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Antihyperlipidemic and Antioxidative Efficacy of *Catharanthus Roseus Linn* [Sadabahar] in Streptozotocin Induced Diabetic Rats

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ABSTRACT

Catharanthus roseus is a well known medicinal plant and is used for curing various human ailments. The dry leaf powder of *C. roseus* [1.5mg/kg b.w and 3.0 mg/kg b.w] was investigated for its antidiabetic, antihyperlipidemic and antioxidative efficacy in male albino Wistar rats. Rats were rendered hyperlipidemic by feeding high fat high cholesterol diet and diabetic by single intraperitoneal injection of freshly prepared streptozotocin [45mg /kg b.w.]. Glibenclamide was used as a standard reference drug. The experimental diets were supplemented for a period of 45 days. High fat-high cholesterol feeding and STZ induced diabetes resulted in significant increase in blood glucose, lipid profile and oxidative stress levels of blood and hepatic tissues of rats. Treatment with *C. roseus* significantly restored the physiological parameters to near normal. The effect of *C. roseus* [3mg/kg b.w] was better than glibenclamide. Results suggest that *C. roseus* possess a significant antidiabetic, antihyperlipidemic and antioxidative effect by attenuating biochemical and physiological alterations in HFHC diet fed animals and STZ induced diabetic rats. *C. roseus* can be used as a prophylactic agent for prevention and progression of lipid abnormalities associated with diabetes in STZ-diabetic rats by virtue of various essential antioxidant, antidiabetic compounds and phytonutrients.

INTRODUCTION

Diabetes mellitus is a metabolic disorder of multiple etiologies characterized by chronic hyperglycaemia with disturbance in carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, action or both. It affected 171 million people worldwide in 2000 and the number is projected to increase to at least 366 million by 2030 [1]. In India the prevalence of the disease has become so high to get for this country the *sobriquet "Diabetes capital of the world"*. Epidemiological studies and clinical trials strongly support the notion that hyperglycaemia is the principal cause of complications. The relationship between diabetes and hyperlipidemia is a well- recognized phenomenon. Accumulation of lipids in diabetes is mediated through a variety of derangements in metabolic and regulatory processes, especially insulin deficiency, thereby rendering the diabetic patient more prone to hypercholesterolemia and hypertriglyceridemia [2]. Hypercholesterolemia characterized by elevated blood cholesterol, particularly LDL cholesterol and VLDL cholesterol is an important factor contributing to the development of atherosclerosis and related diseases [3, 4]. Effective blood glucose control; maintaining normolipidemic levels are the major determinants for the prevention or reversal of diabetic complications to improve the quality of life of diabetics [2].

Insulin and hypoglycaemic drugs are the main ways to treat diabetes and are effective in controlling hyperglycaemia. However, the practical applicability of the majority of these therapeutic agents remained restricted owing to their limited action, pharmokinetic properties, secondary failure rates and accompanying side effects [2, 5]. In addition, these therapies, only partially compensate for metabolic derangements seen in diabetics and do not necessarily correct the fundamental biochemical lesion [6]. This has led to a shift in focus to alternative forms of therapy based on drugs derived from plants [2].

Plants have always been an exemplary source of drug and many of the currently available drugs have been directly or indirectly obtained from plants [7]. Recently the search for appropriate hypoglycaemic agents has been focused on plants used in traditional medicine partly because of leads provided by traditional medicine to natural products that may be better treatments than currently used drugs [8]. Many herbal supplements have been used for the treatment of diabetes, but not all of them have scientific evidence to support their effectiveness [9]. Out of the large number of herbal drugs stated to possess antidiabetic activity in Ayurvedic system of medicine in India, *Catharanthus roseus* is being widely used by traditional practioners to treat diabetes over many centuries.

Catharanthus roseus [Vinca rosea], known as Nayantara, belongs to the family *Apocynaceae*. The plant is indigenous to Madagascar but is now found in tropical regions and cultivated as an ornamental plant in southern Florida, Africa, India, Thailand, Taiwan, Eastern Europe and Australia. *Catharanthus* is an erect ever blooming pubescent herb, 40-80 cm high, woody at the bases. In subtropical areas, it relatively grows to 1m. The leaves are oval to oblong, 2.5 – 9.0cm long and 1.0- 3.5 cm broad, glossy green, hairless, with a pale midrib and a short petiole 1.0- 1.8 cm long; they are arranged in opposite pairs. The flowers are white to dark pink with a dark red centre with five petals like lobes. The plant is known to possess antibacterial, antifungal, antiviral, antioxidant and anticancer activities [10]. It is cultivated mainly for its alkaloids, which are known to have anticancer activity [11] and is also a rich source of tannins. *Catharanthus roseus* produces more than 100 monoterpenoids indole alkaloids [TIA] in different organs [12]. The leaves and stems are the sources of dimeric alkaloids, vinacristine and vinblastine that are indispensable cancer drugs, while roots contain ajmalicine and serpentine which have antihypertensive effects [13]. The leaves are used traditionally in various regions of the world including India, West Indies as well as Nigeria to control diabetes [14]. The leaves have been known to contain 150 useful alkaloids among other pharmacologically active compounds. Significant antihyperglycemic and hypotensive activity of the leaf extracts [hydroalcoholic or dichloromethane-methanol] have been reported in laboratory animals [15]. The present study has been under taken to delineate the biochemical changes triggered by hyperglycaemia; high fat-high cholesterol diet and oxidative stress and its modulation by *Catharanthus roseus* dry leaf powder.

