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Study on the growth factors and hepatoprotective effect of *Beta vulgaris L*. in male Wistar rats

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ABSTRACT

The purpose of this study was to investigate the effect of *Beta vulgaris L*. juice on growth and liver enzymes in male Wistar rats**.** The experiment was conducted on 15 Wistar albino rats, divided into 3 groups. Group 1 was fed with a standard diet (control group), group 2 was fed with a standard diet and 1 ml *Beta vulgaris L*. juice and group 3 was fed with a standard diet and 2 ml *Beta vulgaris L*. juice. Statistically insignificant difference was observed between control and experimental groups for total food consumption. Similarly difference was insignificant for final weight but it has been found that change in weight was slightly more in control group when

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compared to experimental group fed with 2 ml juice although the change in weight was insignificant between control and experimental groups. It has been found that administration of *Beta vulgaris L.* decreased the AST and ALT activities in serum so it can be concluded from this study that beetroot feeding may protect against liver damage.

Keywords: *Beta vulgaris L.*; Liver enzymes; AST; ALT; Liver damage

1. INTRODUCTION

Functional foods and their use in health and disease are continuously gaining significance due to the health benefits of a diet high in fruit and vegetables (Clifford et al., 2015). *Beta vulgaris L.* (Beetroot) is a vegetable characteristic of the Eastern and Central European diet, and is mainly consumed as a cooked vegetable, juice or pickled and canned preserve (Wroblewska et al., 2011). Beetroot juice contains a high level of biologically accessible antioxidants as well as many other health-promoting compounds such as minerals (iron, magnesium, calcium, phosphorus, sodium and potassium), vitamins (niacin, folic acid, vitamin B6) and soluble fibers (Wootton-Beard and Ryan, 2011). It is also a good source of phytochemical compounds that includes phenolic acids, ascorbic acid, carotenoids and flavonoids (Fig 1) (Georgiev et al., 2010 and Wootton-Beard and Ryan, 2011) and contains a group of highly bioactive pigments known as betalains which are synthesized from the amino acid tyrosine into two structural groups: yellow–orange betaxanthins and the red–violet betacyanins (Azeredo, 2009, Vulic et al., 2014) and it possess powerful antiradical and antioxidant activity (Pedreno MA and Escribano J, 2000, and Kekkonen et al, 1999). Studies shows that consumption of *Beta vulgaris L.* presents beneficial physiological effects and is used as a popular folk remedy for several pathologies, such as; hypertension, liver and kidney diseases, atherosclerosis, type 2 diabetes for stimulation

of the immune and hematopoietic systems and dementia (Papas, 1999 , Kapadia et al., 2003 and Georgiev et al., 2010). Liver is the principle organ of biotransformation in the body and so it is very susceptible to damage. Severe acute liver disease has been encountered in clinical practice leading to fulminant or acute liver failure (Finlayson et al., 1999). Chronic liver damage is a worldwide common pathology characterized by inflammation and fibrosis that can lead to chronic hepatitis, cirrhosis and cancer (Kohle et al., 2008). Alcohol and other chemicals, environmental, biologic toxins are the various factors responsible with liver diseases (Kuru, 2014). Researchers are looking for herbal drugs with better hepatoprotective action to cure liver damage. The aim of this study is to find the effect of *Beta vulgaris L*. juice on growth factors and liver enzymes in Wistar rats.

2. MATERIALS AND METHODS

The experiment was conducted on 15 Wistar albino rats aged between 8 to 16 weeks obtained from Experimental Animal Care and Experimental Surgery Center at the Faculty of Medicine, King Saud University, Saudi Arabia. This study is in accordance with the Animal Ethics Committee of the College of Science, King Saud University. The rats were randomly divided by weight into three groups and individually housed in stainless steel cages under controlled temperature (25 ± 2 °C) and relative humidity (50±5%), with a 12-h light/dark cycle.

2.1. Diets formulation and preparation:

Basal diet was obtained from the General Organization for Grain Silos and Flour Mills, Saudi Arabia. Group 1 was fed with a standard diet (control group), group 2 was fed with a standard diet and 1 ml beetroot juice and group 3 was fed with a standard diet and 2 ml beetroot juice. Food and liquid intakes were monitored daily in all groups.

