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Evaluation of Hepatoprotective effects of different doses of Lyophilized Beetroot powder in albino rabbits

Sana Sarfaraz[1], Rahela Ikram[2], Rabia Munawwar[3], Sakina Fatima[3], Humera Anser[3] and Mahwish Fatima[4]

[1]Department of Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi, Karachi, Pakistan
[2]Faculty of Pharmacy, Salim Habib University, Karachi, Pakistan
[3]Faculty of Pharmacy, Jinnah Sindh Medical University Karachi, Pakistan
[4]Indus Hospital, Karachi, Pakistan

Abstract: *Beta vulgaris* L. is a vegetable most commonly consumed in salads and has been shown to possess multiple benefits. This research was carried out to observe the effects of *Beta vulgaris* powder at different doses orally in albino rabbits on liver biochemical parameters and coagulation. The study was carried out on albino rabbits which were divided into three groups designated as Group I (administered distilled water) Group II and III (administered beetroot powder at 500mg/kg and 1000mg/kg dose respectively) orally for 2 month duration. The sample was withdrawn at day 0, 30th and 60th day through cardiac puncture. The results showed that both doses of *Beta vulgaris* were considered safe for use as all the liver parameters were significantly decreased compared to control. Among both doses 500mg/kg dose was considered safer as it reduced the parameters significantly compared to 1000mg/kg dose. Blood coagulation factors at both the doses showed significant increase which was in reference range. *Beta vulgaris* is a highly beneficial dietary product with ample amount of flavonoids and anti-oxidant agents which might help in improving the liver function and also play a role in coagulation by increasing both fibrinogen levels and prothrombin time.

Keywords: Beetroot, coagulation, serum glutamate pyruvate transaminase, serum glutamate oxaloacetate transaminase.

INTRODUCTION

Liver is the main functional organ for metabolism in the body. Liver has many major functions such as it regulates many chemicals and its levels in blood, produces bile, metabolizes fats, carbohydrates and proteins, synthesizes clotting factors and proteins of plasma and carry away waste products like bilirubin, hormones, drugs and cholesterol. It also stores vitamins, minerals and glycogen. Hepatic parameters are important to assess many functional disturbances in the body (Ramaiah, 2007). It is common practice of researchers to evaluate hepatic parameters to calculate the toxicity of any new chemical or drug. These parameters include serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT), Alkaline Phosphatase (ALP) and gamma glutamyl transferase (Materne *et al*., 2002).

High levels of these enzymes indicate liver cells injury or any damage. Biochemical parameters not only detect liver pathology but also the type of liver damage such as membrane damage or necrosis, functional damage or cholestasis that is also known as obstruction in bile production (Horowitz *et al*., 2017).

Beetroot is a nutraceutical product which is used both as a nutrient and for prevention of different diseases. It has a significant value in treating some pathological conditions. It also has advantageous physiological effects. Traditionally beetroot has been used to treat hypertension by reducing both systolic and diastolic pressure (Siervo *et al*., 2013). It is also reported to have anti-inflammatory (Sarfaraz and Najam, 2017), analgesic, diuretic and anti-hyperglycemic effects (Oboh *et al*., 2021). Besides that literature studies have shown its effect in infertility (Sarfaraz *et al*., 2020), hyperlipidemia (Sarfaraz *et al*., 2021) and also neurodegenerative disorders.

Beetroot is an opulent source of flavonoids, ascorbic acid, phenolic acids and carotenoids (Clifford *et al*., 2015). Betalain, the major constituent of beetroot, is the main constituent for treatment of many pathological conditions, because it possesses anti-oxidative and anti-inflammatory effect by reducing cytokines and chemokines and also has a role in cancer by inducing apoptosis (Ninfali and Angelino, 2013). Beetroot also contains rutin, epicatechin and caffeic acid (Lee *et al*., 2014). Rutin and epicatechin are excellent oxidative compounds (Hadipour *et al*., 2020). The present study aimed to assess the hepatoprotective effect of beetroot at different doses by evaluating the liver biomarkers and also considering its effect on coagulation.

