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Evaluation of anti tumour activity of ethanolic extract of beet root (*Beta vulgaris*) against EAC mouse tumor model

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Abstract

Introduction: EAC is referred to as undifferentiated carcinoma, and is originally hyper-diploid. The permeability to water is highest at the initiation of S and progressively decreases to its lowest value just after mitosis. Activation heats for water permeability vary during the cell cycle, ranging from 9–14 k. ca/mole.

Objective: The main aim and objective of my present research work was the extraction, preliminary phytochemical screening of ethanolic extract of beet root (EEBT) and evaluation of *in vivo* anti tumour activity against EAC mouse tumour models.

Methodology: The extraction of phytoconstituents was carried out by reflux condensation and preliminary phytochemical screening was done by various qualitative confirmatory tests. The *in vivo anti* tumour activity of ethanolic extract of beet root (EEBT) was carried out on EAC mouse tumour models.

Results: All five animals were in both 5-FU as well as EEBT group. So mortality was less in both 5-FU and EEBT group. The Hb and RBC count were lower in tumour control group. The WBC counts were significantly increased in tumour control. 5-FU and EEBT mg/kg group decreased the WBC counts approximately near to normal range. The neutrophils were increased and lymphocytes were decreased significantly in tumour control group. The platelet count was also significantly increased in tumour control and EEBT (200 mg/kg) group compared nearly to normal group. EEBT and 5-FU significantly increased the PILS. While 5-FU increased the life span of EAC 90.90% and EEBT increased the life span of EAC 86.36%.

Conclusion: From the present experimental data here we concluded that the EEBT possessed potential anti tumour activity against EAC mouse tumour model which was proved by the assessment of haematological parameters as well as by Percent increase of lifespan (% ILS). The Percent increase of lifespan (PILS) of both standard group as well as EEBT treated group was found to be 90.90% and 86.36%.

Keywords: EAC, carcinoma, anti tumour activity, PILS etc.

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 nut lets.

1.1 Taxonomy

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].

1.2 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].

1.3 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.

1.3.1 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.

1.3.2 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."

1.3.3 Digestion and regularity

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

1.3.4 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.

1.3.5 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 Method

2.1 Drugs and chemicals

Carboxymethyl cellulose sodium (CMC), phosphate buffered saline (PBS) Diethyl ether and 5-FU were used for the study and other chemicals used for the extraction and phytochemical screening were provided by Institutional Store and were of LR and AR grade.

2.2 Animals

Female Swiss albino mice were obtained from the central animal house of C. L. Baid Metha College of Pharmacy, Jyothinagar, OMR, Chennai and they were maintained under standard laboratory conditions throughout the study. The animals were fed with standard rodent pellet feed (Hindustan lever, Bangalore) and water *ad libitum*. Adult mice weighing 20–30 g were used for the experiments. The study protocol was approved by the Institutional Animal Ethics Committee (IAEC, reference number: IAEC/XXIX/09/2016) and all the animal experiments were carried out in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India.

2.3 Cancer cell lines

The initial inoculums of the EAC cells were provided by the Amala Cancer Research Centre, Thrissur, Kerala, India. The EAC cells were then propagated in our laboratory biweekly through intraperitoneal transplantation of 2×10^6 cells per mouse (freshly drawn from a donor Swiss albino mouse, bearing 6- to 7-day-old ascites tumor cells).

2.4 Methodology for extraction [11]

Weigh 20 g of beet root paste (root can be mashed or grinded 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 steam-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 sulphate. Filter and evaporate most of the solvent in vacuum without heating and obtained ethanolic extract of beet root **(EEBT) of *Beta vulgaris*.**

2.5 Phytochemical screening [12-14]

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

2.6 Determination of median lethal doses (LD_{50})

In the present study acute oral toxicity of the **EEBT** was performed by acute toxic class method according to OECD guideline-423 [15]. In this method the toxicity of EEBT was tested using a step wise procedure, each step using three mice of single sex (female/male). The Swiss albino mice were fasted prior to dosing (food but water should be with held) for three to four hours. Following the period of fasting the animal should be weighted and the test compounds were dissolved in 3% CMC, administered intra peritoneal route to the different groups with 2000 mg / kg body weight. Animals were observed individually after dosing at least once during the first 30 min; periodically during the first 24 h with special attention giving during the first 4 h and daily thereafter, for total of 14 days. As know mortality observed with the above dose. Test EEBT dose reduced by specific intervals. The mortality was not observed at the dose 2000 mg / Kg. So 200 mg /Kg body weight was selected for the *in vivo* evaluation (since LD_{50} cut of value is > 2000). Mortality was determined after 24 hours of treatment. The dose at which 50% of the mice survived was considered the LD_{50} value of the test extract.

