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EFFECT OF GREVILLEA ROBUSTA LEAF ON ANTIDIABETIC ACTIVITY

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Abstract

Hypercholesterolemia and the resulting have been implicated the path physiology of coronary heart diseases and myocardial ischemia. Lowering cholesterol level may decrease the risk of CVD, and therefore enormous efforts have been extended achieve this aim. The hypocholesterolaemia activity of ethanol extract of Grevillea Robusta (GRLE) against hypercholesterolemia was monitored on the Lipid profile status, antioxidant status, activities of serum cardiac marker enzyme, and histological changes of liver and heart. In this study the high cholesterol diet (HCD) is used which consists of maize, soya meal, coconut cake, rice polish, groundnut cake and animal tallow which has been used in inducing experimental hypercholesterolemia In the current study, the HCD fed rats showed increased levels of plasma cholesterol (TC), and triglycerides (TG) levels compared to normal control rats. Treatment with GRLE significantly decreased the levels of serum TC, when compared to HCD induced rats. Saponins are also reported to precipitate cholesterol from micelles and interfere with enter hepatic circulation of bile acids making it unavailable for intestinal absorption, this forces liver to produce more bile from cholesterol and hence the reduction in serum cholesterol level. Saponins are also reported to lower triglycerides by inhibiting pancreatic lipoprotein lipase. Similarly in our study also, the presence of both flavonoids and saponins in could have contributed in reducing the levels of lipid status (TC, TG,) elevated levels of serum low density lipoprotein cholesterol (LDL) and very low density lipoprotein cholesterol (VLDL) are often accompanied by premature atherosclerosis and other CVD. FEA low level of high-density lipoprotein cholesterol (HDL) is also an important risk factor for cardiovascular disease.

Keywords:- Hypocholesterolaemia activity of ethanol extract of Grevillea Robusta.

INTRODUCTION:

Cholesterol is a waxy, fat-like substance that's found in al cell s of the body All of them have GQ a similar cyclic nucleus resembles the phenanthrene rings (rings A, B, C) to which a cyclopentanone ring is attached. The parent nucleus is better designated as cyclopentano perhydrophenanthrene. They are divided as sterols, bile acids; sex hormones etc our body needs some cholesterol to make hormones, vitamin GD, and substance that help us to digest foods. Quantity of cholesterol will be 140gms in the body of a man weighing 70 kg. Greater part of the

ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 04, 2021

cholesterol in the body is synthesized whereas 0.3 gram per day is provided by the average diet. Normal concentration of cholesterol in the blood is 140-220 mg per 100 ml of blood. Cholesterol travels through the bloodstream in small packages called lipoproteins. Two kinds of lipoproteins carry cholesterol throughout our body: low- density lipoproteins (LDL) and high-density lipoproteins (HDL).Having healthy levels of both types of lipoproteins are important. LDL cholesterol is cal "bad" cholesterol. A high LDL level leads to a build up of cholesterol in the arteries.

MATERIALS AND METHODS

Experimental animals

Albino wistar rats of either sex weighing between 125-150 were used for the study. The animals were housed in polypropylene cages inside well ventilated room. The room temperature was maintained at 23±2˚C with 12 H: H. The animals were with commercial rat feed pellets and provided with drinking water *labium*. Animal procedures have been approved by ethical committee in accordance with animal experimentation and care.

Drugs and chemicals:

Cholesterol esterase and P-nitro phenyl butyrate), Acetonitrile, taurocholate were purchased from chemicals, Gallic acid, (HFD) food for rats. A torvastatin is used as a standard drug Cholesterol, triglycerides, HDL, LDL, AST, ALP, ALT and LDH levels were determined using standard kits obtained from a gape diagnostics.

Instrument used:

Digital balance (Sartorius Ltd, USA), UV (UV-spectrometry), pH meter and FEGC-MS.

Plant material:

The plant material consists of dried powdered leaves of *Grevillea Robusta* belonging to the family amice.

Plant extraction:

The leaves were separated and dried in shade room temperature, powdered mechanically and sieved through No.20 mesh sieve. Finely powdered leaves were kept in an airtight container until the time of use.

In the cold maceration process, about FE25 gm of the whole or coarsely powdered crude drug is placed in stopper container with the FE70% ethanol and 30% water and allowed to stand at room temperature for period of least 3 days with frequent agitation until the soluble matter has dissolved. On the fourth day the mixture is then strained, the pressed and combined liquids are clarified by filtration or decantation after standing.

Phytochemical screening:

Chemical tests were carried out for the various fractions of *Grevillea Robusta* for the presence of phytochemical constituents.

Test for alkaloids:

ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 04, 2021

Too little of plant leaves extract few drops of Mayer's test reagent was added. Formation of precipitate indicates presence of alkaloids.

Test for flavanoids:
1ml of extract was taken and few drops of very dilute solution of ferric chloride were added. The colour changed to pale green or red brown colour which indicates the presence of flavonoids.

