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Rising trend of Nutraceuticals: Evaluation of lyophilized beetroot powder at different doses for its hypolipidemic effects

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Abstract: A diet comprising of nutrients that would control hypertension as well as hyperlipidemia would be very beneficial over all. This study aimed to assess the effect of lyophilized beet root powder at different doses on lipid profile and hyperlipidemia model. Albino rabbits weighing 1500-2000gms were taken for both studies. Beetroot powder was administered to animals at 500mg/kg and 1000mg/kg doses and after two month dosing the blood samples were withdrawn and lipid profile was assessed. Next a model of hyperlipidemia was created comprising of albino rabbits that were divided into five groups each containing n=6. Group I was considered as control, Group II was marked as Negative control, Group III was taken as standard, whereas Group IV and V were considered as treated and given different doses of beetroot. Blood samples were drawn at baseline, 45th day and at day 60th of study. Highly significant decrease in lipid profile (Cholesterol, LDL and TGS) and significant increase in HDL was observed by both doses after one month. HDL was increased more at 1000mg/kg dose. The presence of flavonoids and saponins in beetroot is responsible for hypolipidemic effect. From our research we came to the conclusion that beetroot powder reduced the lipid profile and could be beneficial in treatment of cardiovascular disease due to atherosclerosis and obesity.

Keywords: High density lipoprotein; hyperlipidemia, low density lipoproteins, triglycerides.

INTRODUCTION

Human civilization has utilized plants in their diets as well as to cure different ailments since traditional times. These plants have played a major role in treating diseases as well as to maintain human body functions and metabolism. Almost 75% population in the world is presently considering plants as the main curing tool for many problems. There are many examples of those plants which are widely used in treating many disorders. Like in India turmeric has an immense significance for treating wounds, rheumatism, acidity, helminthiasis and rhinitis (Prasad and Aggarwal, 2011). Many skin diseases and serious wounds can be easily treated with the plant *Moringa oleifera* in Guatemala (Lopez *et al*., 2018). The people of Pakistan use *Olea ferruginea* fruits for lowering hyperglycemic conditions in diabetes patients (Ahmad *et al*., 2009). There are thousands of researches which reveal that the intake of organic products in daily meals can largely increase the well-being of human and also decrease the advent of diseases and disorders (Vainio and Weiderpass, 2006).

Beta vulgaris belongs to family Amaranthaceae (Christenhusz and Byng, 2016) and is commonly known as table beet, garden beet as well as beetroot. Romans cultivated the beetroot for the first time, now they are mostly cultivated in many countries such as France, Russia, Germany, Europe, United States of America, North America, Central America and Asia (Lefevre & Riviere, 2006). Naturally beetroot is packed full of nutrition. Beetroot is considered as the great source of many vitamins, minerals, carbohydrates, protein and micronutrients. Conferring to The Oxford Dictionary of Food and Nutrition a beetroot weighing 40 grams yields 75 kilojoules (KJ) of energy and contains 1.6gm dietary fiber. The nutrients present in beetroot are presented in following table as reported by McCance and Widdowson's, 1995.

The important chemical constituents present in beetroot are betalains (betacyanins and betaxanthins) (Khan, 2016), N, N, N-trimethylglycine (Day and Kempson, 2016), betavulgarin, betagarin, dihydroisorhamnetin and cochliophilin A (Kujala *et al*., 2002), Besides that the constituent hydroxycinnamic acids such as, ferulic syringic, caffeic acids and gallic (Kazimierczak *et al*., 2014), 4-hydroxybenzoic acid, epicatechin, Catechin hydrate, Rutin and Chlorogenic acid are also found in beet root (Georgiev *et al*., 2010).

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Current studies have highlighted the use of lyophilized beet root in enhancing fertility in experimental animals (Sarfaraz *et al*., 2020), as a diuretic (Sarfaraz and Najam, 2018), as an anti-inflammatory agent (Sarfaraz and Najam, 2017) and as an analgesic (Sarfaraz and Najam, 2019). It also possesses nephroprotective effect (Sarfaraz and Najam, 2019). Its use has also been reported in lowering the blood pressure. Betaine in dose of 6gm OD has been shown to influence release of nitric oxide in human volunteers (Hobbs *et al*., 2012). Literature studies have also shown it to inhibit calcium oxalate crystal formation (Saranya and Geetha, 2014). The current research was designed to assess the effect of different doses of lyophilized beetroot on lipid profile and in lowering the lipid levels in an established hyperlipidemic model.