MATERIALS AND METHODS

Lyophilized Beetroot Powder

The beet root's lyophilized powder was procured from Sun Rise Nutra Chem Group. Its Lot number was Ctc

**Corresponding author:* e-mail: sana.sarfraz@live.com

2015 0320. Zip lock plastic bag was used for packaging and storing the powder. It was further covered with aluminum foil bag to protect from sunlight.

Animal Selection and habitation

Albino rabbits weighing 1.5-2.0 kg's were purchased from animal house of Dow University of Health Sciences and then kept and acclimatized in the animal house of Department of Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi. The rabbits were divided into 3 groups having n=6 animals in each group. Distilled water was given to a group and labeled as control. 500mg/kg and 1000mg/kg lyophilized beetroot was administered to treated group II and III. All animals were dosed orally for a period of 60 days.

Helsinki Resolution 1964 guidelines were followed in handling the rabbits and the research was authorized through Institutional Board of Advanced Studies and Research vide Resol.No10 (P) 18.

Biochemical Testing

Sample was collected by cardiac puncture on 0 day, 30th day and 60thday in clot activator tube for serum collection to analyze effect on hepatic parameters. For coagulation studies the blood sample was drawn in 3.2% sodium citrate tubes.

Evaluation of Hepatic Parameters

After serum separation standard reagent kits of Human Germany were used for measuring SGPT, SGOT and alkaline phosphatase levels. A semi-automatic chemistry analyzer by Human Germany known as Humalyzer 3000 (Model# 16700) was used for sample analysis. Kinetic method was used for determination of alanine amino transferase (ALAT) activity according to International federation of clinical chemistry's (IFCC) expert panel without activating pyridoxilphosphate as stated by Schumann and Klauke (Schumann and Klauke, 2003). For SGOT analysis the methodology stated by Schumann and Klauke was followed (Schumann and Klauke, 2003). Malloy and Evelyn Method was used for analyzing ALP (Ibrahim *et al*., 2021).γ-GT was examined in the serum by means of Calorimetric method (Persijin and Vanderslik, 1976).

Total and Direct Bilirubin Estimation

Total bilirubin is usually defined as combination of direct and indirect bilirubin. Diazotized sulphanilic acid (DSA) is made to react with bilirubin which leads to formation of red azo dye. At wavelength of 546nm its absorbance is read to determine the concentration in the sample. The measurement of bilirubin in serum was determined by photometric test (Jendrassik and Grof, 1938).

Prothrombin Time

Standard reagent kits of Human Germany were used and analysis was done by Humaclot duo Coagulation analyzer (Model # 18650, Human Germany) (Losner *et al*., 1950; Davey *et al*., 1972).

Fibrinogen Estimation

Hemostat fibrinogen (automated and manual determination of Plasma fibrinogen) was used (NCCLS Document H21-A3 1998). The methodology stated by Mackie and colleagues was followed (Mackie *et al*., 2003).

STATISTICAL ANALYSIS

By taking mean and standard deviation of all treated values they were compared with mean and standard deviation of control and among the groups by applying two ways ANOVA (analysis of variance). Multiple comparisons were done by applying post hoc Tukeys test. The P values were considered significant at $p\leq0.05$, $p\leq0.01$ and $p\leq0.001$.

RESULTS

Effect of different doses of Beta vulgaris on Liver Function Parameters in comparison to control

Table 1 showed the effect of different doses of *Beta vulgaris* on liver parameters. Post hoc analysis by Tukey's test showed when comparison was carried out with control both doses of *Beta vulgaris* highly significantly reduced ($p\leq0.001$) the SGOT levels, however the effect by 1000mg/kg dose was insignificant on 60th day. Highly significant ($p\leq0.001$) decrease was noted in SGPT by both dose of *Beta vulgaris* throughout the treated period as compared to control. *Beta vulgaris* (500mg/kg) dose showed highly significant ($p\leq0.001$) decrease in γGT throughout treated period as related to control. Highly significant ($p\leq0.001$) decrease in γ GT was observed by 1000mg/kg dose of *Beta vulgaris* at 30th day, however insignificant difference was observed on 60th day. ALP levels were highly significantly ($p\leq0.001$) reduced by 500mg/kg dose of *Beta vulgaris* throughout treated period whereas 1000mg/kg showed similar significance too but only on 30th day of dosing as compared to control. Highly significant ($p\leq0.001$) increase was observed on 60th day.