Test for saponins:
One ml extract and one ml alcohol diluted with 20 ml distilled water and shaken well for 15 minutes. The formation of 1cm layer of foam indicated the presence of saponins.

Test for Carbohydrates:
Small amount of extract was dissolved separately in 5 ml distilled water and filtered. The filtrate was subjected to mollusc's test. Formation of reddish brown ring indicates the presence of carbohydrate.

Test for Tannins:
- To 5ml of extract solution, 1ml of lead acetate solution was added.
- Flocculent brown precipitate indicates the presence of tannins.

Test for Glycosides:
Small portion of extract was hydrolyzed with hydrochloric acid for few hours on water bath and the hydolysate was subjected to legal's test to detect the presence of different glycosides.

Legal's Test:
To the hydrolysed 1ml of sodium nitroprusside solution was added and then was made alkaline with sodium hydroxide solution. If the produced pink to red colour, indicates the presence of glycosides.

Test for fixed Oils and Fats:
Few drops of 0.5N alcoholic potassium hydroxide were added to small quantity of various extracts along with drop of phenolphthalein. The mixture was heated on water bath for 1-2 hrs. Formation of soaps or partial neutralization of alkali indicates the presence of fixed oils and fats.

Test for Phenols (ferric chloride test):
A fraction of the extract was treated with aqueous 5% ferric chloride and observed for formation of deep blue black colour.

Test for Gums and Mucilage:
About 10ml of extract was added to 25ml of absolute alcohol with stirring and filtered. The precipitate was dried in air and examined for its swelling properties and for the presence of carbohydrates.

Test for Amino acids and Proteins (1% Ninhydrin solution in acetone):

ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 04, 2021

2ml of filtrate was treated with 2-5 drops of Ninhydrin solution placed in boiling water bath for 1-2 minutes and observed for the formation of purple colour.

Gas Chromatography -Mass Spectroscopy Analysis Derivatization procedure:
Two procedures were followed. The crude ethanol ether and ethanol extracts, small amount of concentrated sample was taken in separating funnel and shaken by water and ethyl acetate in the ratio of 1:4. The upper layer was collected and concentrated in rotary evaporator to about 1.5ml. Added 100µl, pyridine and heated at 60°C for 30 minutes. For the layers which are separated from the crude extracts, small amount of extract was taken and evaporated out totally. To this added acetonitrile and filtered into conical flask. The filtrate added 50µl and heated at 60°C in water bath for 30 minutes. Filtered using 0.45µ membrane filter to a vial.

GC-MS Analysis:
GC-MS analysis was carried out on in Elmer Turbo Mass Spectrophotometer (Norwalk, EUSA) which includes Perkin Elmer Auto sampler. The column used was Perkin Elmer light 5 capillary column measuring 30m 0.25mm with film thickness of 0.25mm composed of 95% ethyl polysiloxane. The carrier gas used was Helium at flow rate of keFE0.5ml/min. keFE1µl sample injection volume was utilized. The inlet temperature was maintained as 250°C. The oven temperature was programmed initially at 110°C for 4 min, then an increase to 240°C. And then programmed to increase to 280°C at rate of 20°C ending with 5min. Total time was 90min. The transfer line was maintained at temperature of 200°C. The source temperature was maintained at 180°C. GCMS was analyzed using electron impact ionization Eat 70eV and data was evaluated using total ion count (TIC) for compound identification and quantification. The spectrums of the components were compared with the database of spectrum of known components stored in the GC-MS library. Measurement of peak areas and data processing were carried out by Turbo-Mass-OCPTVS-Demo SPL software [2].

***In vitro* Cholesterol esterase Inhibitory Activity:**
Enzyme inhibition assay is performed in presence of sodium taurocholate with nitro phenyl butyrate as chromomeric substrate. Hydrolysis is performed in the presence of high enzyme concentration and products are identified spectrophotometrically Assay buffer is sodium phosphate buffer (pH-7.0) which made by adding 100 [3].

Selection of dose of the Extract:
LD50 was done as per guidelines fixing the dose for biological Evaluation the 50 of the fractions as per guidelines falls under category 4 values with no signs of acute toxicity at doses of 2000 mg/kg the biological evaluation of the fractions were carried out at dose of 200 mg/kg body weight.

High fat diet fed rats of Hypercholestremia in Rats:
Rat was fed with two dietary regimes such as Normal Pellet Diet and High fat Diet. The rat was feeding either NPD HFD labium, respectively, for the period of 50 days.

Group III:

ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 04, 2021

Rats fed with HCD for 50 days administrated with low dose of plant extract (100mg/kg, body weight/day orally) for last 35 days.

Group IV:
Rats fed with HCD for 50 days administrated with High dose of plant (200mg/kg, body weight/day orally) for last 35 days.
Group V:
Rats fed with normal diet for 50 days administrated with plant extract (200 mg/kg, body weight/day orally) for last 35 days.
Group VI:
Rats fed with HCD for 50 days administered with standard drug (10 mg kg-1 body weight/day orally) for last 35 days.

