EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 3
Issue: 12
311-315
Year: 2016

EVALUATION OF ANTIHYPERLIPEDEMIC ACTIVITY OF VARIOUS EXTRACTS OF BEET ROOT (*BETA VULGARIS*)

Dr. Mukuntha Kumar N.*[1] and Asish Bhaumik[2]

[1]Associate Professor, Department of Pharmaceutical Analysis, Teja College of Pharmacy, Kodad, Nalgonda-508206, Telangana State, India.
[2]Assistant Professor, Department of Pharmaceutical Chemistry, Teja College of Pharmacy, Kodad, Nalgonda-508206, Telangana State, India.

***Corresponding Author: Dr. Mukuntha Kumar N.**
Associate Professor, Department of Pharmaceutical Analysis, Teja College of Pharmacy, Kodad, Nalgonda-508206, Telangana State, India.

Article Received on 26/09/2016 **Article Revised on 16/10/2016** **Article Accepted on 06/11/2016**

ABSTRACT
Hyperlipidemia is abnormally elevated levels of any or all lipids and/or lipoproteins in the blood. It is the most common form of dyslipidemia (which includes any abnormal lipid levels). Lipids (water-insoluble molecules) are transported in a protein capsule. The size of that capsule, or lipoprotein, determines its density. The lipoprotein density and type of apolipoproteins it contains determines the fate of the particle and its influence on metabolism. Hyperlipidemias are divided into primary and secondary subtypes. Primary hyperlipidemia is usually due to genetic causes (such as a mutation in a receptor protein), while secondary hyperlipidemia arises due to other underlying causes such as diabetes. Lipid and lipoprotein abnormalities are common in the general population and are regarded as a modifiable risk factor for cardiovascular disease due to their influence on atherosclerosis. In addition, some forms may predispose to acute pancreatitis. 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 antihyperlipedemic activity. The experimental data was displayed that the various extracts such EEBT, MEBT and CEBT were showing the lipid lowering ability in experimental rats with reference to standard drug lovastatin. The decreased serum lipids profile by various extracts such as EEBT, MEBT and CEBT were found to be 77.24, 73.22 and 78.11 mg/dl (total cholesterol i, e TCH), 110.34, 98.54 and 119.31 mg/dl (TGS), 21.32, 21.74 and 21.31 mg/dl (HDL-C), 32.52, 29.21, and 34.30 mg/dl (LDL-C) and 18.32, 21.32 and 23.11 mg/dl (VLDL-C) etc.

KEYWORDS: Hyperlipidemia, lipoproteins, diabetes, atherosclerosis, antihyperlipedemic etc.

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.

Taxonomy


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

Fig: 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.

MATERIALS AND METHOD

Drugs and chemicals

The standard drug **Lovastatin** 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**.

Principle involve in extraction of phytoconstituents[11]

The extraction of the drug represents either solid-solid or liquid-liquid extraction. Extraction processes for drugs can depends on the partition of compenent between solvent phase and solid residual and dependent on diffusion of component. Solvent volume is used such as the final concentration gradient between miscella and residue has become zero which is an equilibrium stage. The position of the equilibrium depends on properties of the drug nature and type of the drug, quantity, degree of comminution, solvent selectivity, solventquantity and moisture content. Factors which effect the extraction are quantity and nature of drug, degree of size reduction, moisture continent, volume and nature of the solvent, mixing ratios of solvent, method for preparation of solution from intact cells, method for preparation of solution from lysed cells, imbibition by solvent, rate of equilibrium establishment, temperature, P^H of the extracting solvent, interaction between dissolved components, polarity of solvent mixture(s), process governing separation, mixture ratio of solvent and herb, dissolution from lysed cells, penetration of solvent and swelling of drug plant material, movement of constituents out from intact cells and interaction of dissolved components with insoluble support material of plants. The solvent used for extraction are as given below polar: polar-water, ethanol, methanol; nonpolar: benzene, tolune, chloroform, acetonitrile, cyclohexane, petroleumether, diethylether; semipolar: acetone.

