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ANTI-HYPERLIPIDEMIC ACTIVITY OF *SPINACIA OLERACEA* LEAVES EXTRACT AGAINST FRUCTOSE INDUCED HYPERLIPIDEMIA

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ABSTRACT

Hyperlipidemia is the greatest risk factor of coronary heart disease. Currently available hypolipidemic drugs have been associated with number of side effects. Herbal treatment for hyperlipidemia has no side effects and is relatively cheap and locally available. A literature claimsthat flavonoids can able to reduce the hyperlipidemia. The literature available on *Spinaciaoleracea*Leavessuggested for the presence of flavonoid content, therefore the leaves of *Spinaciaoleracea*were selected and the present study was designed to investigate the anti-hyperlipidemic activity of extract of *Spinaciaoleracea* fructose induced Hyperlipidemia. *Spinaciaoleracea*was administered at a dose of 100mg/kg and 200mg/kg per day, (p.o) to fructose induced Hyperlipidemic rats. Atorvastatin was used as reference standard. The statistical analysis was carried out using one way ANOVA followed by Tukey test. *Spinaciaoleracea* showed a significant decrease in the levels of serum cholesterol, triglycerides, LDL, VLDL and a gradual increase in the level of serum HDL at the dose of 200mg/kg/day (p.o) against fructose induced hyperlipidemia. Therefore the study concluded that the extract of leaves of *Spinaciaoleracea* effectively suppressed the hyperlipidemia in rats, suggesting the potential protective role in Coronary heart disease.

KEYWORDS: *Spinaciaoleracea*, Hyperlipidemia, Triglycerides, lipoprotein, fructose.

INTRODUCTION

Hyperlipidemia is defined by abnormally elevated levels of one or more lipids such as cholesterol or triglycerides in the bloodstream. It also involves elevated levels of lipoproteins especially LDL-cholesterol and this is the most common forms of dyslipidemia which comprises a triad of decreased levels of high density lipoprotein (HDL), increased levels of low density lipoprotein (LDL), and elevated levels of triglycerides.

Hyperlipidemia is a disorder of lipoprotein metabolism manifested as hypercholesterolemia, hypertriglyceridemia, or a combination, with elevated plasma apolipoprotein B. Hyperlipidemic is a risk factor for gall stone, pancreatitis and xanthomas, whereas hyperlipidemic is a risk factor for coronary artery disease (CAD), myocardial infarction (MI), hypertension and cerebrovascular accidents. CAD could be considered as the most common cause of death globally, including India, by 2020.

Hyperlipidemia is the result of complex interactions between environmental and genetic factors (Haffner et al, 1999). Hyperlipidemia is the main cause of congestive heart diseases in adulthood. It is also the main cause of atherosclerosis which is the pathophysiological cause of vascular diseases such as angina pectoris, myocardial infarction, and stroke.

*Spinaciaoleracea*is an annual herb belongs to the family Amaranthaceae and it is widely distributed, cultivated in India. The whole plant is medicinally important. Its leaves are commonly eaten as a vegetables, either fresh, frozen, canned, chopped or dehydrated,it is annual plant rarely biennal.

*Spinaciaoleracea*is traditionally use as lowering the cholesterol, boosts immunity and protect skin. Medically *Spinaciaoleracea*is used as lowering the cholesterol level and improve growth and physical activity, spinach is good for digestive and urinary system, *Spinaciaoleracea*is also posses the antibacterial activity, excellent source for vitamin K, A &C, it also plays role in prevention of cancer, cardiovascular diseases, age related muscle degeneration and degeneration of immune and neurological system. Based on high flavonoid content in herbal, *Spinaciaoleracea*was selected and the present study was designed to investigate the antihyperlipidemic activity of ethanolicextract of *Spinaciaoleracea*fructose induced Hyperlipidemia.

MATERIAL AND METHODS

Plant collection, identification and authentication

The specimen was collected from local region of Nanded and identified on the basis of morphological features as *Spinaciaoleracea*belonging to the family Amaranthaceae, and herbarium of the plant specimen has been given for authentication toDr. S. S. Bodke, HOD, Dept. of Botany, YeshwantMahavidyalaya, Nanded.