Comparison between two doses of Beta vulgaris on Liver Function Parameters

Table 1 shows the effect of different doses of *Beta vulgaris on* liver parameters. Post hoc analysis by Tukey's test showed when both doses were compared there was highly significant ($p\leq0.001$) increase in SGOT by 1000mg/kg dose of *Beta vulgaris* as compared to 500mg/kg throughout the treated period. Similarly highly significant ($p\leq0.001$) rise in SGPT by 1000mg/kg dose of *Beta vulgaris* as compared to 500mg/kg was observed throughout treated period. Significant ($p\leq0.05$) increase in γ GT by 1000mg/kg dose of *Beta vulgaris* was seen as

compared to 500mg/kg at 30th day, which was then highly significantly (p≤0.001) increased on 60th day of treatment. There was insignificant difference in ALP levels between the two doses of *Beta vulgaris* at 30th day, however highly significant (p≤0.001) increase was observed on 60th day by 1000mg/kg dose as compared to 500mg/kg.

Effect of both doses of Beta vulgaris on Bilirubin Levels in comparison to Control

Table 2 showed the effect of different doses of *Beta vulgaris on* Bilirubin (Direct Bilirubin and Total Bilirubin). Post hoc analysis by Tukey's test showed very significant (p≤0.01) and highly significant (p≤0.001) decrease in direct bilirubin level by 500mg/kg dose of *Beta vulgaris* at 30thand 60thday of dosing respectively when compared with control. 1000mg/kg dose of *Beta vulgaris* also highly significantly (p≤0.001) decreased direct bilirubin level throughout the treatment period as compared to control. Highly significant (p≤0.001) decrease in TB was seen by both dose of *Beta vulgaris* throughout the treated period as compared to control.

Comparison of both doses of Beta vulgaris on Bilirubin levels

Comparison among treated groups showed highly significant (p≤0.001) decrease in DT by 1000mg/kg dose of *Beta vulgaris* as compared to 500mg/kg at 30th day of dosing which was highly significantly (p≤0.001) increased at 60th day. Comparison among the treated groups showed insignificant difference in TB between the treated groups at 30th day of dosing, however significant (p≤0.05) increase in TB was observed by 1000mg/kg dose of *Beta vulgaris* as compared to 500mg/kg at 60th day.

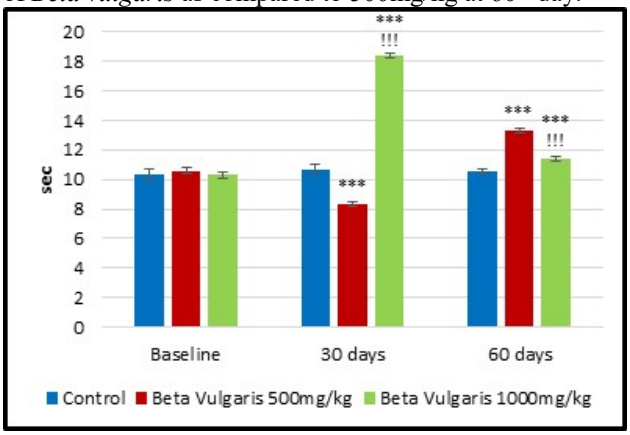

Fig. 1: Effect of different doses of *Beta vulgaris* on Prothrombin Time

Effect of both doses of Beta vulgaris on Prothrombin Time (PT)

Fig. 1 showed highly significant (p≤0.001) decrease in PT by 500mg/kg dose of *Beta vulgaris* at 30th of dosing as compared to control. However on 60th day of dosing extremely substantial (p≤0.001) rise in PT was witnessed by 500mg/kg dose of *Beta vulgaris* as compared to control. Highly significant (p≤0.001) rise in PT was witnessed by 1000mg/kg dose of *Beta vulgaris* throughout treatment period as compared to control. Comparison among the treated groups revealed extremely significant (p≤0.001) rise in PT by 1000mg/kg dose of *Beta vulgaris* as compared to 500mg/kg at 30th day of dosing. However on 60th day of dosing highly significant (p≤0.001) decrease was observed by 1000mg/kg dose as compared to 500mg/kg.