MATERIALS AND METHODS

Plant Material

Catharanthus roseus leaves were collected from Banasthali University, Rajasthan, India. The leaves were shade dried; ground to a fine powder with an auto-mix blender and stored in air tight containers until the time of use.

Chemicals

All the chemicals used in the study were of analytical grade, procured from the credible concerns e.g.: Sigma, Merck, BDH and Qualigens. Chemicals of higher purity and of scarce availability were obtained from M/S chemical Co; St Louis USA. Glibenclamide was provided as a generous gift sample by Hoechst Pharmaceuticals, Mumbai.

Experimental Animals

Healthy male albino rats [Wistar strain, weighing 150-200g] were procured from the small animal house of Chaudhary Charan Singh Haryana Agriculture University Hissar [CCSHAU], India. Animals were given the standard pellet diet [Hindustan Liver Ltd., Chandigarh, India] and water *ad libitum* during acclimatization period of 1 week. The diet contained 20% protein, 5% fat and 5% fiber, 60% carbohydrates and 10% mixture of vitamins and minerals. They were housed individually in the polypropylene cages with sterilized wood chip bedding in a specific pathogen free animal house room under the constant environmental condition with 12 hour light and dark cycle, 22±1 ^{0}C temperature and 50±10% relative humidity. The study protocol was approved by Institutional Animal Ethics Committee [IAEC] of the University constituted as per the directions of the Committee for the Purpose of Control and Supervision of Experiments on Animals [CPCSEA].

Acute Toxicity

Acute oral toxicity study was performed as per Organization for Economic Cooperation and Development (OECD) guidelines 423 [16]. After the oral administration of C. roseus, animals were observed individually at least once during the first 30 minutes and periodically during the first 24 hours, with special attention given during the first 4 hours, and daily thereafter, for a total of 14 days.

Experimental Induction of Diabetes Mellitus

Rats were rendered diabetic by single intraperitoneal injection of freshly prepared streptozotocin [45mg kg^{-1}] in 0.1 M citrate buffer [pH 4.5] in a volume of 1ml kg-^{1} body weight [17]. Normal rats received 1 ml citrate buffer as vehicle. The animals had free access to food and water and were given 5% glucose solution to drink overnight to counter the hypoglycemic shock. After a 48-hours of STZ administration, blood glucose was evaluated in overnight fasting rats. Rats having blood glucose levels ranging between 200-300 mg/dl were considered to be diabetic and were selected for the study.

Experimental Design

Animals were divided into six groups of six animals each. The groups were fed on the following isoenergic [~3600C] diets for a period of 45 days: Group-A, Isoenergic normal fat diet; Group-B, High fat- high cholesterol diet [HFHC]; Group-C, HFHC + *Catharanthus roseus* leaf powder [HFHC-CR] [1.5g/kg]; Group-D, HFHC + *Catharanthus roseus* leaf powder [HFHC-CR] [3.0g/kg]; Group-E, Diabetic + Isoenergic normal fat diet; Group-F, Diabetic +HFHC + *Catharanthus roseus* leaf powder [D+HFHC-CR] [1.5g/kg]; Group-G, Diabetic +HFHC + *Catharanthus roseus* leaf powder [D+HFHC-CR] [3.0.g/kg]; Group-E, Diabetic + Isoenergic normal fat diet+ Glibanclamide [1.25mg/kg] .

Oxidative stress was induced after three weeks of feeding experimental diets by intraperitoneal injections of ammonium acetate at a dose level of 125mg/kg body weight. The gain in body weight was recorded twice a week and food consumption was monitored daily. After the completion of feeding schedule, food was withheld and animals were provided only with water, *adlibitum* for overnight.