Preprarion of plant extract

Spinaciaoleracealeaves were shade dried, leaned and pulverized by hands made to obtain coarse powder of mesh size #40. Coarse powder (1000 g) of SOL was exhaustively defatted using petroleum ether (60-80 ºC) (SOL-PE) and extracted successively with chloroform (SOL-CH), Ethyl Acetate (SOL-EA) and methanol (SOL-ME) using Soxhlet apparatus. All the extracts were collected, filtered through watman filter paper, concentrated and stored in tight desiccator and percentage yield was calculated.

Preliminary phytochemical qualitative screening of SOL Extracts

All the extracts werescreened for presence of phytoconstituents*viz.* alkaloids, flavonoids, tannins, steroids, saponins, triterpenoids, fixed oil and sugars as per standard procedure as given under (Trease& Evans, 1997).

Drugs and Chemicals used

Fructose is used as inducer of hyperlipidemic agent and Atorvastatin as standard drug and other chemicals were obtained commercially and were of analytical grade.

Animals used for the study

Adult wistar rats (150-250 gms) were used for the study and Animals were divided randomly into twelve groups; each group consisting of three rats and were housed in separate cages under controlled conditions of temperature (22 ± 2°c) and humidity (30-70). All animals were given standard diet and water ad libitum. Experiments were carried out as per the rules and regulations of CPCSEA.

Acute toxicity study

Acute oral toxicity studies were performed as per OECD guidelines 423, dosed each animal at the dose of 2000mg/kg b.w.p.o. The animal was observed continuously for 2hrs for gross

behavioral changes and intermittently once every 2hrs and finally at 24 and 72hrs to note any signs of toxicity including death.

Evaluation of antihyperlipidemic activity

Hyperlipidemia in rats is induced by following model, the highly effective model were considered for my studies is

-Fructose induced hyperlipidemic model.

Fructose induced hyperlipidemic model

Animal Grouping

Rats were divided into seven groups (n = 6 for each group). Animals were divided randomly into three groups; each group consisting of three rats and were housed in separate cages under controlled conditions of temperature (22 ± 2°c) and humidity (30-70). All animals were given standard diet and water ad libitum.

Control: DMSO

Standard: Atorvastatin (10 mg/kg)

1. Group A (Negative Control) receive vehicle
2. Group B (Positive Control) receive Fructose solution (10%) and vehicle
3. Group C receive standard drug (Atorvastatin 10 mg/kg)
4. Group D Test group (SOL-EA extract, dose 100 mg/kg)
5. Group E Test group (SOL-EA extract, dose 200 mg/kg)
6. Group F Test group (SOL-ME extract, dose 100 mg/kg)
7. Group G Test group (SOL-ME extract, dose 200 mg/kg)

Induction of Hyperlipidemia

Animals were weighed before the experiment, after fourteen days of fructose administration and after the drug treatment. Group A rats received water as an vehicle and Group B to O received 10% fructose solution throughout the 21 days study period. Treatment (Atrovastatin and plant extracts) was started at 15^{th} day for next seven days.

Collection Of Blood

On 21st day, after 1hr of administration of the last dose, blood samples were collected from overnight fasted rats by retro-orbital puncture. Blood parameters were measured by semi-autoanalyser using commercially available assay kits.

Biochemical Evaluation

Evaluation was carried out over lipid profile parameters as Serum Triglyceride, Serum Total Cholesterol, Serum LDL, Serum HDL, VLDL, etc. by using enzymatic kit procured from Ambika Diagnostics, Parbhani over semi-auto analyzer and morphological parameter viz., body weight.

Statistical Analysis

Data were expressed as the mean ± SEM. The results of the study were subjected to analysis of variance (ANOVA) using graph pad prism followed by Tukey test for multiple comparisons and P <0.05 was considered as statistical significant.

RESULT

Effect of *Spinaciaoleracea on*change in body weight

In present study the effect of SOL-EA andSOL-ET, was studied for itsantihyperlipidemic activity using fructose induced hyperlipidemia where rats were administered with 10% fructose during the treatment for 21 days and the drug treatment was continued after 14th day of fructose treatment up to 21st day. Rats were evaluated over change in body weight and over lipid profile parameters as Serum Triglyceride, Serum Total Cholesterol, Serum LDL, Serum HDL, VLDL.

Table 1: Effect of SOL-EA, SOL-MEon change in body weight in fructose-induced hyperlipidemic rats.

