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EVALUATION OF ANTI-HYPERLIPIDEMIC ACTIVITY OF BOUGAINVILLEA GLABRA "SNOW WHITE LEAVES" EXTRACT

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ABSTRACT

The aim of the present study was to evaluate the Anti- hyperlipidimic activity of Bougainvillea glabra snow white leaves extract. Bougainvillea glabra snow white is cultivated variety of Bougainvillea glabrachoicy which have white bracts with greenish veins. It is an ornamental flowering plant from the genus of Bougainvillea. Family Nyctaginaceae and a native of Brazil. Free radicle reactions of plant have been implicated in the pathology of atherosclerosis. Anti-hyperlipidemic activity was evaluated by triton 100x and carbontetrachloride induced hyperlipidemia model by using hydroalcoholic extract. This extract (50:50) at different doses, was shown significant($p<0.05$) anti-hyperlipidimic activity in both triton 100x and carbontetrachloride induced hyperlipidemia in rats by considering cholesterol, triglycerides, HDL, LDL and VLDL. The histopathological analysis of rats liver was shown that hydroalcoholic extract (50:50) was reduced the excessive deposition of fat in the liver cell and also reduced the mobilization of fats from adipose tissue on the peripheral livert cells and increase the mobilization of free fatty acids from the peripheral depots. The findings of the study suggested that hydroalcoholic extract (50:50) Bougainvillae snow white leaves extract was shown strong Anti- hyperlipidimic activity.

KEYWORD: Antihyperlipidemic activity, Bougainvillae glabra 'snow white', Hydroalcoholic extract, Carbontetrachloride.

INTRODUCTION

The nature has blessed us with numerous gifts in the world. The plants are one of them, which form the basis of life and give us not only food and shelter but also the medicine to cure ourselves. Now a days traditional system of plant medicine continue to be widely practiced all over the world mainly because of increase in population, inadequate supply of drugs, high cost of drugs, side effects of synthetic drugs and development of resistance to currently used synthetic drug by infectious bacteria have thus emphasized for the wide usage of herbs for the treatment of various ailments[1-2] Inspite of tremendous advances in synthetic drugs, and our dependence on modern medicine but still a majority of their population is turning towards herbal medicine mainly because of its safety and less side effects.[3] In most of the developing countries the use of plant material is increase because modern synthetic drugs are beyond reach of three quarters of the total polulationinsipte of spending 40-50% of their total wealth on drugs and health. So inorder to reduce the financial burdern on developing countries, it is quite obvious that the usage of herbal medicine will be followed in new future.

The research for medicinal plants discovered various common plants having distinguishable medicinal properties, among which one is Bougainvillea glabra. It is an ornamental flowering plants from the genus of Bougainvillae; familyNyctaginaceae and a native to Brazil.[4] The genus Bougainvillea has eighteen species of which B.Spectabilis, B.glabra and B.peruvian[5] are horticulture important. B.glabra is a woddy cliber with thorny thin stem and along branches also it has papery bracts and smooth leaves which grows to more 10 meters height.[6] The free radicle reactions of plant have been implicated in the pathology of many human disease including atherosclerosis, ischemic heart disease, The aging process, inflamations, diabetis and other conditions.[7]

From the seventeen major cause of human death, cardiovascular disease rank seventh. Coronary heart disease is the cause of mortality in 50% of people around the world. Hyperlipidimia is characterized by elevated serum total cholesterol, low density lipoprotein(LDL), very low density lipoprotein(VLDL) and decreased high density lipoprotein levels(HDL). Hyperlipidimia associated lipid disorders are considered to causeatherosclerotic cardiovascular disease.[8] Among

these hypercholesterolemia and hypertriglyceridemia are closely related to ischemic heart disease.[9] Hyperlipidimiais considered to be the most influential risk factor for CHD. Therefore, maintaining low blood lipid profile and blood cholesterol is essential for cardiovascular health. A 20% reduction of blood cholesterol level can decrease about31% of CHD incidenc, and 33% of its mortality rate. But present drug have many sideeffect so reduce the side effect need for evaluation of natural drugs.[10]

MATERIALS AND METHODS

Plant Material: The plant material (leaves) was collected from the Balaji Nursery, Jagatpura, Jaipur (Rajasthan), India. The botanical identity of this plant was confirmed by the Dr.N.S. Shekhawat, Head of the Department of Botany, Jai Narayan Vyas University, Jodhpur,(Raj), India. The voucher specimen (JNU/Phcog./004/2009) was deposited in the museum of the Department of Pharmacology, Jaipur National University, Jaipure-302025, (Raj), India.