BIOCHEMICAL ASSAYS

The blood was withdrawn from retrorbital plexus under mild ether anesthesia with heparinized capillary tubes into two prechilled vials, with one containing EDTA [1mg/ml] and the other as such without any added material. The blood samples were mixed thoroughly to prevent clotting. Thereafter the animals were sacrificed by cervical decapitation. The liver was excised, washed with ice cold isotonic saline and weighed. A small part of the hepatic tissue was minced and used for enzyme activity assay and other biochemical evaluation. Samples were stored in vials at -25 ^{0}C until further biochemical analysis. All samples were coded prior to analysis.

Biochemical analysis involved evaluation of glucose, lipid profile and enzyme assay of blood and hepatic tissue. Serum glucose was measured by the O-toluidine method [18]. Insulin level was assayed by Enzyme Linked Immunosorbant Assay [ELISA] kit [19]. Glycosylated haemoglobin [HbA_1C] estimation was carried out by a modified colorimetric method of Karunanayake and Chandrasekharan [20]. Free fatty acids [FFA] [21], phospholipids [22], total cholesterol [TC], triglycerides [TG] and HDL-cholesterol were estimated by using diagnostic

kits [Span Diagnostic]. VLDL and LDL-cholesterol were calculated as per Friedewald's equation [23]:

VLDL-C = TG/5

LDL-C = TC – [HDL-C + VLDL-C]

Serum protein [24] and serum albumin was determined by quantitative colorimetrically method by using bromocresol green; total protein minus the albumin gives the globulin. Serum glutamate pyruvate transaminases [SGPT] and serum glutamate oxaloacetate transaminases [SGOT] were measured spectrophotometrically by utilizing the method of Reitman and Frankel [25] Serum alkaline phosphatase [ALP] was measured by the method of King and Armstrong [26].

For enzyme activity assay, 0.8-1.0g of hepatic tissue was minced and homogenized in 10 times its volume of 0.2M/L tris HCl [pH=8.0] containing 0.5M/L $CaCl_2$ using Potter Elevehjem apparatus at 0-4^{0}C using motor driven Teflon pestle rotated at 3000rpm. The homogenate was centrifuged at 10000x g for 30 minutes at 4^{0}C and 3/4th of the volume was carefully drawn using Pasteur's pipette. Enzyme assay involved, lipid peroxidation [TBARS] [27], red cell and liver reduced glutathione [GSH] [28] and hepatic antioxidant enzymes; glutathione peroxidase [GSHPx] [EC 1.11.1.9] [29]; glutathione reductase [GR] [30], catalase [CAT] [EC 1.11.1.6] [31] and superoxide dismutase [SOD] [EC 1.15.1.1] [32]. The liver supernatant was extracted and used for the estimation of liver glycogen [33] and assay of Hexokinase [34], Fructose - 1, 6- bisphosphatase [35], Glucose -6- phosphatase [36].

STATISTICAL ANALYSIS

Results were expressed as mean ± SEM of 6 rats. Statistical analysis of results involved ANOVA- one way and Student's-'t' test. The values with $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

The study has been undertaken to gain an insight into STZ induced diabetes and diet related increase in lipid and free radical levels due to high fat partaking and its amelioration by *C. roseus*. The results revealed that all the animals in the various groups survived for the entire length of study. HFHC diets and other dietary treatments imposed no toxicity or adverse effect on the apparent growth of rats. Animals fed HFHC diet [Group-B] showed a significant increase in body weight as compared to the ones fed isoenergic normal fat diet [Group-A]. However, animals treated with *C. roseus* enriched diets tended to neutralize the effect though the change was not significant [$p \geq 0.05$] at a lower dose. On the other hand, body weight significantly decreased in diabetic control group and animals treated with *C. roseus* showed a marked improvement in body weight. Relative liver size significantly decreased in diabetic control group. Diets incorporated with *C. roseus* neutralized the effect and significantly increased relative liver size. The drug showed a positive response, however, the effect was less pronounced as compared to the ones supplemented with *C. roseus.* Relative food consumption significantly increased in HFHC diet fed animals and diabetic control group however, supplementation of *C. roseus* enriched diets and drug treatment to diabetics showed a marked decrease in relative food intake. These results were in accordance with the effect of neutraceuticals on HFHC diets [39].

The level of liver glycogen showed a marked increase in HFHC diet fed animals with concomitant decrease in diabetic rats as compared to the ones fed isoenergic normal fat diet. *Catharanthus roseus* enriched diets significantly improved the stored glycogen levels [Table No.1]. The restored liver glycogen level may be considered as the best marker for assessing antihyperglycemic activity of *Catharanthus roseus* dry leaf powder. Similar results have been obtained in other study conducted by Jayanthi *et al*. [53].