MATERIALS AND METHODS

Powder of beetroot

The beet root powder which was freeze-dried was acquired from Sun Rise Nutra Chem Group. Ctc 2015 0320 (lot number). For the storage and packaging of lyophilized beetroot powder Zip lock plastic bag was taken and to protect further from sunlight aluminum foil was used.

Animal selection

Albino rabbits were selected for the study as it is easier to draw blood from them multiple times and they resemble the human physiology. Animals were kept in a well-developed Animal House of Department of Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi. Animals were kept with high monitoring of controlled temperature and humidity. The temperature was maintained at 25±2° C and humidity was kept around 50-65% with half light and dark cycle respectively. The diet and water was also monitored critically and provided as per requirement.

Ethical approval

The guidelines of Helsinki Resolution 1964 were followed for handling the animals and this study was legally approved by Institutional Board of Advanced Studies and Research vide Resol. No.10 (P) 18.

Animal grouping for preliminary analysis

For preliminary analysis the animals were distributed in three groups. The control group was given the distilled water while two groups were made with the doses of 500mg/kg and 1000mg/kg of lyophilized beetroot powder which were marked as group II and group III respectively. The dose was given orally to all the animals for the duration of 60 days.

Animal grouping for hyperlipidemia model

Albino rabbits which were healthy of both sexes were included. The weight of rabbits was around 1500-2000gm and was divided into five groups for the study. There were accommodated and kept at the temperature and humidity as mentioned above.

Dosing scheme

The rabbits were distributed into five groups as follows

Hyperlipidemia induction by cholesterol rich diet

The rabbits were given cholesterol mixed diet to induce hyperlipidemia in them (Fan *et al*., 2015). Vanaspeti ghee along with coconut oil in the ratio of 3:1 v/v was used for cholesterol rich diet (Munshi *et al*., 2014). The diet was made in the form of pellets for comfortable administration. Baseline reading of Lipid profile testing was done at the start of the study (0 day) and then at day 45 and 60th day. On 45th day hyperlipidemia was induced. The drug was started from the day one on both treated group I and II and on 45th and 60th observations were taken 45 minutes after oral dosing.

Collection of sample

The Sample was withdrawn by cardiac puncture on day 0, 30th day and 60th day in a tube called clot activator tube for serum collection to examine effect on lipid factors. Similarly for Hyperlipidemia model sample was collected on Day 0, 45 and 60.

Analysis of lipid profile

A semi-automatic chemistry analyzer by Human Germany known as Humalyzer 3000 (Model# 16700) was used to separate the serum from clotted blood samples within 3 hours. Lipid biomarkers were investigated using Human standard kits.

The estimation of cholesterol was done after hydrolysis and enzymatic oxidation. The indicators used for the determination of cholesterol were phenol and peroxidase hydrogen peroxide and 4-amino phenazone form quinoneimine. This method is known as oxidase/ peroxidase aminophenazone (CHOD-PAP) method of enzymatic colorimetric testing and it involves using monoreagent with lipid clearing factor (LCF) (Sarfaraz *et al*., 2017). For the evaluation of HDL levels phospha-tungstic acid and magnesium chloride was used which helps in precipitation of Very low density lipoprotein (VLDL) and Low density lipoprotein (LDL). Supernatant fluid was obtained after centrifugation which contained HDL and was measured enzymatically (Warnick *et al*., 2001). The low density lipoprotein concentration can be calculated from total cholesterol (TC), HDL and Triglyceride concentration (TG) as stated by Friedwald and co-workers (Larrson *et al*., 2018). Enzymatic colorimetric (Liquicolor) GPO-PAP method with LC (Pundir and Narwal, 2018) was used for the analysis of triglycerides. Friedwald's formula (Fukuyama *et al*., 2007) was used for the estimation of VLDL.

Phytochemical evaluation

Using standard procedures Evans, 2009, Phytochemical analysis of beetroot was performed (Evans, 2009).