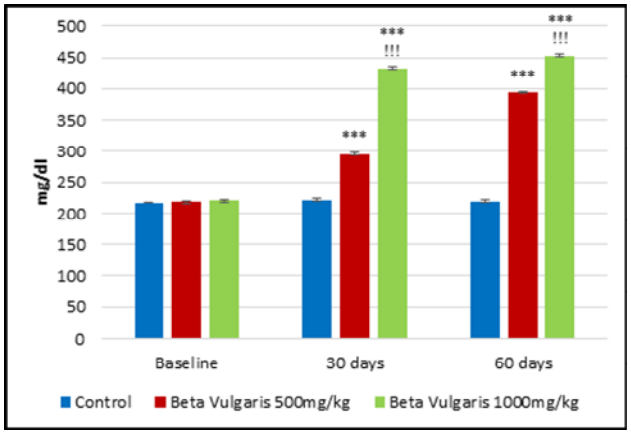

Fig. 2: Effect of different doses of *Beta vulgaris* on Fibrinogen

Effect of both doses of Beta vulgaris on Fibrinogen Level

Fig. 2 showed highly significant (p≤0.001) increase in fibrinogen by both doses of *Beta vulgaris* throughout treatment period as compared to control. Comparison among the treated groups showed highly significant (p≤0.001) increase in fibrinogen level by 1000mg/kg dose of *Beta vulgaris* as compared to 500mg/kg dose throughout the treated period.

DISCUSSION

Liver is the site of biotransformation and metabolism for a large variety of medications and external agents, many substances can cause hepatic damage. Genuine underlying ailments can be found by rise in major biomarkers of hepatotoxicity such as alanine transaminase (ALT) and aspartate transaminase (AST) (Singh *et al*., 2011). The enzymes ALT and AST are also referred as serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxaloacetate transaminase, (SGOT) respectively.

SGPT levels are more specific for determination of hepatic abnormalities, since SGPT is mainly found in hepatocytes. When these cells are destroyed, this enzyme is released into the bloodstream, where it is measured. SGOT is similar to SGPT in that it is found in hepatocytes as well. It is increased in acute liver damage and is also found in erythrocytes, cardiac and skeletal muscle. It is not only specific to the liver (Shuvaev *et al*., 2009).

Table 1: Effect of different doses of *Beta vulgaris* on Hepatic Parameters

Groups	Days	SGPT (U/L)	SGOT (U/L)	Gamma GT (U/L)	Alkaline Phosphatase (U/L)
Group I (Distilled Water)	Baseline	90.4± 1.81	51.7± 1.49	5.4 ± 0.52	39.7 ± 1.15
	One month	94.7 ± 0.92	56.7 ± 1.41	6.0 ± 0.81	42.2 ± 1.03
	Two month	98.3 ± 1.40	62.1± 1.91	5.8 ± 0.78	41.5 ± 1.43
Group II (500mg/kg lyophilized beetroot powder)	Baseline	94.1 ± 1.1	55.6 ± 1.17	5.5 ± 0.84	42.9 ± 1.19
	One month	35.7 ± 1.4***	24.7 ± 1.15***	2.5 ± 1.08***	27.8 ± 1.05***
	Two month	28.4 ± 1.20***	23.6 ± 0.69***	3.1 ± 0.74***	30.4 ± 1.07***
Group III (1000mg/kg lyophilized beetroot powder)	Baseline	99.0± 1.05	66.9 ± 1.09	6.0 ± 0.82	43.5 ± 1.26
	One month	44.3 ± 1.3$^{***!!!}$	53.8 ± 1.03$^{***!!!}$	3.1 ± 0.74$^{***!}$	27.6 ± 1.64***
	Two month	85.0 ± 1.5$^{***!!!}$	62.2 ± 1.05$^{!!!}$	6.1 ± 0.73$^{!!!}$	47.5 ± 1.27$^{***!!!}$