Measurement of Body weight, Food intake and Water Consumption:
In addition, the food intake and water consumption of rats in all the groups were noted down.

Estimation of Malondialdehyde (MDA):
One ml of the homogenate was combined with 2 ml of TCA-TBA-HCl reagent (15% tricolored acetic acid (TCA) and 0.375% thiobarbituric acid (TBA) in 0.25N HCl) and boiled for 15 min. precipitate was removed after cooling by centrifugation at 1000g for 10 min and absorbance of the sample was read at 535nm against a blank without tissue homogenate. The levels of MDA were calculated using extinction coefficient calculation. The values are expressed as moles/min/mg brain tissue [4].

Determination of Enzymatic Antioxidants:

Assay of Catalyse:
The reaction mixture contained 2.0 ml of homogenate and 1.0 ml of 30 hydrogen peroxide (in 50M phosphate buffer, pH 7.0) System devoid of the substrate (hydrogen peroxide) served asked control. Reaction was started by the addition of the substrate and decrease in absorbance mentioned at 240 nm for 30 seconds at 25°C. The difference in absorbance per unit time was expressed as the activity and three parallel experiments were conducted. One unit is defined as the amount of enzyme required to decompose 1.0 of H_2O_2 per minute at pH 7.0 and 25°C [5].

Estimation of Glutathione Peroxidise:
The reaction mixture consists of 0.2 ml of 0.4 buffers, 0.1 ml of 1.0 sodium aside, 0.1 ml of 0.042% hydrogen peroxide, 0.2 ml of 200 M glutathione and 0.2 ml of brain tissue homogenate supernatant incubated at 37°C for 10 min. The reaction was arrested by the addition 0.1 ml of 10% TCA and the absorbance was taken at 340 nm. Activity was expressed as moles/min/mg brain protein [6].

Assay of Superoxide Dismutase (SOD):
The assay mixture contained 1.2 ml of sodium pyrophosphate buffer (0.052 M, pH 8.3), 0.1 ml of 186µm Phenazonium sulphate, 0.3 ml of 300µm nitro blue tetrazolium chloride, 0.2 ml of

ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 04, 2021

780µm NADH, 1.0 ml of homogenate and distilled water to final volume of 3.0 ml. reaction was started by the addition of NADH and incubated at 30°C for 1 min. the reaction was stopped by the addition of 1.0 of glacial acetic acid and the mixture was stirred vigorously. 4.0 ml of n-butane was added to the mixture and shaken well. The mixture was allowed to stand for 10 min, centrifuged, the butanes layer was taken out and absorbance was measured at 560 nm against butanes blank system devoid of enzyme served as the control and three parallel experiments were conducted.

Estimation of Glutathione Reductive (GSSH):
The enzyme activity was determined spectrophotometrically by the decrees absorbance of NADPH at 340nm. The reaction mixture contained 2.1ml of 0.25m potassium phosphate buffer pH 7.6, 0.1 ml. 0.2ml of 0.0165 M oxidized glutathione and 0.1 ml of bovine serum albumin (10 mg/ml). The reaction was started by the addition of 0.02 ml of brain tissue homogenate with mixing and the decrease in absorbance at 340nm was measured for 3 min against blank Glutathione reductive activity was expressed as moles/min/mg brain protein at 30°C [7].

Determination of Non-Enzymatic Antioxidants Estimation of Reduced Glutathione (GSH):
Brain was homogenized in 10% w/v cold 20M EDTA solution on ice. After deproteinization with 5% TCA, an aliquot of the supernatant was allowed to react with 150µm DTNB. The product was detected and quantified spectrophotometrically at keFE416 nm. Pure GSH was used as standard for establishing the calibration curve and three parallel experiments were conducted [8].

Histopathological Studies:
The portions of heart and liver were immersed in 10% formalin for 24h for histopathological examination. The specimens were cleared in xylems and embedded in paraffin. Paraffin bees wax blocks were prepared and cut into 5µm thick sections. The obtained tissue sections were mounted on glass slides and stained with haematoxylin and eosin for histopathological examination using a light microscope.

Statistical Analysis
The results would be statistically analyzed by one way-ANOVA followed by Turkey's test using Graph Pad Instate software. The values would be expressed as mean ± SEM. $P<0.05$ would be considered statistically significant when compared to standard and negative control.

RESULT & DISCUSSION
Phytochemical screening of powdered leaves of *Grevillea Robusta* showed the presence of alkaloids, flavonoids, saponins, tannins, sterols, oil fat, phenol compound, protein and amino acid, gums and mucilage's, Carbohydrates, and glycosides.