2.2. Assessment of body weight and food consumption

2.2.1. Growth:

Body weight was recorded in the non-fed state at the beginning of study (initial weight) and at time before slaughter (final weight).

Weight gain = final body weight (g) − initial body weight (g) and

Growth rate =total weight gain (g) /100 days study period

2.2.2. Food Consumption:

Food consumption was analyzed daily in all experiments by calculating the difference between the diet provided (before consumption) and the diet consumed using a calibrated scale with 0.01mg precision. The calculation was performed as follows:

Food consumption per day (g) = diet provided (gm) - diet consumed (g).

2.2.3. Food efficiency:

Food efficiency was calculated by the following formula:

Food efficiency: gain weight / total food consumption

2.3. Plasma liver function test:

The parameters measured include alanine transaminase (ALT) and aspartate transaminase (AST). All parameters (ALT and AST) were analysed by using kit provided by United Diagnostic Industry, Dammam, Saudi Arabia (REF 007 and REF 015 respectively) and measured spectrophotometrically.

2.4. Sampling and sample storage method:

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After 27 days, rats were food deprived over-night; 10 ml of blood was collected from rat via orbital sinus in vacutainer heparinized tube and centrifuged at 1000 rpm for 5 min at 4 °C to obtain the plasma and was stored in an eppendorf tube at 4 °C for further analysis.

2.5. Statistical analysis:

Data were analyzed using SPSS statistical software package (version 22) and expressed as mean ± standard deviation. The differences among treatment groups were analyzed by ANOVA at a significance level of $P \leqslant 0.05$; if significant differences were found, Post-hoc analysis using Duncan's multiple range tests was performed.

3. RESULTS AND DISCUSSIONS

3.1. Dietary intake and growth rate

Table 1 shows food consumption and growth by the control and experimental Wistar rats. Statistically insignificant difference was observed between control and experimental groups for total food consumption but difference was slight significant for food efficiency. Similarly difference was insignificant for change in weight but change or increase in weight was least in experimental group consuming 2 ml of juice. Wroblewska et al., 2011 in their study found that the experimental dietary application of the beetroot crisps did not affect the final body weight of the animals. The weight gain data corroborates the food consumption data because the food consumption was lower in experimental group as compared to control group.

3.2. Biochemical parameters

AST and ALT serum levels were performed to assess liver function. AST and ALT levels remain the most useful tests for the detection hepatic cell damage, because both are present in high

concentrations in hepatocytes. If hepatocytes or their cell membranes get damaged, then these enzymes leak into the circulation (Kew, 2000). High levels of AST indicate liver damage, ALT catalyses the conversion of alanine to pyruvate and glutamate and is released in similar manners, therefore, ALT is more specific to liver and is thus a better parameter for detecting liver damage (Willianson et al., 1996). No significant alterations were detected in AST in the animal groups treated with the *Beta vulgaris L*. during 3 weeks, when compared to the control group but the significant was slight significant for ALT (Table 2). In a study by Kuzniak et al., 2012, pretreatment with beetroot juice significantly decreased ALT, SDH and GGT activities elevated by N-nitrosodiethylamine. Similar result was obtained by Sadeek., (2011), Szaefer et al., (2014) and Rabeh, (2015).

4. CONCLUSION

To summarize, in this study; the *Beta vulgaris L*. does not significantly lowered the liver enzyme AST but significantly lowered the ALT activity. It also didn't exhibit any major effect on weight. This might be due to short study period. From this study it can be conclude that beetroot feeding may protect against liver damage.

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Table. 1. Growth indicators of males Wister rats fed with beetroot juice.

	Control	1 ml juice B1	2 ml juice B2
Initial weight	110.34 ± 1.58^{b}	103.26 ± 4.46^{a}	118.8 ± 2.28^{c}
Final weight	210.02 ± 9.21^{a}	215.16 ± 4.86^{a}	213.86 ± 19.67^{a}
Change in weight	99.68 ± 10.55^{a}	111.9 ± 8.38^{a}	95.06 ± 18.28^{a}
TFC	784.00 ± 196.42^{a}	667.36 ± 35.76^{a}	708.57 ± 27.85^{a}
FE	0.132 ± 0.03^{a}	0.167 ± 0.02^{b}	0.13 ± 0.03^{a}

Small alphabet letters in each row indicates significant difference among dietary treatment groups separately as indicated by ANOVA followed by Duncan's multiple range tests.