Groups	Change in Body Weight (gm)	
	Day 14	Day 21
Control	02.54±1.88	03.17±0.73
Positive Control	25.44±3.17	31.33±0.72
Atorvastatin	23.13±3.03	1.11±0.53**
SOL-EA 100	24.11±3.72	19.33±0.32**
SOL-EA 200	24.72±3.19	16.55±0.41**
SOL-ME 100	24.53±3.32	13.67±0.27**
SOL-ME 200	23.93±3.43	4.84±0.37**

Values are expressed as Mean±SEM. (n=6), ANOVA followed by Tukey test. *p<0.05 significant difference, **p<0.001 highly significant difference when compared with Positive-control. #p>0.05 non-significant difference when compared with standard; **SOL-*Spinaciaoleracealeaves***extract, **EA-** ethyl acetate, **ME- M**ethanol.

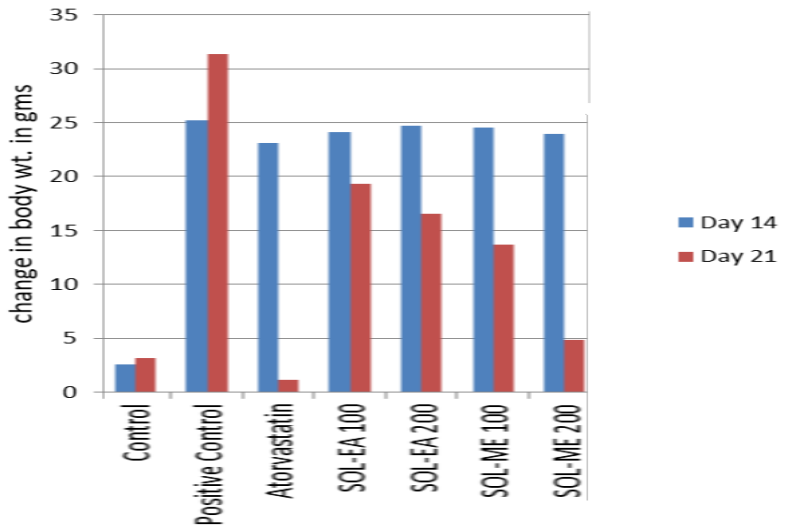

Figure No. 1: Effect of SOL-EA & SOL-ME on change in body weight in fructose-induced hyperlipidemic rats.

Effect of *Spinaciaoleracea*on total Cholesterol levels

In the normal rats the total cholesterol levels were to be found be79.93±0.62. Treatment with fructose caused a significant rise in the levels of cholesterol (184.84±3.73). Administration of various doses of the plant extract after the treatment with fructose resulted in the lowering of Cholesterol levels in a dose dependent manner. The total cholesterol levels of groups treated with 100 and 200 mg/kg cholesterol level produced by 200mg/kg methanolic extract was significant at ($p<0.05$).

Effect of *Spinaciaoleracea*on Triglyceride levels

Induction of hyperlipidemia resulted in significantly raised triglyceride levels (185.83±0.32) compared to the normal (62.51±0.49). Administration of various doses of the plant extract was able to produce a dose dependent decrease in the triglyceride levels. The respective triglyceride values for rats treated with 100 and 200 mg/kg of extract weresignificant at 200mg/kg of methanolic extract was significant at ($p<0.05$).

Effect of *Spinaciaoleracea*on serum LDL levels

The LDL levels in normal rats were found to be 67.51±0.17. Administration of fructose resulted in a rise in LDL levels (113.14±3.43). In Atorvastatin group the LDL was reduced to 64.14±3.43, whereas groups treated with 100 and 200 mg/kg of methanolic extract showed a dose dependantdecrease in the LDL levels (73.54±3.79**, 50.43±3.73$^{**\#}$ respectively).

Effect of *Spinaciaoleracea*on serum VLDL levels

The VLDL levels in normal rats were found to be 12.5±0.98. Administration of fructose resulted in a rise in VLDL levels (37.16±0.88). In Atorvastatin group the VLDL was reduced to 24.26±0.61**, whereas groups treated with 100 and 200 mg/kg of methanolic extract showed a dose dependant decrease in the VLDL levels (33.56±0.67**, 27.51±0.22** respectively).

Effect of *Spinaciaoleracea*on serum HDL levels

The HDL levels in normal rats were found to be 59.93±3.65. Administration of fructose resulted in a fall in HDL levels (34.53±3.48). In Atorvastatin group the LDL was elevated to 66.54±3.58**, whereas groups treated with 100 and 200 mg/kg of methanolic extract showed a dose dependant increase in the HDL levels (57.34±3.83*, 62.81±3.53**#respectively).