Table: 1 Phytochemical Screening

S.No	Phytochemical	Interpretation
1	Alkaloid	+
2	Flavanoids	+

ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 04, 2021

3	Saponins	+
4	Tannins	+
5	Sterols	+
6	Oil and Fat	-
7	Phenol compound	+
8	Protein and Amino acid	+
9	Gums and mucilage	+
10	Carbohydrates	+
11	Glycosides	+

GC-MS Analysis

Gas chromatography mass spectroscopy analysis was carried out in crude leaf ethanol extract of *Grevillea Robusta*. The total ion chromatogram (TIC) of ethanol extract of .*Robusta* showing GC-MS profile the com-pounds were identified. The in the chromatogram were integrated and were compared with the database of spectrum of known components stored in the GC-MS library. The detailed tabulations of GC-MS analysis of the extracts are given below. Phytochemical analysis by GC-MS analysis of the plant extract revealed the presence of different fatty acids, heterocyclic compounds etc.

***IN VITRO* STUDIES:**

Cholesterol Esterase Inhibitory Activity:

The ethanol leaf extract of *Grevillea Robusta* was studied or its cholesterol esterase inhibitory activity concentrations ranging from 20, 40, 80, 160, and 320 µm/ml. The absorbance of the mixture was measured at 405nm. Was observed that there is dose dependent increase in the percentage inhibition from the concentration 10µg/ml to 320 µm/ml and the values are shown Table-7. C50 values the extract was calculated and compared with the standard Gallic acid.

For the extract of GRLE C50 value was calculated and was found to be 46.68±11.66µg/ml. Gallic acid is used as a reference standard and C50 value was found to be 45.68±10.069µg/ml.

Table: 2 Cholesterol Esterase Inhibitory Activity of GRLE

Concentration (µg/ml)	**% Inhibition**			**Mean ± SEM**	**IC_{50}(µg/ml)**
	I	**II**	**III**		
5	3.74	26.64	51.51	27.31±13.804	45.68±10.069
10	6.03	38.70	57.75	34.21±15.102	
20	43.1	23.80	46.74	37.866±7.106	
40	20.12	38.72	56.97	38.573±10.652	
80	28.5	32.41	69.04	43.366±12.884	

ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 04, 2021

160	26.25	62.24	65.55	51.303±12.614	
320	37.11	52.44	69.21	53.056±9.166	

Table: 3 Cholesterol Esterase Inhibitory Activity of GRLE

Concentration(µg/ml)	% Inhibition			Mean ± SEM	IC_{50}(µg/ml)
	I	II	III		
5	16.09	40.06	58.14	38.096±12.188	46.68±11.66
10	20.87	50.90	58.76	43.54±11.550	
20	71.27	58.18	24.84	51.43±13.825	
40	34.71	52.29	61.88	49.61±7.942	
80	42.01	49.07	60.34	50.46±5.335	
160	25.5	59.92	64.48	50.10±12.173	
320	37.64	53.52	69.33	53.51±9.148	

Table: 4 Observations Done For the Acute Oral Toxicity Study of GREL

	Parameters observed	0 h	0.5 h	1h	2h	4 h	Day 2&3	Day 4&5	Day 6&7	Day 8&9	Day 10&11	Day 12&13	Day 14
Respiratory	**Dyspnea**	-	-	-	-	-	-	-	-	-	-	-	-
	Apnea	-	-	-	-	-	-	-	-	-	-	-	-
	Nostril discharges	-	-	-	-	-	-	-	-	-	-	-	-
Motor activity	**Tremor**	-	-	-	-	-	-	-	-	-	-	-	-
	Hyper activity	-	-	-	-	-	-	-	-	-	-	-	-
	Hypo activity	-	-	-	-	-	-	-	-	-	-	-	-
	Ataxia	-	-	-	-	-	-	-	-	-	-	-	-
	Jumping	-	-	-	-	-	-	-	-	-	-	-	-
	Catalepsy	-	-	-	-	-	-	-	-	-	-	-	-
	Locomotor activity	-	-	-	-	-	-	-	-	-	-	-	-
Reflexes	**Corneal reflex**	+	+	+	+	+	+	+	+	+	+	+	+

ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 04, 2021

	Pinna reflex	+	+	+	+	+	+	+	+	+	+	+	+
	Righting reflex	+	+	+	+	+	+	+	+	+	+	+	+

Table:5 Acute toxicity Studies and Selection of Dose for *In vivo* Studies

Parameters observed		**0 h**	**0. 5h**	**1 h**	**2 h**	**4 h**	**Day 2&3**	**Day 4&5**	**Day 6&7**	**Day 8&9**	**Day 10&11**	**Day 12&13**	**Day 14**
Convulsion	**Tonic and clinic convulsion**	-	-	-	-	-	-	-	-	-	-	-	-
Muscle tone	**Hypertonic**	-	-	-	-	-	-	-	-	-	-	-	-
	Hypotonic	-	-	-	-	-	-	-	-	-	-	-	-
Ocular sign	**Lacrimation**	-	-	-	-	-	-	-	-	-	-	-	-
	Meiosis	-	-	-	-	-	-	-	-	-	-	-	-
	Mydriasis	-	-	-	-	-	-	-	-	-	-	-	-
	Ptosis	-	-	-	-	-	-	-	-	-	-	-	-
Skin	**Edema**	-	-	-	-	-	-	-	-	-	-	-	-
	Skin and fur	-	-	-	-	-	-	-	-	-	-	-	-
	Erythematic		-	-	-	-	-	-	-	-	-	-	-
Cardiovascular signs	**Bradycardia**	-	-	-	-	-	-	-	-	-	-	-	-
	Tachycardia	-	-	-	-	-	-	-	-	-	-	-	-
Piloerection	**Contraction of erectile tissue of Hair**	-	-	-	-	-	-	-	-	-	-	-	-
Gastrointestinal signs	**Diarrhea**	-	-	-	-	-	-	-	-	-	-	-	-

