (**EEBT, MEBT & CEBT) of *Beta vulgaris* had shown very** good analgesic activity at 200 mg/kg b. w.

4. Conclusion

The results obtained from the ***in-vivo*** studies displayed that the various extracts of **Beta vulgaris (EEBT, MEBT and CEBT**) possessed a very good analgesic activity and it had been concluded that **EEBT, MEBT and CEBT**, all were exhibiting the potential capability to reduce pain and

inflammation when compared with standard drug Pentazocine.

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