Table No.1: Effect of *Catharanthus roseus* and *high fat-high cholesterol* on nutritional parameters of albino rats [Values are mean ± SEM of 6 rats in each group]

Dietary Groups	Body weight [g]		Relative liver size [liver wt g/100g body wt]	Relative food consumption [g/100g body wt]	Glycogen [mg/g wet tissue]
	Initial	Final			
A	179.1±7.70	262.5±17.97	7.5±0.54	3.1±0.15	46.8 ± 0.24
B	178.8±8.73	322.5±13.76^a	7.6±0.38^{NS}	4.1±0.28^a	57.6±0.38^a
C	179.1±7.70	291.6±10.53^{NS}	7.3±0.28^{NS}	3.9±0.24^{NS}	47.4±0.30^b
D	179.1±7.70	270.8±13.56^b	7.4±0.30^{NS}	3.6±0.11^{NS}	52.2 ± 0.37^b
E	179.2±5.32	191.5±14.27^a	6.2±0.22^a	5.8±0.13^a	22.5 ± 0.61^a
F	178.6±6.18	286.6±15.13^c	7.4±0.27^c	3.7±0.24^c	39.4±0.27^c
G	179.1±7.70	276.6±17.86^c	7.7±0.31^c	3.5±0.41^c	42.2 ± 0.41^c
H	178.4±6.25	262.2±14.51^c	6.8±0.28^{NS}	4.3±0.83^c	34.2±0.74^c

Groups B and E are compared with Group A; Groups C and D are compared with Group B;
Groups F, G and H are compared with Group E

$^a p \leq 0.05$: Significantly different from Normal Control [A]
$^b p \leq 0.05$: Significantly different from HFHC [B]
$^c p < 0.05$: Significantly different Diabetic Control [E]
NS : Non Significant [$p \geq 0.05$]

Table No.2: Effect of *Catharanthus roseus* and *'high fat-high cholesterol'* diets on serum glucose, insulin and glycosylated haemoglobin status of albino rats [Values are mean ± SEM of 6 rats in each group]

Dietary Groups	Glucose [mg/dl]	Insulin [g/dl]	HBA_1C [%]
A	74.3 ± 2.04	19.5± 0.27	3.6 ± 0.04
B	106.3 ± 2.28[a]	23.2±2.21[a]	3.8 ±0.08[NS]
C	97.1±2.04[NS]	14.2 ± 0.45[b]	3.9 ± 0.49 [NS]
D	92.1± 1.84[b]	16.9 ± 0.57[b]	3.8±0.57 [NS]
E	225.3 ± 2.58[a]	4.31 ± 0.14[a]	11.4 ± 0.49[a]
F	92.6±2.33[c]	18.7±1.10[c]	7.1 ± 0.32[c]
G	87.1 ± 2.58[c]	20.8 ± 0.29[c]	5.3 ± 0.53[c]
H	102.2± 0.57[c]	14.3 ± 0.98[c]	7.3 ± 0.49[c]

Groups B and E are compared with Group A; Groups C and D are compared with Group B;Groups F, G and H are compared with Group E
[a]p ≤0.05 : Significantly different from Normal Control [A]
[b]p≤0.05 : Significantly different from HFHC [B]
[c]p<0.05 : Significantly different Diabetic Control [E]
NS : Non Significant [p=0.05]

Table No. 3 : Effect of *Catharanthus roseus* and *high fat-high cholesterol* on serum lipid status of albino rats [Values are mean ± SEM of 6 rats in each group]

Dietary Groups	Free fatty acids [mg/100 ml]	Triglycerides [mg/100 ml]	Phospholipids [mg/100ml]
A	12.3± 0.26	74.3 ± 0.69	129.0± 0.90
B	18.3± 0.34[a]	157.1± 0.67[a]	145.5± 0.65[a]
C	13.6± 0.14[b]	131.4 ± 1.07[b]	124.7± 0.96[b]
D	12.7± 0.15[b]	102.5 ± 0.86[b]	133.1± 0.63 [b]
E	21.4±0.19[a]	169.4 ± 0.90[a]	153.6±0.84[c]
F	15.2±0.18	78.4±0.48[c]	130.7±0.64 [c]
G	12.7± 0.17[c]	76.1 ± 0.45[c]	124.5± 0.73[c]
H	14.2±0.15[c]	81.5 ± 0.65[c]	133.8±1.85[c]

Groups B and E are compared with Group A; Groups C and D are compared with Group B;Groups F, G and H are compared with Group E
[a]p≤0.05 : Significantly different from Normal Control [A]
[b]p≤0.05 : Significantly different from HFHC [B]
[c]p<0.05 : Significantly different Diabetic Control [E]
NS : Non Significant [p≥0.05]

