Anti-nociceptive potential of lyophilized *Beta vulgaris* L. (Beet root) powder

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Abstract: Pain is a very common symptom and pain management is most important challenge to alleviate the suffering and for improving the quality of life of those living with chronic pain. Beet root is a vegetable consumed as salad and present study is designed to evaluate the analgesic activity of its lyophilized extract. The study was conducted in 2016 (March) on albino mice of both sexes weighing 18-25 gm, divided into groups comprising of 10 animals each. Group I was taken as control and administered 0.1ml distilled water orally. Group II was labeled as Treated and administered lyophilized beet root in the dose of 1000mg/kg. Group III was taken as Standard and was given acetyl salicylic acid 300mg/60kg. Analgesic activity was evaluated using Hot plate apparatus, Tail Flick method and Writhing method. Results showed significant ($p<0.001$) analgesic effect by beet root as compared to control. Beet root also showed significant ($p<0.001$) effect as compared to standard but the effect was not seen from the beginning indicating its role in the second phase of analgesia. This study suggests that beet root powder possesses analgesic potential and can be used for central as well as for peripheral analgesia.

Keywords: Analgesic, beet root, *Beta vulgaris* L., lyophilized, nociception, pain.

INTRODUCTION

Pain is essential for the maintenance and preservation of body and gives multidimensional experience. It alerts the body to trauma and injury and warns of danger. An unpleasant response that occurs due to tissue damage is pain which has been defined by IAPS (International Association of study of pain) (www.ninds.nih.gov).

The feeling and unpleasant sensation of pain occurs due to the activation of insula (limbic sensory) and cingulate (limbic motor cortices) besides that somatosensory cortice is also thought to play a role (Coull *et al*., 2005). The classification of pain is based on organization of nerve fibers. There are two types of nerve fibers: Fiber type C includes nerve fibers which are unmylelinated, are small in diameter hence conduct the nerve impulse slowly whereas Fiber type A delta are highly myelinated nerve fibers having a larger diameter that can conduct nerve impulses at faster rate. The sensation of pain is divided into two phases an epicritic pain which is sharp, fast pain that occurs initially and a protopathic pain that occurs at later stage and is dull, slow and long lasting (Hashmi, 2010). Acute and chronic pains are different clinical entities. A normal sensation that alerts us to possible injury is an acute pain whereas pain lasting longer than 12 weeks is known as chronic pain (Grichnik and Ferrante, 1991). There are two main types of ascending pain pathways i.e. the spino-parabrachial pathway and the spino-thalamic pathway that are involved in the transmission of pain impulse from spinal cord to brain stem and thalamus. In the thalamus the pain impulse is directed to multiple areas for processing as the brain does not have a discrete pain center (Farquhar-Smith, 2007). For treating acute pain the prescription pattern normally involves non-steroidal anti-inflammatory drugs (NSAIDs), opioid analgesics and skeletal muscle relaxants. Acetaminophen and aspirin are the frequently used over the counter medicines (Bernstein *et al*., 2004; Lou *et al*., 2004). Chronic pain is difficult to treat based on the vast strategies available (Flor *et al*., 1992). An inclusive treatment plan for chronic pain management includes multiple approaches as use of medication, physiotherapy, ergonomic training and patient education (Vlaeyen and Morley, 2005). Allopathic drugs while efficacious, usually produce side effects on long-term use, compared to that dietary supplements and plant extracts are thought to be safer and cheaper alternative treatment that can be used for chronic pain (Maroon *et al*., 2010).

Vegetables are normally consumed in our daily life as a source of nutrition, they have been known to be used as therapeutic agent too based on the constituents present in them. *Beta vulgaris* commonly referred to as garden beet or beet root belongs to family Chenopodiaceae. Beetroot is herbal, bi-annual plant having a height of 1-2 inch, with leafy stems. The roots are red in color mostly (Carmen, 2008).

Beta vulgaris L. has been used as source of nutrition since traditional times. According to Oxford dictionary of Food and Science beet root weighing 40gm provides 75 KJ of energy and 1.6gm of dietary fiber (McCance and Widdowson, 1995). Beet root is very rich in minerals too. The mineral content vary depending on how beet root is

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consumed. Raw beet root contains 66 gm Sodium (Na), 380 gm Potassium (K), 11 gm Magnesium (Mg^{+2}), 51 gm Phosphorous (P), 1 gm Iron (Fe) and 0.4 gm Zinc (Zn). Vitamin content of 100 gm of raw beet root consists of folic acid 150 mg of beetroot, 20 mg of carotene, 5 mg of vitamin C, pantothenate varies between 0.12-0.10 mg of beetroot, vitamin B_6 between 0.03-0.04 mg, thiamine between 0.01 mg-0.02 mg, riboflavin between 0.01 mg-0.03 mg and niacin 0.1mg. Minute concentrations of vitamin E and biotin are also present but vitamin D, vitamin B_{12} and retinol are absent (Heinerman, 1994).