STATISTICAL ANALYSIS

Statistical analysis was done by taking the mean of all treated values and they were compared with the mean and

Table 1: Nutrients in Beet root

Energy	154 KJ	Zinc	0.4mg
Water	87.1gm	Manganese	0.7 mg
Carbohydrates	7.6gm	Magnesium	11mg
Proteins	1.7gm	Chloride	59mg
Fats	0.11gm	Folate	150µgm
Sodium	66mg	Ascorbic acid	5mg
Potassium	380mg	Pantothenic acid	0.12 mg
Calcium	17mg	Pyridoxine	0.04mg
Phosphorus	51mg	Thiamine	0.02 mg
Iron	1 gm	Riboflavin	0.1mg
Niacin	0.33 mg	Carotene	20µgm

Table 2: Dosing Scheme of Hyperlipidemia Model

Group I	Control group	Only distilled Water
Group II	Negative Control group	Cholesterol rich diet + distilled water
Group III	Standard	Atorvastatin 2.1 mg/kg + Cholesterol rich diet
Group IV	Treated I	Cholesterol rich diet+ 500mg/kg lyophilized *Beta vulgaris* (beetroot) powder (Jain *et al*., 2011)
Group V	Treated II	Cholesterol rich diet +1000mg/kg lyophilized *Beta vulgaris* (beetroot) powder.

Table 3: Effect of different doses of Beetroot powder on VLDL in Hyperlipidemia Model

Groups	Days	VLDL (mg/dl)
Control (Distill water)	Baseline	13.7 ± 1.15
	45 days	13.6 ± 2.11
	60 days	13.9 ± 1.37
Negative Control (Distill water + Cholestrol rich diet)	Baseline	13.5 ± 1.16
	45 days	$75.3 \pm 1.17^{***}$
	60 days	$83.6 \pm 0.96^{***}$
Standard (Atorvastatin 2.1mg/kg +Cholesterol rich diet)	Baseline	14.0 ± 0.98
	45 days	$21.1 \pm 1.12^{***\$\$\$}$
	60 days	$14.9 \pm 1.91^{\$\$\$}$
Treated I *Beta vulgaris* 500mg/kg + Cholestrol rich diet	Baseline	13.65 ± 1.35
	45 days	$11.3 \pm 0.94^{***\ \$\$\$\ \#\#\#}$
	60 days	$7.4 \pm 0.97^{***\ \$\$\$\ \#\#\#}$
Treated II *Beta vulgaris* 1000mg/kg + Cholestrol rich diet	Baseline	13.6 ± 0.67
	45 days	$15.1 \pm 0.73^{***\ \$\$\$!!!\ \#\#\#}$
	60 days	$14.1 \pm 1.19^{\$\$\$!!!}$

Values are shown as X ± S.D where ***, *represent p≤0.001 and p≤0.05 as compared to control, $^{\$\$\$}$ represent p≤0.001 as compared to negative control, $^{\#\#\#}$ represent p≤0.001 as compared to standard and $^{!!!}$represent p≤0.001 when compared among treated groups.

Table 4: Phytochemical analysis of beetroot

Phytochemical Constituent	Type of Test	*Beta vulgaris*
Carbohydrate	Molisch Test	+
	Iodine Test	-
	Seliwanoff's Test	+
	Barfoed Test	-
Flavonoids	Powder solution+ Ferric chloride	+
Tannins	Powder solution+ Ferric chloride	+
Phenols	Powder solution+ Lead acetate	+
Terpenoids	Powdersolution+ Chloroform+ Conc.H_2SO_4	+
Triterpenoids	Liberharmann-Burchard's Test	+
Steroids	Salkowski Test	+
Saponins	Foam Test	+
Anthraquinone	Borntrager's Reagent	+
Cardiac glycoside	Keller-Killiani Test	+

the standard deviation of control and standard readings by relating two ways analysis of variance (ANOVA). Multiple contrasts were done by the method of applying post hoc Turkey's test. The P values were considered significant at $p \leq 0.05$ and $p \leq 0.001$. The comparisons were done with respect to control (***), negative control ($^{\$\$\$}$), standard ($^{\#\#\#}$) and among treated groups ($^{!!!}$).

RESULTS

Fig. 1-5 shows the effect of different doses of *Beta vulgaris on* Lipid Profile. The readings were observed before starting treatment and at 30 and 60 days of treatment. Figure 6-9 and Table 3 show the effect of different doses of *Beta vulgaris* on Hyperlipidemia model. The readings were observed before starting treatment, at 45 days (when hyperlipidemia was induced) and at 60th day of treatment.