Methodology for extraction[12]

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 screening[13-15]

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.

Protocol for the study of acute oral toxicity of EEBT, MEEBT and CEBT

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.[16]

Screening methodology for antihyperlipidemic activity[17-18]

A. Experimental Design: Animal: Wister rats, **Sex:** Either Sex, **Weight:** 150-170 gm

On the day of the experiment, the animals were divided randomly into Six groups of three animals each.

Group I: Normal (1% Tween 80; 10ml/kg, p.o)

Group II: Control (Cholesterol (25 mg/kg/day) in oil

Group III: **Test** (**EEBT**; 100 mg/kg in 1% Tween 80) along with cholesterol in oil
Group IV: Test (MEBT; 100 mg/kg in 1% Tween 80) along with cholesterol in oil
Group V: Test (CEBT; 100 mg/kg in 1% Tween 80) along with cholesterol in oil
Group VI: Standard (Lovastatin (10 mg/kg/day) along with cholesterol in oil

Rats were fed daily with standard diet supplied by Pranav Agro Industries, Sangli. cholesterol in oil was given by oral route at 10 am and Test drugs or **Lovastatin** was given by oral route at 3 pm daily, to respective groups, for a period of 10 days. The normal control group was treated with vehicle instead of drugs. At the end of the experimental study, animals were fasted for 12 hr and blood was collected by retro-orbital puncture and serum TC, LDL-C, VLDL-C, TAG were determined using Analyser.

RESULTS AND DISCUSSION

Acute oral toxicity study

(i) Acute oral toxicity studies were performed according to the OECD guideline 423 method.

(ii) 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.
(iii) 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.
(iv) 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.
(v) Since no sign of toxicity observed at 2000 mg/kg b. w. to the group of animals, the $\mathbf{LD_{50}}$ value of the **extract** expected to exceed 2000 mg/kg b. w. and represented as class 5 (2000 mg/kg < LD50 < 2500 mg/kg).
(vi) 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 $\mathbf{LD_{50}}$ 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

Screening methodology for antihyperlipidemic activity

The present experimental data (**Table-2**) were expressed as mean ± standard error mean (SEM). The data were analyzed by using Graph pad software version5 by one way analysis of variance (ANOVA). The test was followed by Dennett's't'-test, p values less than 0.05 were considered as significance. The experimental data of table 2 was displayed that the various extracts of beet root showing the lipid lowering ability in experimental rats with reference to standard drug lovastatin. The declined serum lipids profile by various extracts such as EEBT, MEBT and CEBT were found to be 77.24, 73.22 and 78.11 mg/dl (total cholesterol i, e TCH), 110.34, 98.54 and 119.31 mg/dl (TG_S), 21.32, 21.74 and 21.31 mg/dl (HDL-C), 32.52, 29.21, and 34.30 mg/dl (LDL-C) and 18.32, 21.32 and 23.11 mg/dl (VLDL-C) etc.

Table 2: Effects of Test drugs on lipid profile in rats

Groups	TCH (mg/dl)	TG_S (mg/dl)	HDL – C (mg/dl)	LDL – C (mg/dl)	VLDL – C (mg/dl)
Normal-I	65.7±6.19	69.6±6.50	22.51±2.20	28.41±2.79	13.31±1.49
Control-II	88.19±7.10	126.51±9.50	18.11±1.80	36.34±3.18	23.50±1.86
EEBT-III	77.24±7.23	110.34±7.34	21.32±2.45	32.52±2.30	18.32±1.61
MEBT-IV	73.22±5.41	98.54±5.54	20.74±1.82	29.21±3.25	21.32±1.50
CEBT-V	78.11±6.33	119.31±5.60	21.31±3.81	34.30±2.55	23.11±1.39
Lovastatin-VI	58.91±2.80	71.32±5.30	22.3±1.59	29.35±3.20	14.45±1.31