Table No.2: Effect of SOL-EA &SOL-ME on lipid profile level in Fructose-induced hyperlipidemic rats.

Groups	Serum Triglycerides (mg/dl)	Serum Total Cholesterol (mg/dl)	Serum LDL Cholesterol (mg/dl)	Serum HDL Cholesterol (mg/dl)	Serum VLDL Cholesterol (mg/dl)
Control	62.51±0.49	79.93±0.62	07.51±0.17	59.93±3.65	12.5±0.98
Positive Control	185.83±0.32	184.84±3.73	113.14±3.43	34.53±3.48	37.16±0.88
Atorvastatin	121.31±0.43**	126.23±3.33**	35.44±3.29**	66.54±3.58**	24.26±0.61**
SOL-EA 100	184.44±0.39	180.41±3.57	103.55±3.73	40.02±3.42	36.88±0.63
SOL-EA 200	171.72±0.63*	178.22±3.43	92.26±3.34*	51.63±3.58*	34.34±0.88*
SOL-ME 100	167.81±0.34**	164.41±3.67*	73.54±3.79**	57.34±3.83*	33.56±0.67**
SOL-ME 200	136.51±0.53**	140.71±3.44**#	50.43±3.73**#	62.81±3.53**#	27.51±0.22**

Values are expressed as Mean±SEM. (n=6), ANOVA followed by Tukey test. *p<0.05 significant difference, **p<0.001 highly significant difference when compared with Positive-control. #p>0.05 non-significant difference when compared with standard;**SOL-*Spinaciaoleracealeaves***extract**.**

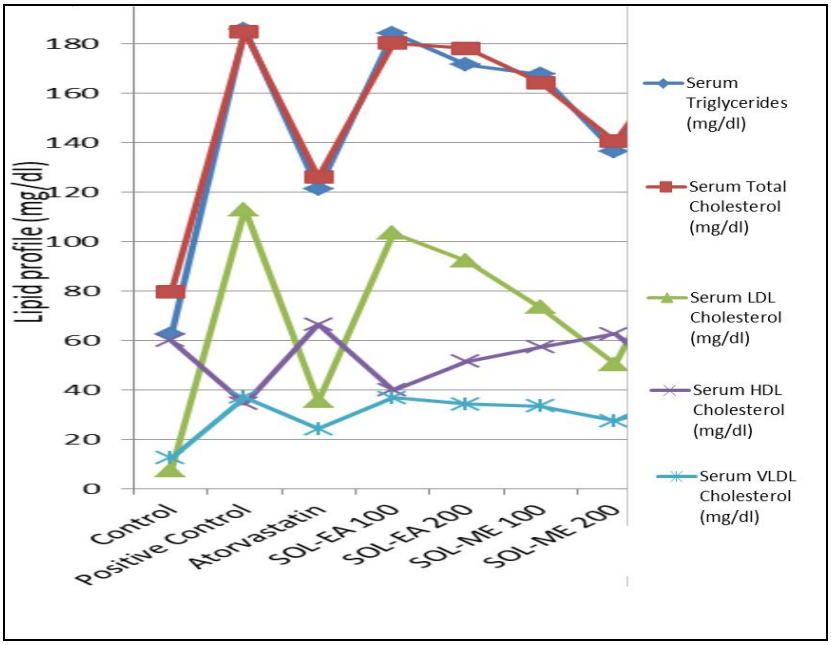

Figure No.2: Effect of SOL-EA & SOL-ME on lipid profile level in Fructose-induced hyperlipidemic rats.

DISCUSSION AND CONCLUSION

The present study was designed to investigate the antihyperlipidemic activity of *Spinacia oleracea leaves* extract in fructose induced hyperlipidemic rats. Administration of fructose 10% solution to rats caused an elevation of total cholesterol, total triglycerides, VLDL and LDL and reduction in HDL levels. *Spinacia oleracea* was administered at various doses 100, 200 mg/kg day, (p.o) to fructose induced hyperlipidemic rats. Atorvastatin was used as reference standard. Treatment with plant extract was able to significantly ($p<0.05$) decrease the levels of TC, TG, VLDL and LDL. Also, the extract was found to cause a significant ($p<0.05$) increase in the HDL levels.

Therefore, it can be concluded that *Spinacia oleracea* leaves extract is able to effectively suppress fructose induced hyperlipidemia in rats. Therefore, 200mg/kg methanolic extract of is more effective than 100mg/kg extract, and comparatively more significant then ethyl acetate extract.

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