Different parts of *Beta vulgaris* L. (especially roots and leaves) have been used in traditional medicines for many ailments. Ancient Romans used it for treating fever and constipation (De Azeredo *et al*., 2009 a). Hippocrates used beet leaves for wound healing. In Middle Ages beet juice was considered as aphrodisiac. Beet root has been widely used in treating different systemic ailments such as gastro-intestinal, pulmonary, cardiac, hepatic, blood and neurological disorders. It is also used in cosmetics and for decorative purposes (De Azeredo, 2009 b).

Betalains are the main constituents of beet root which give it red colour (Lechner *et al*., 2010). It also contains sugars (34% sucrose), 13% proteins and betaine (trimethyl glycine 1.0%). By HPLC it was found that beet root also contains 5,5,6,6-tetrahydroxy-3,3-biindolyl, cyclo-DOPA (Dihydroxyphenylalanine), L-tryptophan, P-coumaric acid, ferulic acid, ferulyl glucose, dihydroxyindole carboxylic acid, flavonoids, betavulgarin and phenolic amides (Bender and Bender,1995). Besides these, cyclo-DOPA -5-o glycoside has also been reported (Neelwarne, 2012).

Literaure studies have shown the use of beetroot as inhibitor of calcium oxalate crystal as well as diuretic (Saranya and Geetha, 2014). Traditionally it has been referred to as blood building tonic (Leiva *et al*., 2014). Beet root has also shown to possess antiproliferative activity (Das and Ramanathan, 1992). According to researches betaine exhibits anticoagulant response and also influences release of nitric oxide in human volunteers when administered in dose of 6gm OD (Iqbal *et al*., 2006). Beet root extract has been shown to inhibit inflammation at 1000mg/kg dose too. Lyophilized aqueous extracts of beetroot at 250mg and 500 mg/kg dose has been shown to reduce cholesterol and increase HDL levels (Al-Dosari *et al*., 2011). The current study was designed to assess the analgesic effect of lyophilized beet root using different methods.

MATERIALS AND METHODS

Animal Selection

The study was conducted in 2016 (March). 18-25gm of albino mice of either sex were used. The animals were arranged into three groups each comprising of 10 mice. The rodents were housed in the animal house of Department of Pharmacology, University of Karachi at room temperature of 25±2°C. The mice were given food (standard diet) and water ad libitum.

The study was approved by Karachi University Board of Advanced Studies and Research vide Resolution No10 (P) 18. As per the specification provided by Hubrecht and Kirkwood 2010, the animals were handled.

Lyophilized powder

The beet root lyophilized powder was procured having lot # Ctc 2015 0320 from Sun Rise Nutra Chem Group. The Powder was packaged and stored in zip log Plastic bag which was further covered with aluminum foil to protect from sunlight.

Dosing protocol

0.1 ml distilled water was given to Group I labeled as control. 1000mg/kg *Beta vulgaris* lyophilized powder was given to Group II labeled as treated (Jain *et al*., 2011). 20 gm beetroot powder was dissolved in 120ml distilled water to prepare a stock solution. On the basis of body weight the dose was calculated for each animal and administered orally daily. 300mg/60kg acetyl salicylic acid was given to Group III and marked as standard. Oral dose was administered daily, based on the body weight of animal.

Hot plate method

The hot plate apparatus comprises of a temperature regulator (thermo-regulator) which controls the temperature surrounded by a glass cylinder to subdue the animal on the plate and prevent escape. The heating temperature is 51±2°C. Each rodent is placed on the plate one at a time and time is noted till pain response is observed to thermal stimuli i.e paw licking. Emotional response along with pain which is a more elaborated effect is observed by jumping behavior (Khan *et al*., 2010).

Tail Flick Method

The animals were taken out from home cages one at a time and the experiment was conducted recording the time taken to withdraw the tail from hot water. About 3 cm of the mouse tail was immersed in water bath containing water maintained at 51±2°C and the time taken by the mouse to flick its tail was noted. This shows the pain response at which animal reacts to thermal stimulus (Gorzalczany *et al*., 2011).

Writhing test

Acetic acid is used for the induction of writhes in this chemically induced pain model. 30 minutes after dose of beetroot was administered 1% acetic acid was given to mice by intra-peritoneal (i/p) route. The number of writhes were noted in animal for 30 minutes after 5 minutes of i/p administration (Duarte *et al*., 1988).