The above experimental data were expressed as mean ± standard error mean (SEM). The data were analyzed by using Graph pad software version5 by one way analysis of variance (ANOVA). The test was followed by Dennett's't'-test, p values less than 0.05 were considered as significance. The experimental data of table 2 was displayed that the various extracts of beet root showing the lipid lowering ability in experimental rats with reference to standard drug lovastatin. The declined serum lipids profile by various extracts such as EEBT, MEBT and CEBT were found to be 77.24, 73.22 and 78.11 mg/dl (total cholesterol i, e TCH), 110.34, 98.54 and 119.31 mg/dl (TG_S), 21.32, 21.74 and 21.31 mg/dl (HDL-C), 32.52, 29.21 and 34.30 mg/dl (LDL-C) and 18.32, 21.32 and 23.11 mg/dl (VLDL-C) etc.

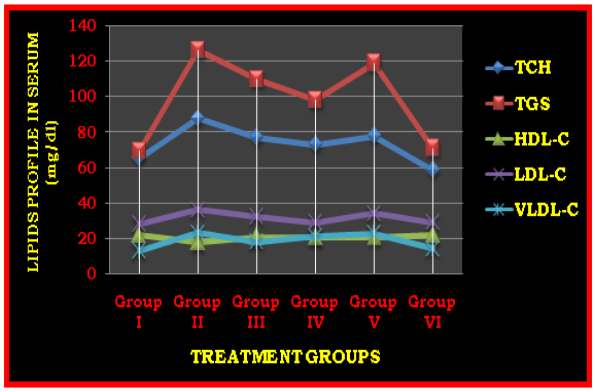

Fig 1-Effects of Test drugs and extracts on lipid profile in rats

CONCLUSION

From the present experimental data here we concluded that the various extracts of beet root (EEBT, MEBT and CEBT) had the potential ability to lower the plasma lipids profile in experimental rats and this research work given an overview to treat the disease associated with the metabolism of lipids.

REFERENCES

1. "A synopsis of Chenopodiaceae subfam. Betoideae and notes on the taxonomy of Beta; USDA PLANTS" (PDF). Willdenowia.
2. "Spinach, Beet and Swiss Chard - Notes - HORT410 - Vegetable Crops - Department of Horticulture and Landscape Architecture - Purdue University". Hort.purdue.edu. Retrieved 2010-09-12.
3. http://www.avrdc.org/pdf/seeds/beet.pdf.
4. "Sugar beet". Agronomy.unl.edu. Archived from the original on March 29, 2007. Retrieved 2010-09-12.
5. "Integrative Biology 335: Systematics of Plants". Life.illinois.edu. Retrieved 2010-09-12.
6. Zeldes, Leah A. (2011-08-03). "Eat this! Fresh beets, nature's jewels for the table". Dining Chicago. Chicago's Restaurant & Entertainment Guide, Inc. Retrieved 2012-08-03.
7. Hanelt, Peter; Büttner, R.; Mansfeld, Rudolf; Kilian, Ruth (2001). Mansfeld's Encyclopedia of 8. Agricultural and Horticultural Crops. Springer. pp. 235–241. ISBN 3-540-41017-1.
8. Grubben, G.J.H. & Denton, O.A. (2004) Plant Resources of Tropical Africa 2. Vegetables.
9. Dr. G. Devala Rao, A Manual of Practical Biochemistry, pp 17.
10. Jaswant Kaur, PV Chemistry of Natural Products, 2010 edition, PP-113-114, 116, 344-346, 381.
11. "OECD guidelines – 423" for testing of chemicals, 2001; 1-14.
12. Hirunpanich V, Utaipat A, Mo rales N P, Bunyapraphatsara N, Sato H, Herunsale A & Suthisisang C Hypocholesterolemic and antioxidant effects of aqueous extracts from the dried calyx of Hibiscus sabdariffa L. in Hypercholesterolemic rats, J Ethnopharmacol, 2006; 103: 252.
13. Chang-Che Chen, Jeng-Dong Hsu & San-Fa Wang, Hibiscus sabdariffa extract inhibits the development of atherosclerosis in cholesterol fed rabbits, J Agric Food Chem, 2003; 51: 5472.