Table: 6 Phase Observation live Animals

Live phase animals	**Observations**
1. Body weight every day	Normal
2. Food consumption daily	-
3. Water consumption daily	-
4. Home cage activity	-

Table: 7 Mortality Record for GRLN Acute Oral Toxicity

Group	**Group1 (5 mg/kg)**	**Group 2 (50 mg/kg)**	**Group 3 (300mg/kg)**	**Group 4 (2000mg/kg)**

ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 04, 2021

No. of animals	1	1	1	1	2
Body weight (g)	150	183	180	200	195
Sex	Female	Female	Female	Female	Female
30 min	-	-	Nil	-	Nil
1h	-	Nil	-	-	-
2h	Nil	-	-	Nil	-
3h	-	Nil	-	-	-
4h	-	Nil	Nil	-	Nil
Day 1	Nil	-	-	-	-
Day 2	-	Nil	-	-	-
Day 3	-	-	-	-	Nil
Day 4	Nil	-	-	-	-
Day 5	-	-	Nil	-	-
Day 6	-	-	-	-	-
Day 7	-	-	-	-	-
Day 8	Nil	-	-	-	-
Day 9	-	-	-	-	-
Day 10	-	-	-	-	-
Day 11	Nil	-	-	-	-
Day 12	-	-	-	-	-
Day 13	-	-	-	-	-
Day 14	-	-	-	-	-
Mortality	**0/1**	**0/1**	**0/1**	**0/2**	

Effect of the Extract of *G. Robusta* on Body Weight:

The comparison of body weight between the first day of the study and day, showed significant increase in the body weight in all groups And the HCD group is increases when compare to the other groups. Statistical analysis of the results using Student's paired test showed that the values obtained are signify- cant values $P<0.05$.Values are expressed as mean ±SEM (n=6). Denotes $P<0.05$ When compared to the initial readings (Student's Paired' -test)

Average Food Intake:

Quantities of food consumed by rats in group were more when compared remaining groups, this was because rats were fed with extra HFD for to 25 days during hyerlipidemia induction. The quantities of food intake were the same in all groups, although body weight gain differed significantly between control groups, standard and treated groups. This difference was probably due to the different doses of extracts seems to exert protective effect against overweight in treated group as compared to control group. The use of average feed intake were tabulated in Table No 8.

Table 8: Effect of GRLE on Food Intake in HFD Induced Hyperlipidemic Rat

ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 04, 2021

Groups	Treatment	Average food intake 1-25 days(gm)	Average food intake 25-50 days(gm)
Group I	Normal control	17.28±0.302	15.83±0.215
Group II	Negative control	10.42±0.374	16.64±0.648
Group III	GRLE low dose (100mg/kg)	10.04±0.425	16.62±0.693
Group IV	GRLE high dose (200 mg/kg)	9.99±0.473	16.95±427
Group V	GRLE alone (200 mg/kg)	15.20±0.55	14.50±0.35
Group VI	Standard (10 mg/kg)	10.25±0.240	17.29±0.500
Groups	Treatment	Average water in-take E1-25	Average water intake 25-50 days(ml)
Group I	Normal control	100.50±3.0	120.10±8.50
Group I	Negative control	106.8±1.41	141.89±1.02
Group III	GRLE low dose (100mg/kg)	187.04±1.23	222.04±1.14
Group IV	GRLE high dose (200 mg/kg)	141.68±2.11	281.82±2.13
Group V	GRLE alone (200 mg/kg)	110.12±5.60	130.58±60
Group VI	Standard(10 mg/kg)	45.95±2.22	74.15±1.33

Table No: 9 effect of GRLE on water consumption in HFD Induced Hyperlipidaemia Rats
Serum Biochemical Parameters

	Treatment	**Lipid Serum profile (mg/dl)**								
		TC	TG	ALP	LDH	SGOT	SGPT	HDL-C	LDL-C	VLDL-C
Group	**Vehicle control**	122.8±2.16	164.9±6.53	0.26±.015	1.33±0.18	3.65±0.77	3.004±0.82	3.51±0.32	54.3±2.94	28.38±0.74
Group II	Negative Control	193.4±2.64	394.3±2.36	2.46±0.15	2.13±0.006	16.41±4.9	10.62±1.81	2.13±0.006 #	101.0±3.3	68.81±0.92
Group II	GRLE low dose (100 mg/kg)	193.4±2.65	178.51±9.55	0.64±0.03	0.45±0.02	2.14±0.29	0.025±0.00	0.26±0.07	98.85±3.95	64.20±0.59
Group IV	GRLE high	180.3±3.22	144.14±9.19	0.68±0.04	0.48±0.19	3.178±1.18	0.03±0.003	0.35±0.07	60.51±2.67	34.62±0.90

ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 04, 2021

	dose (200 mg/kg)									
Group V	GRLE alone (200 mg/kg)	119.5±1.90^{ns}	208.5±24.12^{ns}	0.40±0.03^{ns}	1.63±0.18 ns	8.71±1.78^{ns}	2.55±1.16^{ns}	2.38±0.56^{ns}	50.40±1.86^{ns}	25.40±0.56^{ns}
Group VI	Standard (10 mg/kg)	129.4±2.2	206.01±14.66	0.52±0.10	0.46±0.10	3.92±0.871	0.03±0.004	3.46±0.07	58.01±1.38	32.90±0.55

Values are expressed in Mean ± SEM (n=6) one way NOVA followed by Tukey's test. Where, denotes P<0.05 and notes P<0.001 when compared to vehicle control rats. Denotes P<0.05, denotes P<0.01 and denotes P<0.001 when compared to negative control. Denotes P>0.05 when compared to vehicle control group.

Effect of GRLE on Total Protein and MDA:

There was significant (P<0.05decrease in the level of total protein and MDA in Negative control group when compared to the normal control groups. (Table.16)

When the treated groups shows significant (P<0.05) in the Total protein and MDA in the tissues (heart and liver) homogenate in the dose100 and 200mg/kg when compared to the Negative control group (HFD).GRLE alone treated groups shows non-significant (P>0.05) when compare to the normal control groups.

High at Diet animals shows significant (P<0.05) decrease in the Enzymatic antioxidants like catalyse, superoxide dismutase, glutathione peroxidise and glutathione reductive and the non-enzymatic antioxidant reduced glutathione in the tissues (heart and liver) homogenate when compared to normal control. (Table No16.)

Table: 10 Effect of GRLE on enzymatic and non-enzymatic antioxidants in control and experimental groups

GROUPS	Total protein	MDA	SOD	CAT	GSH	GSSH	GP_X
Normal control	14.51±0.56	0.41±0.098	2.660±0.310	2.498±0.219	1.81±0.40	1.581±0.243	3.06±0.416
Negative control	7.95±1.07	0.014±0.004	0.028±0.004	0.073±0.010	0.043 ±0.005	0.023±0.003	0.054±0.003
AML low dose(100mg/kg)	2.45±1.39	0.253±0.055	1.54±0.2183	1.388±0.202	1.75±0.167	1.095±0.020	1.504±0.403
AML high dose(200 mg/kg)	9.02±0.71	0.37±0.070	1.595±0.236	1.404±0.230	1.948±0.23	0.9054±0.0206	1.895±0.187
GRLE alone(200 mg/kg)	12.44±1.0^{ns}	0.191±0.017 ns	1.892±0.166^{ns}	1.604±0.238^{ns}	1.73±0.355 ns	1.518±0.2437^{ns}	1.97±0.1941^{ns}
Standard(10 mg/kg)	7.23±1.57	0.306±0.012	2.24±0.021	1.995±0.390	1.36±0.340	1.06±0.0092	1.55±0.244

ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 04, 2021

Protein moles/min/mg, CAT moles/min/mg wet protein, moles/min/mg protein, SOD moles/min /mg protein, GSSH μ moles/min/mg protein, GSH moles/min/mg protein, and MDA μ moles/min/mg protein; Values expressed in Mean SEM (n=6) one way ANOVA followed by Tukey's test. Where, denotes <0.05 and denotes P<0.001 when compared to vehicle control rats. Denotes P<0.05, denotes.

Table: 11 Effect of GRLE on Lipid per oxidation and Antioxidant enzymes in Heart

GROUPS	Total protein	MDA	SOD	CAT	GSH	GSSH	GP_X
Normal control	12.51±06	0.351±0.090	2.77±0.393	2.946±0.271	2.276±0.306	1.88±0.278	2.74±0.324
Negative control	76.95±07	0.034±0.005	0.049±0.013	0.070±0.010	0.031±0.0048	0.0380±0.009	0.090±0.02
GRLE Low dose(100mg/kg)	1.45±1.39	0.330±0.07	1.832±0.281	1.44±0.210	1.962±0.259	1.766±0.157	1.435±0.174
GRLE High dose(200) mg/kg)	5.02±0.71	0.634±0.070	1.72±0.20	1.419±0.216	2.101±0.422	1.642±0.4622	1.611±0.32
GRLE alone (200 mg/kg)	10.44±1.0	0.247±0.017	2.055±0.1791	2.0994±0.23	1.686±0.306	1.3188±0.16	1.97±0.25
Standard(10 mg/kg)	6.23±1.57	0.312±0.024	2.156±0.2110	2.079±0.37	1.7006±0.27	1.678±0.331	1.66±0.2620