Table 1: Analgesic activity of *Beta vulgaris* L. by hot plate method

Drugs	Pre Drug (0 min)	30 min	60min	90min	120min	150min	180min	240min
Control (0.1ml distill water)	9.3±0.82	7.3±0.949	7.8±0.789	11.8±0.789	13.2±1.2	13.3±1.16	10.9±0.79	10.5±1.34
Beta vulgaris(1000mg/kg)	9.5±0.53	18.5±0.52 *** ###	18.1±0.87 *** ###	17.8±1.03 *** ###	16.5±0.52 *** ###	15.8±0.789 *** ###	17.7±0.48 *** ###	20.5±0.52 *** ###
Aspirin (300mg/60kg)	9.4±0.96	21.7±0.67	23.9±1.59	20±0.81	14.6±2.45	12.9±1.91	10.7±0.67	10.4±0.96

Table 2: Analgesic activity of *Beta vulgaris* L. by tail flick method

Drugs	Pre Drug (0 min)	30 min	60min	90min	120min	150min	180min	240min
Control (0.1ml distill water)	1.0±0.04	1.1±0.05	1.0±0.08	1.1±0.06	1.2±0.08	1.1±0.11	1.0±0.02	1.0±0.05
Beta vulgaris (1000mg/kg)	1.0±0.08	2.4±0.15 *** ###	4.4±0.24 ***###	3.9±0.43 ***###	5.1±0.21 ***###	9.5±0.35 ***###	8.5±0.30 ***###	4.5±0.84 ***####
Aspirin (300mg/60kg)	1.1±0.02	7.0±0.81	6.4±0.51	6.7±0.72	6.8±1.2	4.1±0.11	2.5±0.39	2.2±0.31

Table 3: Analgesic activity of *Beta vulgaris* L. by acetic acid induced writhing

Drugs	5 min	10 min	15 min	20 min	25 min	30 min
Control (0.1ml distill water)	22± 0.81	29.7± 1.1	25.9± 1.37	25.4 ± 1.34	23.7 ± 1.41	22± 0.94
Beta vulgaris (1000mg/kg)	13.4± 1.17 *** ###	22.3± 1.15 *** ###	14.4± 1.26 *** ###	10.9 ± 0.99 *** ###	5.9± 0.73 *** ###	2.4 ± 0.84 *** ###
Aspirin (300mg/60kg)	7.3± 0.67	24.9± 0.43	20.4± 0.96	18± 0.81	13.1± 1.19	8.3± 1.41

n=10, values are Mean ± S.D using Statistical analysis SPSS 20. Using Two way Anova and Post hoc Tukeys test applied according to which P values

***$p \leq 0.001$ is considered as significant as compared to control respectively.

###$p \leq 0.001$ is considered significant as compared to standard

STATISTICAL ANALYSIS

SPSS 20 was used for statistical evaluation**.** Mean ± S.D (n=10) was used for presenting the data. Statistical analysis was performed using Two-way Anova (analysis of variance) followed by Post hoc Tukey's test and multiple pair wise comparisons.

RESULTS

Table 1 shows the analgesic activity by hot plate method. Our results show significant increase in paw licking time in treated group given *Beta vulgaris* 1000 mg/kg as compared to control throughout test duration from 30 min to 240 min. table 1 also shows significant ($p<0.001$) reduction in time to lick the paw by treated group at 30 and 60 minutes as compared to acetyl salicylic acid (standard) which was then significantly increased after 90 minutes.

Table 2 indicates analgesic effect by Tail flick test. Our results show significant ($p<0.001$) increase in latency to flick the tail in treated group (*Beta vulgaris* 1000 mg/kg) throughout the test period from 30 minutes to 240 minutes.

It also shows significantly ($p<0.001$) reduced latency to flick tail in treated group as compared to acetyl salicylic acid (standard) from 30 min to 120 minutes. There was significant increase in latency in tail flick after 150 minutes in treated group.

Table 3 shows analgesic activity by acetic acid writhing. The data shows significant ($p<0.001$) reduction in wriths in treated group as compared to control throughout the testing period of 30 minutes. It also shows significant ($p<0.001$) reduction in writhes in treated group as compared to acetyl salicylic acid (standard) after 5 minutes till 30 minutes.

DISCUSSION

Pain is often described as an obnoxious response to tissue damage (Sana *et al*., 2017). Relief from pain is referred to as analgesia. When tissue damage occurs the body releases pro-inflammatory substances such as prostaglandins that are thought to play a very important role in mediating pain and inflammation. Hence by reducing the production of these prostaglandins analgesic effect can be produced.