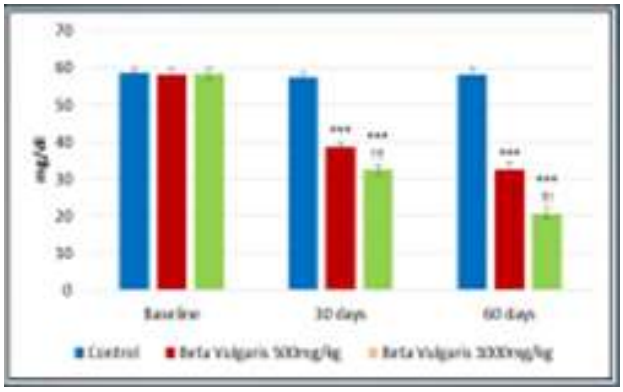

Fig. 1: Effect on Cholesterol level mg/dl

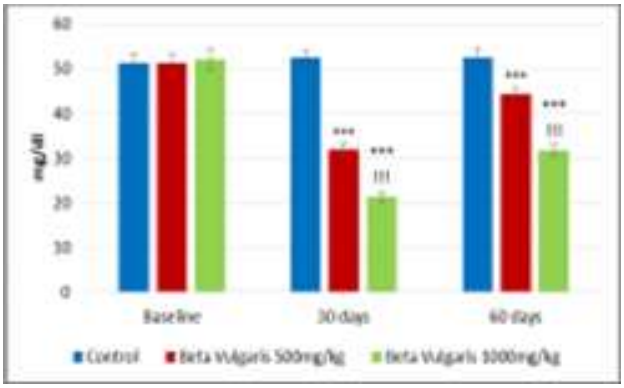

Fig. 2: Effect on TGs level mg/dl

Effect on the level of Cholesterol

Post hoc analysis by Tukey's test displayed significant decline ($p \leq 0.001$) in cholesterol level by beetroot (500mg/kg and 1000mg/kg) throughout study period as compared to control. When comparison was done between 500mg/kg and 1000mg/kg dose notable decline in cholesterol level by 1000mg/kg dose of beetroot was observed throughout the study period.

In the hyperlipidemia model analysis showed notable drop ($p \leq 0.001$) in cholesterol level by 500mg/kg and 1000mg/kg doses throughout treatment period as compared to control. However notable incline in cholesterol level was observed by negative control group in relation to control. When related with negative control, beetroot (both doses) and standard displayed significant decline in cholesterol levels throughout the treated period. When compared with standard 500mg/kg dose of beetroot displayed notable drop where as non-significant effect was observed as compared to 1000mg/kg dose. Comparison among treated groups displayed notable decline in cholesterol level by 500mg/kg dose of *Beta vulgaris* as compared to 1000mg/kg throughout treatment period.