HFHC diet and STZ induced diabetes elicited a significant rise in blood glucose from 74.3±2.04 to 225.34±2.58 mg/dl [p ≤ 0.05] and a significant decrease in plasma insulin level from 19.51 to 4.31 [p≤0.05].On the contrary, diabetic rats treated with *Catharanthus roseus* dry leaf powder exhibited decreased blood glucose and increased the plasma insulin significantly [Table No.2]. The hypoglycemic effect of *C. roseus* may induce insulin release from pancreatic cells of diabetic rats. Earlier many plants have been studied for their hypoglycemic and insulin release stimulatory effects [37]. Diabetic rats showed significant increase in glycosylated haemoglobin [HbA_1C] level as compared to the normal control group fed isoenergic normal fat diet. The C. roseus treated rats showed a significant decrease [p ≤ 0.05] in glycosylated haemoglobin levels. Glycosylated haemoglobin determination are self monitoring of blood glucose therefore play important complementary roles for the management of diabetes mellitus [38, 45].

The levels of serum lipid profiles and lipoprotein fractions [LDL-C and VLDL-C] in HFHC diet fed animals and STZ induced diabetic rats showed a significant increase as compared to normal rats. The *C. roseus* and glibenclamide treated rats showed a significant decrease [p ≤ 0.05] in the content of lipid profiles when compared with HFHC diet fed animals and diabetic controls. On the other hand HDL-C showed a significant decrease in both the groups with concomitant increase in groups reared on *C. roseus* incorporated diets [Table No.3 and Table No.4]. The results are in accordance with previous study conducted by

Table No.4: Effect of *Catharanthus roseus* and *'high fat-high cholesterol'* diets on serum total and lipoprotein cholesterols of albino rats [Values are mean ± SEM of 6 rats in each group]

Dietary Groups	TC [mg/dl]	LDL – C [mg/dl]	VLDL – C [mg/dl]	HDL – C [mg/dl]
A	92.1 ± 1.6	24.1 ± 0.98	14.8 ± 1.31	53.2 ± 1.93
B	158.7 ± 2.7[a]	92.5 ± 1.47[a]	31.4±1.79[a]	34.8±2.93[a]
C	116.3 ± 3.4[b]	35.8 ± 3.11[b]	26.2 ± 1.62[b]	54.3 ± 2.92[b]
D	102.3 ± 1.2[b]	23.7 ± 1.23[b]	20.5 ± 1.39[b]	58.1 ± 3.14[b]
E	166.2 ± 2.6[a]	100.3 ± 2.86[a]	33.8 ± 2.45[a]	32.1 ± 2.61[a]
F	108.5 ± 2.8[c]	40.4 ± 3.12[c]	15.6 ± 2.87[c]	52.5±2.83[c]
G	91.5 ± 1.8[c]	26.0 ±1.88[c]	15.2 ± 1.37[c]	50.3 ± 1.63[c]
H	96.2± 1.9	30.4 ± 2.11[c]	16.3 ± 1.64[c]	49.5 ± 1.92[c]

Groups B and E are compared with Group A;
Groups C and D are compared with Group B; Groups F, G and H are compared with Group E
[a]p=0.05 : Significantly different from Normal Control [A]
[b]p=0.05 : Significantly different from HFHC [B]
[c]p=0.05 : Significantly different Diabetic Control [E]
NS : Non Significant [p=0.05]

Table No.5: Effect of *Catharanthus roseus* and *high fat-high cholesterol* on hepatic lipid status of albino rats [Values are mean ± SEM of 6 rats in each group]

Dietary Groups	Free fatty acids [mg/100g]	Triglycerides [mg/100g]	Total cholesterol [mg/100g]	Phospholipids [mg/100g]
A	0.69±0.012	426.2± 1.02	361.9± 1.55	1827.5± 0.64
B	0.85± 0.003 [a]	509.7± 1.04 [a]	433.9± 1.24 [a]	2203.3± 1.96 [a]
C	0.71± 0.014 [b]	448.4± 1.63 [b]	369.2± 1.13 [b]	1899.9± 2.23 [b]
D	0.71± 0.015 [b]	435.6± 0.79 [b]	376.3± 1.24 [b]	1883.6± 1.84 [b]
E	0.91±0.017 [a]	523.7 ±1.83 [a]	445.2±1.64 [a]	2231.0±1.48 [a]
F	0.69± 0.005 [c]	442.4 ± 2.33 [c]	383.3± 1.56 [c]	1923.5±2.17 [c]
G	0.67±0.002 [c]	433.7 ± 2.75 [c]	376.8±2.13 [c]	1884.6± 0.83 [c]
H	0.78±0.004 [c]	487.5 ± 2.46 [c]	393.1±1.27 [c]	2010.1±1.64 [c]