DISCUSSION AND CONCLUSION:

Hypercholesterolemia and the resulting have been implicated the path physiology of coronary heart diseases and myocardial ischemia. Lowering cholesterol level may decrease the risk of CVD, and therefore enormous efforts have been extended achieve this aim. The hypocholesterolemic activity of ethanol extract of *Grevillea Robusta* (GRLE) against hypercholesterolemia was monitored on the Lipid profile status, antioxidant status, activities of serum cardiac marker enzyme, and histological changes of liver and heart. In this study the high cholesterol diet (HCD) is used which consists of maize, soya meal, coconut cake, rice polish, groundnut cake and animal tallow which has been used in inducing experimental hypercholesterolemia In the current study, the HCD fed rats showed increased levels of plasma cholesterol (TC), and triglycerides (TG) levels compared to normal control rats. Treatment with GRLE significantly decreased the levels of serum TC, when compared to HCD induced rats.

Saponins are also reported to precipitate cholesterol from micelles and interfere with enter hepatic circulation of bile acids making it unavailable for intestinal absorption, this forces liver to produce more bile from cholesterol and hence the reduction in serum cholesterol level. Saponins are also reported to lower triglycerides by inhibiting pancreatic lipoprotein lipase. Similarly in our study also, the presence of both flavanoids and saponins in could have contributed in reducing the levels of lipid status (TC, TG,) elevated levels of serum low density lipoprotein cholesterol (LDL) and very low density lipoprotein cholesterol (VLDL) are often accompanied by premature atherosclerosis and other CVD. FEA low level of high-density lipoprotein cholesterol (HDL) is also an important risk factor for cardiovascular disease. The cardio protective effects of FEHDL have been attributed to its role FE in reversing cholesterol transport, its effects on endothelial cells, and FE its antioxidant activity. Flavanoids can increase HDL-C and also decreases oxidation of FELDL- cholesterol. High cholesterol diet increases serum LDL levels and oxidative stress which results in the production of increased oxidized LDL

ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 04, 2021

and thereby increases atherosclerotic plaque formation. From our present study it is evident that FEHCD induced rats showed increased serum LDL and FEVLDL levels with the concomitant reduction in FE serum HDL level, when compared to normal rats. Supplementation with GRLE reduced the serum LDL and FEVLDL levels and increased the FE serum FEHDL level which could be due to reduction in plasma total cholesterol and increasing FELDL receptor activity by the flavanoids and phytosterol present in the plant extract. Also it FE could be presumed that the reduction of total cholesterol by GRLE could have been associated with a reduction of its LDL fraction, which is the target of FE several hypolipidemic drugs. Oxidative stress is believed to contribute to the pathogenesis of hypercholesterolemia atherosclerosis enhance, various antioxidant compounds are being evaluated for potential anti-hypercholesterolemia effects. A high diet brings about remarkable modifications in the antioxidant defence mechanisms of rat tissues by the process of lipid per oxidation. Several reports have shown that hypercholesterolemia diminishes the antioxidant defence systems by producing free radicals and thereby elevating the lipid peroxide products, resulting in the production of toxic intermediates. Superoxide dismutase is the first enzyme in antioxidant defence that protects tissues against oxygen free radicals by catalyzing the removal of superoxide radical (O2), which damages the membrane and biological structures. Catalyse has been shown to be FE responsible for the detoxification of significant amounts of Hydrogen peroxide.(50) SOD and FE FECAT are the two major scavenging enzymes that remove the toxic free radicals. From our study we observed that there was a reduction in the activity of hepatic FESOD and CAT in HCD induced rats when compared to control rats FE, this may be due to the enhanced production of Reactive Oxygen Species (ROS) by FEHCD. This free radical affects FE the antioxidant activity and hence resulted FE in the decreased activity of FESOD and CAT. Treatment with AMLE restores the HCD induced alteration in FE the activity of the SOD and CAT to near control due to its free radical FE scavenging activity. GP has been shown to be responsible for the detoxification of H2O2. Glutathione reductive is responsible for the reduction of oxidised glutathione to glutathione (reduced). The increased oxidant stress in hypercholesterolemia conditions exhausts the GSH pools. Activities of FE hepatic antioxidant enzymes viz., Glutathione peroxidise (GPx) and Glutathione Reductive (GR) enzymes and Glutathione contents were significantly decreased in FEHCD induced rats. On oral administration with GRLE, the FE activities of these antioxidant enzymes in liver were reverted back FE to normal levels. FE earlier it FE has been reported that GRLE has FE an antioxidant activity owing to the presence of its saponins, flavanoids and phytosterol. Lipid per oxidation is regarded as one of the basic mechanisms of cellular damage caused by free radicals. The relationship between LPO and hypercholesterolemia is FE well recognized, a cholesterol rich diet results in increased LPO by the induction of free radical production. Hypercholesterolemia and lipid per oxidation are believed to be critically involved in development of atherosclerosis. In our study we found that significant increase in LPO levels were observed in HCD fed groups when compared to the control group. GRLE brought down the level of LPO to near normal. It has FE been FE already reported that GRLE has antioxidant activity owing to the presence of its saponins, flavanoids and phytosterol, thus it decrease the concentration of free radicals, which might terminate the initiation and propagation of FELPO. Several reports. Showed that high cholesterol level can cause cardiac damage Lavation in the levels of diagnostic marker enzymes such as Alanine transaminase, Aspartame transaminase, Lactate dehydrogenise in serum of HCD induced rats is due to peroxide formation induced by hypercholesterolemia in the form of ROS. This ROS FE production increases cellular membrane

ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 04, 2021

permeability, intracellular fluid transfers onto intercellular space, resulting in muscle and cardiac. Damage which leads to the leakage or release of marker enzymes from cardiac tissue to serum and hence the level of marker enzymes are raised in HCD fed rats. There was a significant elevation in the levels of marker enzymes such as SGOT, SGPT, LDH were observed in HCD induced rats when compared with control rats. Treatment with GRLE significantly reduced the activity of SGOT, SGPT, and LDH to near normal levels. Macroscopic observation of liver and heart showed that tenacity of vessel in control group was better than that of hypercholesterolemia rats. Furthermore, surface of FE intimae in FE rats fed with normal rat chow was smooth and glossy, and there is no thickening of FE the intimae or migration of smooth muscle cells to the intimae, whereas surface of intimae of HCD induced rats showed a typical plaque characterized by thickening of FE the intimae, FE migration of smooth muscle cells to the intimae, infiltration of macrophages, appearance of foam and lamellar calcification under the endothelium. From our result we observed that there was an increased foam cell formation which leads to intimae thickening in HCD induced group. These foam cell counts were reduced in GRLE FE treated FE group which FE could FE be due FE to its inhibition activity on cholesterol ester and thereby reduces the specific binding sites of acetyl LDL hence can reduce the foam cell formation and leads to less thickening of FE intimae. The result obtained in this study suggests that the ethanol extract of *G. Robusta* has beneficial effects in preventing hypercholesterolemia by lowering lipid status, improving antioxidant status ask
Well as protecting the heart and liver morphology. The present experimental data therefore suggest that ethanol extract of *G.Robusta* has an atheroprotective potential. Further studies using various other models have to be carried out to confirm these findings.

REFERENCE:

- Thomas E, Aneesh T, Thomas D, Anandan R. GC-MS analysis of Photochemical Compounds present in the rhizomes of Nervilia aragoana Gaud. Asian Journal of Pharmaceutical and Clinical Research.2013; Vol. 6(3):68-74.

- Markus P and Gut show M. Alternate substrate inhibition of cholesterol esterase by the no [2, 3-d] [1, 3] oxazin-4-ones.The Journal of Biological. Chemistry 2002; 27:24006-13.

- Kamesh V, Sumathi T. Anti hypercholesterolemia effect Bacopa monniera linn. On high cholesterol diet induced in rats. Asian Pacific Journal of Tropical Medicine.2012; 949-55.

- Adeneye A and Crooks P. Weight losing, antihyperlipidemic and cardio protective effects of the alkaloid fraction of Hunteria umbellate seed extract on normal and triton-induced hyperlipidemic rats. Asian Pacific Journal of Tropical Biomedicine.2015; 5(5): 387-94.

- Lowry OH, Rosenberg NJ, Farr AL. and Randall RJ. Protein measurement with folin phenol reagent. Journal of Biological Chemistry.1951; 193: 265-7.

- Nichans WG and Samuelson B. Formation of MDA from phospholipids arachidonate

ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 04, 2021

during microtonal lipid per oxidation. European Journal of Biochemistry.1986; 6:126-30.

- Aebi H. Catalase in vitro: Methods in enzymologist. Packer L .Academic Press, New York.1984; 121-6.

- Evans WC. Trease and Evans Pharmacognosy; 16thed Churchill Livingston. British Librarian 2005.

- Srivastava Kshama , Dubey Anubhav ,Tiwari Mamta ,Dubey Anurag, To evaluate the synergistic effect of pinitol with glimepride in diabetic wistar rats;7,(13)2020, 2058-2062
-
- Dubey Anubhav, Tiwari Mamta, Kumar Vikas, Srivastava, Kshama Singh. Akanksha. Investigation of anti-hyperlipidemic activity of vinpocetine in wistar rat International Journal of Pharmaceutical Research 2020;12(02):1879-1882

- Akshay Tiwari, Shalini Singh, Anubhav Dubey and Yatendra Singh. “A preliminary study on anti-hyperlipidemic activity of cinnamon oil in wistar rat”, 2021. International Journal of Current Research, 13, (03), 16741-1674

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