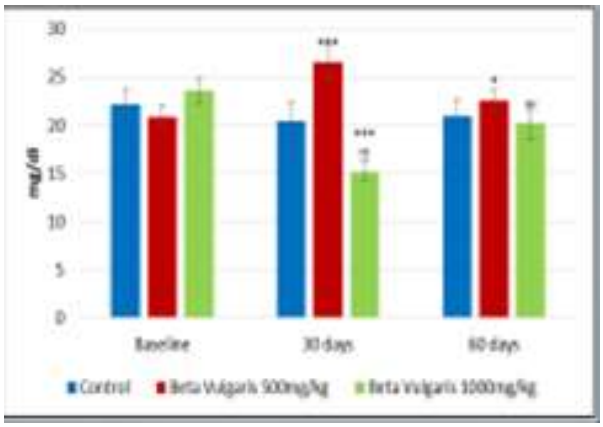

Fig. 3: Effect on HDL level mg/dl

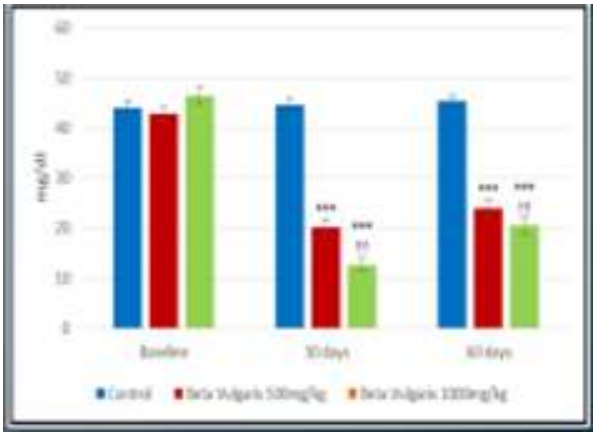

Fig. 4: Effect on LDL level mg/dl

Effect on Triglycerides (TG) Level

Post hoc analysis by Tukey's test displayed notable ($p \leq 0.001$) drop in TG'S by both doses of beetroot powder as compared to control throughout the study period. Comparison among the treated groups showed notable decline in TG'S level by 1000mg/kg dose of *Beta vulgaris* as compared to 500mg/kg throughout treatment period.

In hyperlipidemia model Post hoc analysis by Tukey's test displayed notable drop ($p \leq 0.001$) in TG level by beetroot (500mg/kg and 1000mg/kg) throughout treatment period as compared to control. However notable

rise in TG level was observed by negative control in relation to control throughout treatment period. When compared with negative control, both doses of beetroot and atorvastatin displayed noteworthy drop in TG levels throughout the treated period. When compared with standard notable drop in TG's was observed by 500mg/kg beetroot dose throughout the treatment period whereas 1000mg/kg dose showed noteworthy effect only on day 45th. Comparison among treated groups showed notable decline in TG level by 500mg/kg dose of *Beta vulgaris* as compared to 1000mg/kg throughout treatment period.

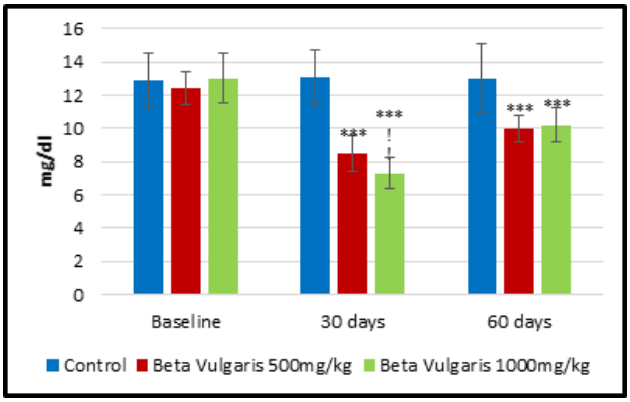

Fig. 5: Effect of different doses of *Beta vulgaris* on VLDL level (mg/dl)

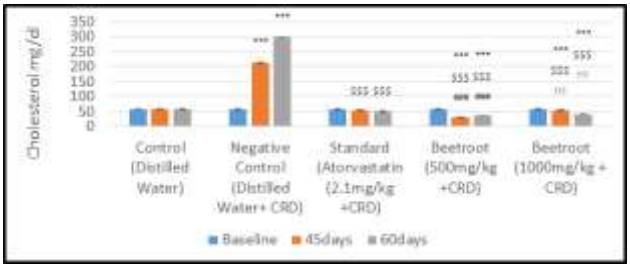

Fig. 6: Effect of different doses of *Beta vulgaris* on Cholesterol level (mg/dl) in Hyperlipidemia Model

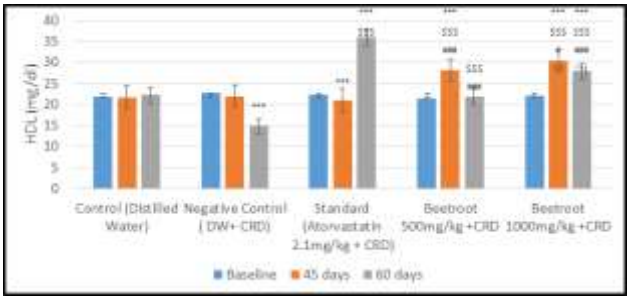

Fig. 7: Effect of different doses of *Beta vulgaris* on HDL (mg/dl) in Hyperlipidemia Model

Effect on High density lipoprotein (HDL)

Post hoc analysis by Tukey's test reported substantial rise in HDL by 500mg/kg dose of beetroot on 30th day and 60th as compared to control. 1000mg/kg dose of beetroot notable declined HDL level at 30th day of dosing as compared to control. When treated groups were equated the results displayed notable decline in HDL level by 1000mg/kg dose of beetroot as compared to 500mg/kg throughout treatment period.