Groups B and E are compared with Group A;
Groups C and D are compared with Group B; Groups F, G and H are compared with Group E
[a] $p \leq 0.05$: Significantly different from Normal Control [A]
[b] $p \leq 0.05$: Significantly different from HFHC [B]
[c] $p < 0.05$: Significantly different Diabetic Control [E]
NS : Non Significant [$p \geq 0.05$]

Chauhan *et al.* [39] demonstrating that bioactive substances or neutraceuticals from natural food stuffs have hypolipidemic effect, thereby preventing the onset and progression of coronary heart diseases and other chronic diseases.

A similar trend was observed in hepatic lipid profile, indicating hyperlipidemia in HFHC diet fed animals and STZ induced diabetes. *C. roseus* enriched diets restored the levels [Table No.5]. Raised lipid profiles are usually seen in diabetics and such elevation represents risk factor for coronary heart diseases [40]. The hypolipidemic effect may be due to inhibition of fatty acid synthesis [41], enhanced excretion or lowered absorption of cholesterol [42]. In normal metabolism insulin activates the enzyme lipoprotein lipase and hydrolyses triglycerides and the deficiency in insulin results in inactivation of these enzymes thereby causing hypertriglyceridemia. The significant reduction of serum lipid levels in diabetic rats after *C. roseus* treatment may be directly attributed to improvements in insulin levels. Results are in accordance with previous study conducted by Maruthupandian and Mohan [45].

Table No.6: Effect of *Catharanthus roseus* and *'high fat-high cholesterol'* diets on serum proteins transaminases and alkaline phosphatase status of albino rats [Values are mean ± SEM of 6 rats in each group]

Dietary Groups	Protein [g/dl]	Albumin [g/dl]	Globulin [g/dl]	A/G ratio	SGPT [u/l]	SGOT [u/l]	ALP [u/l]
A	7.3 ±0.28	4.1± 0.86	3.2 ± 0.84	1.2:1.0	13.2 ± 1.14	17.4 ±1.99	153.3 ± 4.89
B	6.3±0.41 [a]	3.8±0.34 [NS]	2.5± 0.28 [a]	1.5:1.0 [NS]	29.7±3.43 [a]	23.4±2.20 [a]	204.3±3.87 [a]
C	6.9 ±0.51 [NS]	3.9 ±0.22 [NS]	3.0 ±0.78 [NS]	1.3:1.0 [NS]	22.7±2.10 [b]	18.4±1.90 [b]	196.3±8.88 [b]
D	7.5 ±0.71 [b]	3.8 ±0.16 [NS]	3.6 ±0.23 [b]	1.0:1.0 [b]	19.1±2.65 [b]	17.3±1.23 [b]	168.4±7.98 [b]
E	5.2 ±0.31 [a]	3.1 ±0.19 [a]	2.0 ±0.34 [a]	1.5:1.0 [NS]	32.8±4.34 [a]	29.2±1.32 [a]	298.4±6.98 [a]
F	7.2±0.42 [c]	3.9±0.21 [NS]	3.3±0.53 [c]	1.1:1.0 [c]	18.4±2.87 [c]	21.3±1.67 [c]	194.5±4.87 [c]
G	7.8 ±0.31 [c]	4.3 ±0.08 [c]	3.5 ±0.90 [c]	1.2:1.0 [NS]	15.3 ± 2.67 [c]	18.6±2.12 [c]	189.4±8.65 [c]
H	7.6 ±0.11 [c]	3.9 ±0.62 [c]	3.6±0.45 [c]	1.0:1.0 [c]	16.3 ± 1.78 [c]	14.7±1.04 [c]	164.2±6.39 [c]

Groups B and E are compared with Group A; Groups C and D are compared with Group B;
Groups F, G and H are compared with Group E
[a] $p = 0.05$: Significantly different from Normal Control [A]
[b] $p = 0.05$: Significantly different from HFHC [B]
[c] $p = 0.05$: Significantly different Diabetic Control [E]
NS : Non Significant [$p = 0.05$]

Table No.7: Effect of *Catharanthus roseus* and *'high fat-high cholesterol'* diets on the blood and hepatic oxidative stress status of albino rats [Values are mean ± SEM of 6 rats in each group]