2.7 Study design

An investigational study was designed to evaluate the *in vivo* antitumor activity of EEBT on EAC mouse tumour models. Study was carried out with EAC cell line induced malignant ascites on mouse models. The dose of EEBT 200 mg/kg were chosen based on the results of a toxicity study done previously. The animals were divided into four different groups as follows:

The animals were divided into four different groups (each group contain 5 mice) as follows:

A. Group I: Normal Control Group [only the vehicle (1 ml/kg/day of 1% CMC orally)]

B. Group II: T. Control (1% CMC orally + EAC = 2×10^6 i. p.)

C. Group III: Standard (EAC = 2×10^6 i. p + 5-FU 25 mg/ml inj.)

D. Group IV: EEBT (EAC = 2×10^6 i. p + 200 mg/kg orally)

Table 1 for designing of experiment

Days	Activity was carried out	No. of mice / group (5)
Day 1	Collection of 0.3 ml of blood sample	Group-I
Day 2	Tumour cell injection, EAC = 2×10^6 i. p.	Group-II-IV
Day 3-12	Treatment of CMC	Group-II
	Treatment of std. drug 5-FU	Group-III
	Treatment of EEBT	Group-IV
Day 15	Collection of 0.3 ml of blood sample	Group-II-IV
Day 16-35 follow up	Observed till death/35th day	Group-II-IV

2.8 Experimental procedure [16]

On day 1, blood collection from retro-orbital plexus was carried out and the samples (0.3 ml) in EDTA were used for the assessment of haematological parameters such as haemoglobin (Hb) content, red blood cell (RBC) count, total white blood cell (WBC) count, DLC and platelet count. On day 2, tumour fluid was withdrawn from the stock animals for EAC and the tumour cell count was done using Neubauer chamber under the light microscope. The PBS was added to make a concentration of 1×10^6 cells in 0.1 ml. For tumour induction in study each experimental animal (Group-II to Group-IV) was injected with 2×10^6 EAC cells i.e. 0.2 ml intra peritoneal route. After 24 h of the tumour cells inoculation, the animals were treated according to their respective groups once daily for next 10 days. On day 15, the retro-orbital blood collection was done again for haematological assessment, if the animal was alive. The animals were followed till death or up to 35-45 days. The parameters for antitumor activity in study were recorded as followed. Determination of the percentage increase in life span (PILS): It is calculated from the mean survival time (MST) values [17]. The MST for each group was calculated as:

MST (days) =Total number of days survived by all animals in the group/Number of animals in the group

For each group, Percent increase of lifespan (% ILS) was determined by the following formula:

PILS (%) =

[(MST of treated group/MST o f control group) −1] × 100

3. Result and Discussion

Assessment of haematological parameters: The haematological parameters of all surviving animals such as haemoglobin, RBC, WBC, neutrophils, lymphocytes and platelets were assessed for all. A group of five normal mice was studied for assessing their haematological parameters. These normal (control) values were used for comparisons. The tumour bearing animals alive at the end of the study were sacrificed by cervical dislocation.

Table 2: for the assessment of haematological parameters

Group	Treatment	Hb (g/dl)	$RBC_S(1 \times 10^6/mm^3)$	$WBC_S(1 \times 10^3/mm^3)$
I	N. Control	14.1	9.45	63.25
II	T. Control	5.9	6.41	75.73
III	5-FU	9.8	9.11	64.12
IV	EEBT	9.7	8.99	64.22

Table 3: for the assessment of haematological parameters

Group	Treatment	Neutrophils (%)	Lymphocytes (%)	Platelets $(1 \times 10^3/mm^3)$
I	N. Control	13.4	87.9	453.6
II	T. Control	82.9	12.9	1226.2
III	5-FU	13.8	84.89	459.9
IV	EEBT	13.6	84.99	463.5

3.1 Effect on the survival

EEBT and 5-FU significantly increased the PILS. While 5-FU increased the life span of EAC 90.90% and EEBT increased the life span of EAC 86.36%.