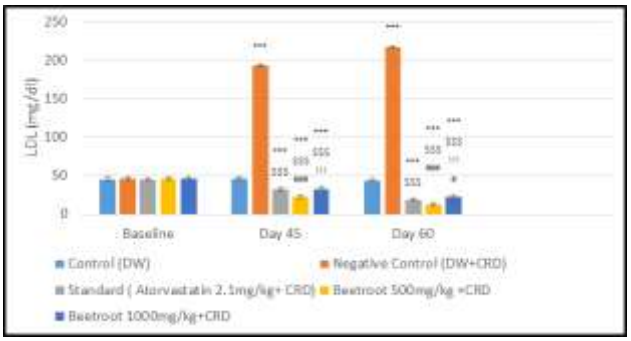

Fig. 8: Effect of different doses of *Beta vulgaris* on LDL (mg/dl) in Hyperlipidemia Model

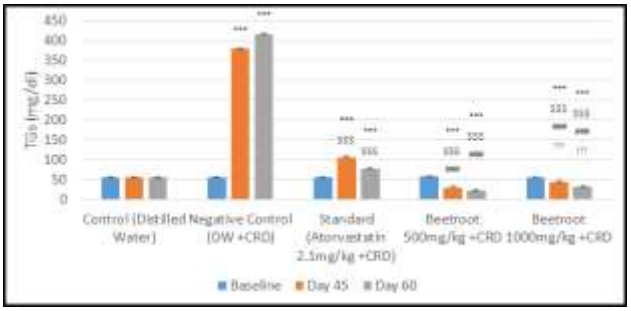

Fig. 9: Effect of different doses of *Beta vulgaris* on TG's (mg/dl) in Hyperlipidemia Model

In hyperlipidemia model Post hoc analysis by Tukey's test displayed noteworthy incline in HDL by 500mg/kg dose of *Beta vulgaris* at 45th day of dosing which was then non-significant on day 60th as compared to control. Notable rise was observed by 1000mg/kg dose of *Beta vulgaris* throughout treated period as compared to control. Noteworthy decline in HDL was also observed by negative control on day 60th as compared to control. When compared with negative control, both doses of *Beta vulgaris* displayed notable incline in HDL throughout the treated period, whereas standard showed increase on day 60th only. When compared with standard noteworthy rise was observed on day 45th when compared with both doses of beetroot which was then significantly decreased on day 60th. Comparison among treated groups displayed noteworthy rise in HDL by 1000mg/kg dose of beetroot in relation to 500mg/kg throughout treatment period.

Effect of different doses of Beta vulgaris on Low Density Lipoprotein (LDL)

Analysis displayed substantial decline in LDL levels by both doses of beetroot powder throughout the treatment period as compared to control. When the treated groups were equated noteworthy decline in LDL levels by 1000mg/kg dose of beetroot was seen as compared to 500mg/kg throughout treatment period.

In Hyperlipidemia model analysis by Tukey's test displayed substantial drop in LDL by both doses of beetroot throughout the study period as compared to control. However substantial rise in LDL was observed by negative control as compared to control. When compared with negative control, Standard as well as both doses of beetroot displayed substantial drop in LDL throughout the treated period. When compared with standard noteworthy drop in LDL was observed by 500mg/kg dose throughout the treatment period. Comparison among treated groups displayed substantial decrease in LDL by 500mg/kg dose of beetroot as compared to 1000mg/kg throughout treatment period.

Effect of different doses of Beta vulgaris on Very Low Density Lipoprotein (VLDL)

Statistical analysis displayed noteworthy decline in VLDL levels by both doses of beetroot throughout the treatment period as compared to control. Comparison among the treated groups displayed significant decline in VLDL levels by 1000mg/kg dose of beetroot as compared to 500mg/kg on 30^{th} day of treatment. The difference was insignificant at 60^{th} day of dosing.