Table. 2. Liver enzymes of males Wister rats fed with beetroot juice

	Control	1 ml juice B1	2 ml juice B2
ALT	5.75 ± 5.27^{b}	0.354 ± 0.79^{a}	1.768 ± 2.17^{ab}
AST	11.49 ± 12.12^{a}	1.768 ± 1.443^{a}	1.547 ± 1.509^{a}

Small alphabet letters in each row indicates significant difference among dietary treatment groups separately as indicated by ANOVA followed by Duncan's multiple range test.

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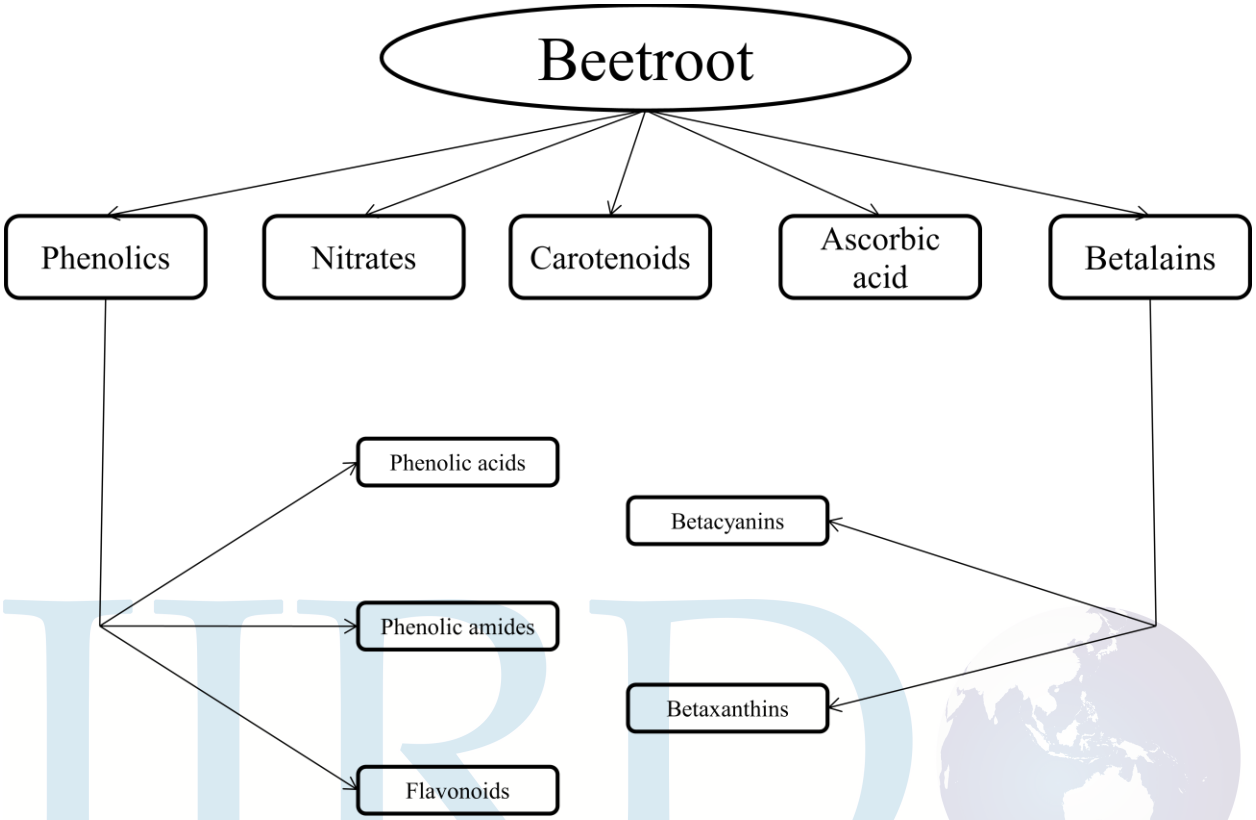

Fig 1. Flowchart of potentially bioactive compounds in beetroot (Kujala et al., 2002 and Georgiev et al., 2010)