Dietary Groups	Blood/Serum			Hepatic	
	GSH [mM/100ml]	TBARS [nM/100ml]	GR [nM/100ml]	GSH [mM/100g]	TBARS [nM/mg protein]
A	45.8±0.27	27.3±0.27	16.83±0.78	384.5±3.56	0.77±0.038
B	27.8±0.57^{a}	43.5±0.28^{a}	11.24±0.54^{a}	273.0±3.19^{a}	0.85±0.044^{a}
C	30.7±1.87^{NS}	37.9±0.25^{NS}	13.45±0.56^{NS}	341.2±4.75^{b}	0.75±0.016^{b}
D	33.4±1.02^{b}	32.2±0.42^{b}	14.14±0.45^{NS}	333.4±5.47^{b}	0.79±0.018^{NS}
E	24.3±1.71^{a}	47.9±0.34^{a}	10.6±0.87^{a}	251.0±4.21^{a}	0.93±0.014^{a}
F	40.5±2.02^{c}	31.4±0.41^{c}	13.8±0.42^{NS}	338.4±5.31^{c}	0.81±0.001^{c}
G	42.7±0.25^{c}	29.3±0.25^{c}	14.7±0.32^{c}	342.3±5.10^{c}	0.79±0.021^{c}
H	36.4±2.22^{c}	32.3±0.18^{c}	13.6±0.34^{NS}	321.5±5.43^{c}	0.89±0.037^{NS}

Groups B and E are compared with Group A; Groups C and D are compared with Group B;
Groups F, G and H are compared with Group E
$^{a}p \leq 0.05$: Significantly different from Normal Control [A]
$^{b}p \leq 0.05$: Significantly different from HFHC [B]
$^{c}p < 0.05$: Significantly different Diabetic Control [E]
NS : Non Significant [$p \geq 0.05$]

A significant reduction in serum protein, albumin and globulin were observed in STZ induced diabetic rats [Group E] when compared to control [Group A] and glibenclamide treated rats [Group H] probably because insufficient insulin leads to increased protein degradation and decreased protein synthesis. Supplementation of *C. roseus* enriched diets to the diabetic rats restored the levels of protein, albumin and globulin to near normal [Table No.6]. These results were in accordance with the effect of *Wattakaka volubilis* [43] and *Pterocarpus marsupium* [45] in diabetic rats.

The effect of HFHC diet and STZ induced diabetes on the activity of the hepatic marker enzymes in serum revealed that the levels of SGPT and SGOT were elevated in Group B and Group E. It may be due to leaking out of enzymes from the tissues and migrating into the circulation by the adverse effect of STZ. *C roseus* dry leaf powder regulated the activity of SGPT and SGOT in liver of rats intoxicated with STZ and high fat diets. The effect of glibenclamide on the recovery of hepatic enzyme activity in serum was very similar to that of the earlier study [44]. The restorations of SGPT and SGOT to their respective normal levels after treatment with both glibenclamide and *C. roseus* further strengthen its antidiabetogenic effects. Moreover, SGPT and SGOT levels also act as indicators of liver function and restoration of normal levels of these parameters indicate normal functioning of liver [45]. Since STZ can also affect the liver by free radical mechanism. In addition to the assessment of SGPT and SGOT levels during diabetes the measurement of enzymatic activities of phosphatases such as acid phosphatase [ACP] and alkaline phosphatase [ALP] is of clinical and toxicological importance as changes in their activities are indicative of tissue damage by toxicants [46]. Serum ALP increased considerably [$p \leq 0.05$] in HFHC diet fed animals and STZ induced diabetic rats. Elevated level of this enzyme in diabetes may be due to extensive damage to liver in the experimental animals by STZ. Treatment with *C. roseus* and glibenclamide produced a significant [$p \leq 0.05$] decline in ALP levels [Table No.6].

Table No.7 shows increased lipid peroxidation [LPO] in serum and hepatic tissue in HFHC diet fed animals and STZ induced diabetic rats. Earlier studies have shown increased lipid peroxidation in high fat fed and diabetic rats [39, 46, 47, 48]. A significant reduction was observed after the supplementation of *C. roseus* and glibenclamide indicating that dry leaf powder can inhibit oxidative damage due to the antiperoxidative effect of bioactive substances. This could be correlated with previous studies with *Cassia auriculata* flower [49], *Scoparia dulcis* [50], *Wattakaka volubilis* [43] *and Pterocarpus marsupium* [45].

The levels of superoxide dismutase [SOD], catalase [CAT] glutathione peroxidase [GPx] reduced glutathione [GSH] and glutathione reductase [GR] significantly [$p \leq 0.05$] reduced in HFHC diet fed animals and STZ induced rats. The upward and adverse changes were reversed to near normal values in C. roseus treated rats [Table No.8]. It is well known that CAT, SOD and GSHPx play an important role as protective enzymes against free radical formation in tissues [39, 51]. The results reveal the protective role of leaves in decreasing lipid peroxidation and by normalizing antioxidant system [45].