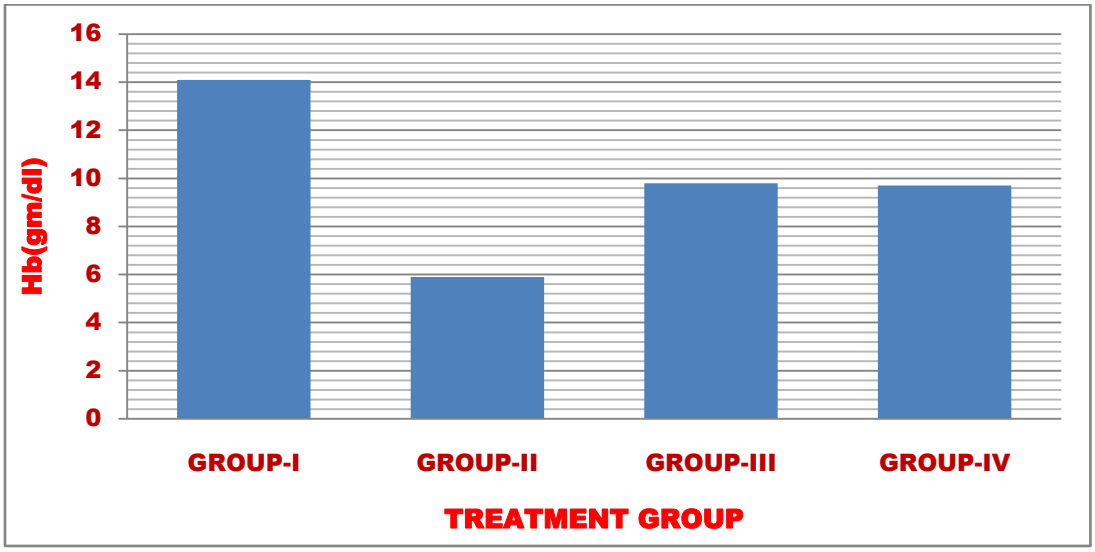

Fig 1: Status of haemoglobin level in different treatment groups

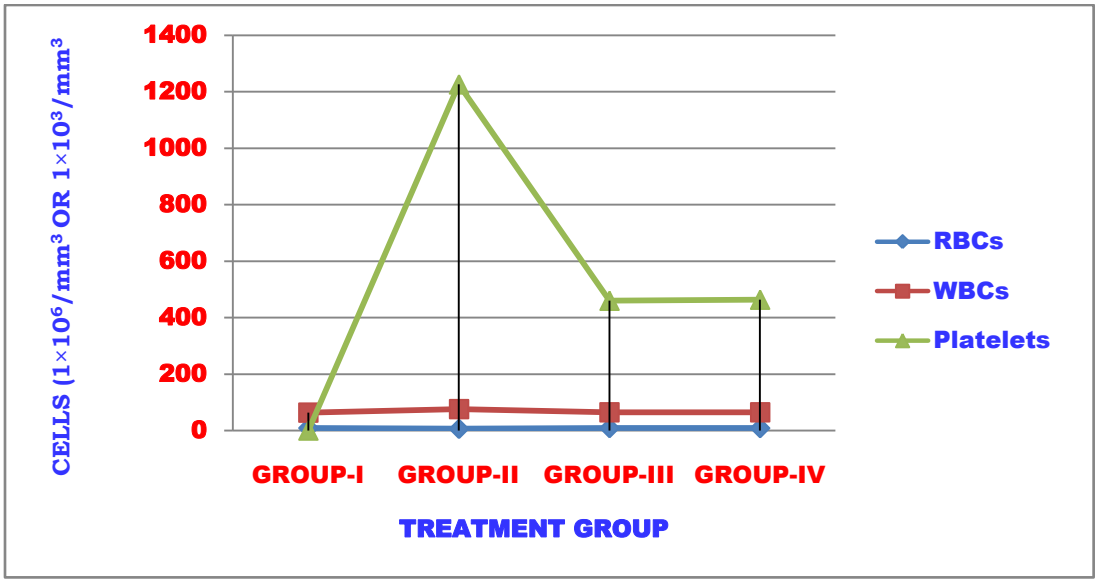

Fig 2: Status of blood cells in different treatment groups

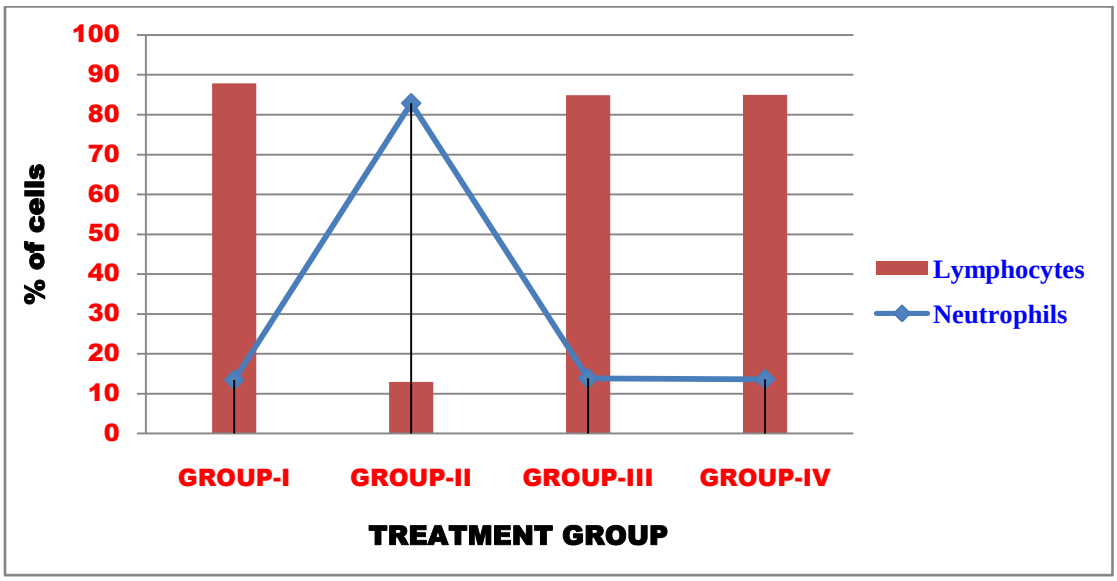

Fig 3: Status of percentage of neutrophils and lymphocytes in different treatment groups

Table 4: for Percent increase of lifespan (% ILS)

Group	Treatment	MST(days)	PILS (%)
II	T. Control	4.4	-
III	5-FU	8.4	90.90
IV	EEBT	8.2	86.36

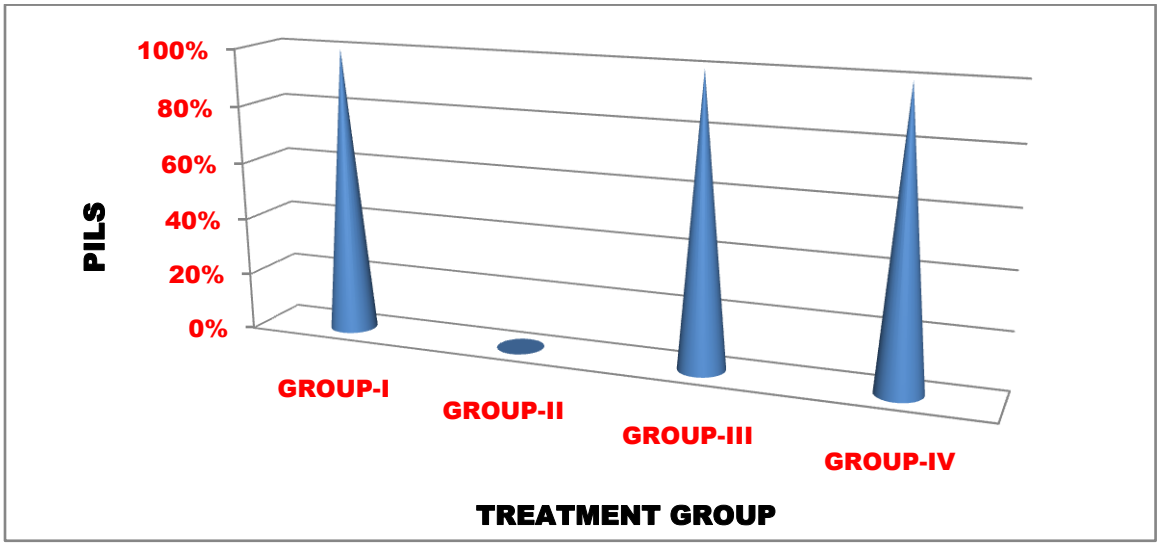

Fig 4: Percent increase of lifespan (% ILS) in different treatment groups

3.2 Effect on the haematological parameters

All five animals were in both 5-FU as well as EEBT group. So mortality was less in both 5-FU and EEBT group. The Hb and RBC count were lower in tumour control group. The WBC counts were significantly increased in tumour control. 5-FU and EEBT mg/kg group decreased the WBC counts approximately near to normal range. The neutrophils were increased and lymphocytes were decreased significantly in tumour control group. The platelet count was also significantly increased in tumour control and EEBT (200 mg/kg) group compared nearly to normal group.

4. Conclusion

From the present experimental data here we concluded that the EEBT possessed potential anti tumour activity against EAC mouse tumour model which was proved by the assessment of haematological parameters as well as by Percent increase of lifespan (% ILS).

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