Preparation of Plant Extract: The plant material was dried in shade and crush in the grinder. The dried powder was weighed in sufficient quantity. The dried powdered material was initially defatted with pet. Ether (60-80^{0}c) in a soxhlet apparatus for 72 hrs according to successive solvent extraction. The pet. Ether extract was dried and collected. The mark was dried and successively extracted with acetone and hydro-alcohol (50:50) for 72hrs. The extracts were filtered while hot and the solvent was removed by distillation under reduced pressure.

Successive Solvent Extraction- Petroleum Ether (Defatting) (60^{0}c -80^{0}c)

↓

Acetone

↓

Ethanol + water (50:50)

Phyto-chemical Evaluation

Preliminary Phyto-chemical Evaluation

Preliminary phytochemical screening was carried out by using standard procedures.[11]

Qualitative Analysis of Extracts

Alkaloids

Dragendorff's Test: To 2mg of each extract in separate test tubes + 5ml of distilled water was added in each test tube + 2M Hydrochloric acid was added. To the above solution 1ml of Dragendroff's reagent was added. Formation of orange or orange red precipitate was indicated the presence of alkaloids.

Hager's test: To 2mg of the each extract was taken in a separate test tube and few drops of Hager's reagent were added. Formation of yellow precipitate was indicated the presence of alkaloids.

Wagner's test: To 2mg of each extract was acidified with 1.5 per cent v/v of hydrochloric acid in a separate test tube and a few drops of Wagner's reagent were added. Formation of yellow or brown precipitate was indicated the presence of alkaloids.

Mayer's test: Few drops of the Mayer's reagent and 2mg of each extract were added in a separate test tube. Formation of white or pale yellow precipitate was indicated the presence of alkaloids.

Carbohydrates (Molisch's test): In a test tube containing 2mg of each extract in a separate test tube + 2drops of freshly prepared 20 per cent alcoholic solution of α- naphthol were addedin each test tube. Then 2ml of conc. Sulphuric acid was added so as to form a layer below the mixture. Red- violet ring appeared, indicating the presence of carbohydrates which disappeared on the addition of excess of alkali.

Flavanoids (Shinoda's test): In a separate test tube, containing 0.5 mg of the each extract + 10 drops of dilute hydrochloric acid followed by a small piece of magnesium turning was added. Formation of pink, reddish or brown colour was indicated the presence of flavonoids.

Proteins

Biuret test: To 1ml of each hot extract + 5 to 8 drops of 10 per cent w/v sodium hydroxide solution, followed by 1 or 2 drops 3 per cent w/v copper sulphate solution were added in a separate test tube. Formation of a violet red colour was indicated the presence of proteins.

Millon's test: 1mg of each extract was dissolved in 1ml of distiiled water in separate test tube and 5 to 6 drops of Millon's reagent were added. Formation of white precipitate which turns red on heating was indicated the presence of proteins.

Glycosides

Legal's Test: Each extract with CHCl3 was shaken well in a separate test tube and treated with pyridine, sodium nitroprusside+NaOH, and then pink colour was observed if the glycosides were present.

Ferric Chloride Test (Fecl3): Each extract was treated with CHCl3 and FeCl3 in a separate test tube, and then green colour will appear if glycosides were present.

Tannins: To 1-2mg of the each extract, few drops of 5 percent w/v FeCl3 solution were added in a separate test tube. A green colour was indicated the presence of gallotannins, while brown colour was indicated the presence of pseudotannins.

Steroids

Liebermann-Burchard's test: 2 mg of each dry extract was dissolved in acetic anhydride, heated to boiling, cooled and then 1ml of concentrated sulphuric acid was

added along the sides of the separate test tube. Formulation of green colour was indicated the presence of steroids.