The changes in the activities of hepatic enzymes hexokinase, glucose 6-phosphatase and fructose- 1, 6- bisphosphatase of experimental animals are shown in Table No.9. Enzymes of glucose metabolism markedly altered in diabetic control group indicating pathogenesis resulting from diabetic complications. Hexokinase enzyme activity significantly decreased in HFHC diet fed animals and diabetic rats. On the contrary, *Catharanthus roseus* treated rats showed a significant increase in its activity [$p \leq 0.05$] leading to increased glycolysis and utilization of glucose for enzyme production [52, 53]. On the contrary the activity of the glucose 6-phosphatase and fructose- 1, 6- bisphosphatase increased in both the groups. However, treatment with *Catharanthus roseus* dry leaf powder brought the activities of the enzymes to near normal [$p \leq 0.05$]

Table No.8: Effect of *Catharanthus roseus* and *'high fat-high cholesterol'* diets on the hepatic antioxidant enzymes activity of albino rats [Values are mean ± SEM of 6 rats in each group]

Dietary Groups	Glutathione peroxidase [g of GSH utilized/min/mg protein]	Catalase [values x 10^{-3} units/mg protein]	Superoxide dismutase [units/mg protein]
A	7.6±1.24	66.7±0.99	3.0±0.25
B	6.1±2.86[a]	49.9±0.73 [a]	1.9±0.15 [a]
C	8.5±3.93 [b]	59.7±1.21 [b]	2.9±0.21 [b]
D	8.4±1.72 [b]	59.0±1.18 [b]	3.1±0.23 [b]
E	5.8±2.45[a]	34.6±1.14[a]	1.1±0.21[a]
F	8.9±3.17 [c]	62.2±0.95 [c]	3.0±0.26 [c]
G	8.2±1.56[c]	69.3±0.97[c]	3.4±0.17[c]
H	6.6±3.12[NS]	51.4±0.48[c]	2.3±0.24[c]

Groups B and E are compared with Group A; Groups C and D are compared with Group B;
Groups F, G and H are compared with Group E
[a]$p \leq 0.05$: Significantly different from Normal Control [A]
[b]$p \leq 0.05$: Significantly different from HFHC [B]
[c]$p < 0.05$: Significantly different Diabetic Control [E]
NS : Non Significant [$p \geq 0.05$]

Table No.9: Effect of *Catharanthus roseus* and *'high fat-high cholesterol'* diets on activities of hexokinase, fructose 1, 6 – bisphosphatase, glucose 6 – phosphatase in liver of albino rats [Values are mean ± SEM of 6 rats in each group]

Dietary Groups	Hexokinase [µ moles of glucose - 6 – phosphate formed/h/mg protein]	Fructose 1, 6 –bisphosphatase [nmoles of phosphorous liberated/h/mg protein]	Glucose 6 – phosphatase [n moles of phosphorous liberated/h/mg protein]
A	187.2 ± 7.51	489.2 ± 4.61	1043.2 ± 9.64
B	156.4±7.12[a]	601.4±3.49[a]	1183.4±4.81
C	184.6 ± 7.64[b]	483.2±5.82[b]	990.2 ± 1.38[b]
D	184.8±6.48[b]	479.9 ± 5.54[b]	947.5±1.54[b]
E	130.3 ± 1.51[a]	770.1 ± 7.53[a]	1260.5 ± 2.77[a]
F	171.1 ± 8.43[c]	553.8 ± 1.04[c]	1163.6 ± 6.41[c]
G	178.3±6.45[c]	514.3 ± 5.17[c]	1098.5±3.21[c]
H	166.2±5.32[c]	573.1 ±3.54[c]	1121.8±5.74[c]

Groups B and E are compared with Group A; Groups C and D are compared with Group B;
Groups F, G and H are compared with Group E
[a]$p \leq 0.05$: Significantly different from Normal Control [A]
[b]$p \leq 0.05$: Significantly different from HFHC [B]
[c]$p < 0.05$: Significantly different Diabetic Control [E]
NS : Non Significant [$p \geq 0.05$]

CONCLUSION

The study clearly demonstrate that that *Catharanthus roseus* leaf powder containing diets have been denting at hyperlipidemia, hyperglycemia and peroxidative effect induced by high fat, high cholesterol diet and steptozotocin. The bioactive components in *Catharanthus roseus* leaves can be a promising therapy for the prevention and treatment of nutritionally linked illnesses and serve as a good adjuvant in the present armamentarium of antidiabetic and antihyperlipidemic drugs.

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