Table 2: Effect of different doses of *Beta vulgaris* on Bilirubin

Groups	Days	Direct Bilirubin(mg/dl)	Total Bilirubin(mg/dl)
Control	Baseline	0.066± 0.008	0.43 ± 0.04
	30 days	0.071 ± 0.010	0.49 ± 0.03
	60 days	0.072± 0.009	0.48 ± 0.02
Beta vulgaris 500mg/kg	Baseline	0.065 ± 0.007	0.44 ± 0.05
	30days	0.061 ± 0.005**	0.04 ± 0.009***
	60 days	0.014± 0.004***	0.03 ± 0.01***
Beta vulgaris 1000mg/kg	Baseline	0.069 ± 0.006	0.41 ± 0.01
	30 days	0.018 ± 0.004$^{***!!!}$	0.05 ± 0.02***
	60 days	0.04 ± 0.003$^{***!!!}$	0.08 ± 0.04$^{***!}$

Where $^{*}p\leq0.05$, $^{**}p\leq0.01$, $^{***}p\leq0.001$ = significant, very significant and highly significant as compared to control
$^{!}p\leq0.05$, $^{!!}p\leq0.01$, $^{!!!}p\leq0.001$ = significant, very significant and highly significant, when compared among treated groups

Alkaline phosphatase is found only in the cells that line the biliary duct and is involved in the transfer of a variety of metabolites. ALP levels rise in infiltrative liver disorders.

Excessive productions of ALP lead to Hepatobiliary injury and cholestasis. Gamma GT is found in the cell membranes of a variety of tissues, including the bile duct, gall bladder, heart, brain, and kidney. Elevated level of both ALP and GGT is a specific indication of hepatobiliary injury, especially biliary effects and cholestasis (Ramaiah, 2007). Bilirubin is a yellow breakdown product produced by normal heme catabolism. It's also been discovered in plants. It is eliminated through the bile and urine (Pirone *et al*., 2009). To monitor gall bladder or liver issues, total or direct bilirubin is frequently measured (Panda *et al*., 2015).

Beta vulgaris showed reduction in all hepatic biomarkers i.e (SGPT, SGOT, Gamma GT, ALP and bilirubin) however the effect was more pronounced at 500mg/kg dose. Comparison among groups showed significant variation in 1000mg/kg dose as compared to 500mg/kg however the values were within the normal range (Suckow *et al*., 2012). Literature studies have shown the positive effect on hepatic biomarkers is because of its anti-oxidant property which is due to the presence of phenols and flavanoids present in *Beta vulgaris*. Phenols possess anti-oxidant, free radical scavenging and anti-carcinogenic properties. Flavanoids possess hepatoprotective and anti-inflammatory activity. Flavanoids are usually polyphenolic compounds and due to presence of phenolic hydroxyl group they have the ability to scavange the reactive oxygen specie (ROS) as well as inhibit enzymes such as aldose reductase and xanthine oxidase making them strong anti-oxidant (Rose *et al*., 2014).

Beta vulgaris at both the doses showed increase in prothrombin time, it is the measure of extrinsic and common pathway factors. This increase in prothrombin time is also confirmed with the results of fibrinogen levels which are increased at both the doses. Fibrinogen is one of the common factors of clotting as it is converted into fibrin which form clots (Dahlback, 2000). This activity shown by *Beta vulgaris* is due to the presence of two flavonoids rutin and epicatechin (Kuntic *et al*., 2011). This can be beneficial in People who have deficiency of platelets so clotting ability by the body can be improved by giving beetroot. *Beta vulgaris* at 500mg/kg showed more significant rise as compared to 1000mg/kg, this is because of presence of ascorbic acid as it is vastly present in *Beta vulgaris*. Literature surveys indicated that ascorbic acid reduces anticoagulant effect in warfarin (Sattar *et al*., 2013) so in 1000mg/kg, the quantity of ascorbic acid increase which slightly inhibits anticoagulation activity of

rutin and epicatechin. It is also noted that rutin produce its anticoagulant effect on lower doses (Ganeshpurkar and Saluja, 2017).

CONCLUSION

Beta vulgaris is a highly beneficial dietary product with ample amount of flavonoids and anti-oxidant agents which might help in improving the liver function in patients of jaundice and hepatitis. It could also play a role in those patients whose coagulation profiles are affected by liver disorders as it can increase both fibrinogen levels and prothrombin time.

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