In Hyperlipidemia model analysis represented noteworthy decline in VLDL by 500mg/kg dose of beetroot throughout treatment period as compared to control. Substantial decline was also observed by 1000mg/kg dose of beetroot at 45^{th} day which was then insignificant at 60^{th} day of dosing as compared to control. However noteworthy rise in VLDL was observed by negative control as compared to control throughout treated period. In equation with negative control, both doses of beetroot and standard showed substantial drop in VLDL throughout the treated period. In comparison to standard noteworthy decline was observed by 500mg/kg dose of beetroot throughout the treatment period whereas significant decline was observed by 1000mg/kg only at 45^{th} day. Comparison among treated groups showed substantial decrease in VLDL by 500mg/kg dose of beetroot as compared to 1000mg/kg throughout treatment period.

Phytochemical analysis

Table 4 showed the presence of Carbohydrates, flavonoids, tannins, phenols, terpenoids, triterpenoids, saponins, anthraquinines and cardiac glycosides in beetroot.

DISCUSSION

Many Cardiovascular diseases and atherosclerosis are due to the increase plasma lipid levels (Upadhyay, 2015). The condition of hypercholesterolemia is caused by the production of free oxygen radical's specie (ROS) which can also lead to cardiovascular diseases (Wu *et al*., 2002). The development of atheroma plaque is because of high levels of cholesterol. Many researches have shown that number of problems like cardiac diseases, diabetes, atherosclerosis and cancer may be due to high levels of cholesterol while it is also indicated that lowering the levels of cholesterol may lower the risk of prostate cancer (Solomon *et al*., 2009). Low density lipoprotein (LDL) and high density lipoprotein are marked as most particular and sensitive biomarkers in the cardiac diseases and HDL is advantageous in the protection against the formation of atherosclerosis (Guyton and Hall, 2006). Our study has shown that the beetroot, in both doses remarkably declines all lipid biomarkers hence *Beta vulgaris* was further tested in pathological model by developing hyperlipidemia.

The elevation in the levels of cholesterol, LDL, VLDL and triglycerides appeared to indicate the condition of hyperlipidemia whereas HDL levels were decreased. Hyperlipidemia is the disorder or the problem associated with disturbed levels of lipoprotein metabolism or overproduction of lipoproteins (Adam, 2005). Hyperlipidemia is the basis and the key risk factor in the advancement and growth of heart diseases like angina, strokes, heart attacks and peripheral arterial disease are also caused due to the condition known as atherosclerosis (Bisht *et al*., 2015).

The results of our study have shown decline in all the biomarkers of hyperlipidemia with both the doses of *Beta vulgaris*. However, 500mg/kg dose had more significant effects in lowering the levels of lipoproteins. The chronic dosing of 500mg/kg dose decreased the overall cholesterol level including HDL although its value was within limit but the chronic dosing of 1000mg/kg, increased the HDL levels significantly too. Rabbits were chosen for the study because rabbits and humans have same cholesteryl ester protein and the results would not be compromised. Beetroot has the property of lowering lipid metabolism and production due to the presence of flavonoids and saponins. Saponins are the constituents which contain aglycone molecule linked by glycosidic bond to sugar residue. Studies in the past have reported that the saponins due to the presence of aglycone majorly effect the lowering of cholesterol and levels of triglycerides (Atamanova *et al*., 2005). The mechanism of saponins in lowering the cholesterol level is by inhibiting the enzyme pancreatic lipase which is involved in the breakdown and hydrolysis of 50%-70% dietary fats (Marrelli *et al*., 2016).

Hyperlipidemia further worsen the condition by causing the oxidative stress which is due to the inhibition of enzymes which are beneficial for anti-oxidation of tissues and also increase formation of free radicals like superoxide which ultimately leads to the heart diseases (Al-Dosari *et al*., 2011). Some studies have shown that the extract of beetroot is very helpful at 250mg/kg and

500mg/kg by causing a rise in anti-oxidation in myocardial condition as well as also by causing an increase in endogenous hepatic anti-oxidation (Ninfali and Angelino, 2013). This anti-oxidation effect of *Beta vulgaris* might be due to the presence of flavonoids because flavonoids are useful in subduing the ROS formation and also defeating ROS with the help of anti-oxidant defenses. Therefore *Beta vulgaris* can act as a cardio protective agent (Kumar and Pandley, 2013).

CONCLUSION

From our study we came to the conclusion that low dose beetroot can be incorporated as a dietary nutrient in the lifestyle modification therapy of patients suffering from obesity, atherosclerosis and other cardiovascular disorders as it would lower the lipid profile and act as prophylaxis in development of diseases due to hyperlipidemia.

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