For the evaluation of anti-nociceptive activity three different models of pain were considered. For evaluation of central pain hot plate and tail flick models were selected. These tests are usually indicative for analgesic drugs of opioid origin where pain mediates from spinal

region (Gupta *et al*., 2003). These are based on response to thermal stimuli where hot plate indicates supraspinal reflex whereas tail flick is for spinal reflex (Arsalan and Bektas, 2010). It has been suggested that the spinal mechanism involves the μ2- and δ-opioid receptors, whereas supraspinal analgesia is mediated by μ1/μ2-opioid receptors (Jinsmaa *et al*., 2005). Literature studies have shown that thermal stimuli responds normally by binding to transient receptor potential receptors (TRP) on primary afferent neurons. Heat and acid can activate TRPV1 (Transient receptor potential vanilloid receptor 1) which leads to discharge in central neurons and spinothalamic tract cells causing sensation of itching and burning. Peripheral nociceptor sensitization causes primary hyperalgesia whereas central nociceptor sensitization causes secondary hyperalgesia. Enhanced response to excitatory aminoacids and decreased response to inhibitory amino acids increases central sensitization (Willis, 2009). Hence any substance that increases the inhibitory amino acid and decreases the excitatory amino acid may have affect at this level. *Beta vulgaris* root contain different phenolic acids (caffeic acid 0.35mg/gm dry extract)(Ben Haj *et al*., 2014) which is postulated to increase the activity of Gabaergic neurotransmission hence potentiating analgesic effect (Pereira *et al*., 2006).

Both of these methods are simple and inexpensive. The hot plate method is more reliable and scientific than that of tail immersion method. Our study shows analgesic effects after 30 minutes in treated group as compared to control, however analgesic effect was better than standard (acetyl salicylic acid) and was observed after 90 minutes of administration in animals that received 1000 mg /kg Beta vulgaris. This shows that *Beta vulgaris* root possesses analgesic effect and its effect is mediated through central action.

Acetic acid-induced writhing also referred to as abdominal contraction response is a well recommended method in evaluating medicinal agents for their analgesic property due to its high sensitivity and ability to detect at minute doses (Tanko *et al*., 2008). In this model, sensation of pain is produced in response to localized inflammatory response due to release of endogenous substances such as histamine, substance P, bradykinin, serotonin and Prostaglandins (PG's) as well as lipooxygenase products in the peritoneal fluids (Divya *et al*., 2009). The postulated mechanism by which prostaglandins produce pain is stimulation of C fibers which cause increase release of substance P and neurokinins which ultimately cause pain sensation. Substance P binds to its receptor NK causing activation of phospholipase C leading to formation of Inositol-triphosphate (IP3) and Diacylglycerol (DAG) which ultimately cause influx of calcium ions which are thought to play a role in neural processing of pain sensation and its transmittance to the brain (Mamun *et al*., 2017). It is known that abdominal writhing is mediated by local peritoneal receptor (Muhammad *et al*., 2012). Increase capillary permeability and vasodilation is also one of the postulated mechanism by which PG's produce pain (Anosike *et al*., 2012). So any substance which is capable of inhibiting prostaglandin and leukotrienes synthesis, can also inhibit writhing. Acetic acid induced writhing therefore is indicative of both peripheral and central anti-nociceptive effect (Andrade *et al*., 2007).

Our results showed reduction in writhing's in test group when compared with control as well as with standard (acetyl salicylic acid). This shows that *Beta vulgaris* root can also produce analgesia through peripherally mediated anti-nociceptive actions. The centrally mediated effects are further confirmed.

Caffeic acid, betalain, rutin and epicatechin are the various constituents of beetroot known for their anti-oxidant effect (Wootton and Ryan, 2011). Rutin is a bioflavanoid which produces a central analgesic effect by acting on ventrolateral periaqueductal grey matter (VLPAG) which is a descending pathway mediated by opioid mechanism (Hernandez *et al*., 2016). Another possible mechanism is that prostaglandin and ecosanoid synthesis are also targeted by flavanoids (Jothimanivannan *et al*., 2010).

Prostaglandin sysnthesis is inhibited by betalain by inhibition or suppression of Cyclooxygenase-2 (COX-2) production (Vidal *et al*., 2014). Besides its role in central analgesia, anti-oxidant and anti-inflammatory effects of caffeic acid may also play a role in reducing pain (Olthof *et al*., 2001). It is thus established that beetroot possesses both central and peripheral analgesic effect by above postulated mechanism.

CONCLUSION

From above study we can conclude that lyophilized powder of *Beta vulgaris* L. root possess marked analgesic activity by acting both centrally and peripherally. However further studies are required to establish exact mechanism of action.

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