Salkowski reaction: 2mg of each dry extract was shaken with chloroform, to the chloroform layer sulphuric acid was added slowly by the sides of the separate test tube. Red colour was indicated the presence of steroids.

Saponins: In a separate test tube containing about 5ml of each extract, a drop of sodium bicarbonate solution was added. The test tube was shaken vigorously and left for 3 min. Formation of honeycomb like froth was indicated the presence of saponins.

Pharmacological Activities

Animals: Adult albino rats of either sex weighing 150-200g bred in animal house of in School of Pharmaceutical Sciences, Jaipur Natinal University, Jaipur. The animals had free access to standard commercial diet and water *ad libitum* and were housed in cages under standard laboratory conditions i.e., 12:12 hour light or dark cycle at 25±20C. The experiments were carried out as per the guidelines and ethical norms, approved by Ministry of Social Justice and Empowerment, Government of India and study was got approved from Institutional Animal Ethical Committee (IAEC), (**004/2009/IAEC/JNU)(Approval No. 1054/ac/07/CPCSEA)** of committee for the purpose of control and supervision of experiments on animals(CPCSEA).

Acute Toxicity Studies: The acute toxicity test of the extract was determined according to the OECD guidelines No. 420. Adult albino rats (180-200g) of either sex were used. Starting dose of 2000mg/kg (P.O) of extract was given to three groups (n=6). The treated animals were monitored for 14 days for mortality and general behavior. No death was observed till the end of study. The extract was safe up to the dose of 2000mg/kg and from the results suitable dose was chosen for each activity in each extract for further experimentation.

Antihyperlipidemic Activity

Triton-100X Induced Hyperlipidemia in Rats[12-14]

Rats were divided into five groups of six rats each. The I group (control) was given standard pellet diet, water and orally administered with 5% CMC.in the II, III, IV and V groups single dose of triton was administered at a dose of 100mg/kg (I.P). After 72 hours of triton injection, II group (negative control group) received a daily dose of 5% CMC (p.o) for 7 days. III group received 200mg/kg (p.o) of hydroalcoholic extract (50:50) of *Bougainvillea glabra* for 7 days daily. IV group received 400 mg/kg (p.o) of hydroalcoholic extract (50:50) of *Bougainvillea glabra* for 7 days daily. V group received 65mg/kg of standard drug fenofibrate (p.o) for 7 day daily. The hydroalcoholic extract (50:50) was suspended in 5% CMC.

Collection of Blood[15]

On the 8th day, blood was collected by reto-orbital sinus puncture, under mild ether anesthesia. The collection samples were centrifuged for 10 minutes at 2500rpm/min. Then serum samples were collected and used for various biochemical experiments. The animals were then sacrificed and the liver collected for histopathologicl analysis.

Biochemical Analysis[16]

The serum and liver extract were assayed for total cholesterol, triglycerides, high density lipoprotein (HDL), low density lipoprotein(LDL), very low density lipoprotein(VLDL) using standard protocol methods.

Preparation of Triton 100X Injection

The triton 100X was weighed and dissolved in normal saline solution (0.9%) w/v. According to the body weight the dose was determined.

Carbon Tertra Chloride Induced Hyperlipidemia in Rats[17]

Rats, each weighing 150-200g were divided into four groups of six animals in each group. Group I (Normal Control) animals were administered with a single daily dose of tween 80 (1ml/kg body weight, i.p) as 1:1 dilution with liquid paraffin after every 72 hours. Simultaneously group II received single daily dose of tween 80 (1ml/kg body weight, p.o), groups III-IV (test groups) were administered with *Bougainvilla glabra* hydroalcoholic extract(50:50), of 200 & 400 mg/kg body weight, p.o (for administration the extract was suspended in 2% tween 80) once daily and group V received 65mg/kg body weight (p.o) dose of fenofibrate for 14 days. On the 15th day the blood was collected and biochemical analysis was done.

Assessment of antihyperlipidemic activity

The blood samples were collected separately by retro orbital sinus and allowed to clot, for 30 minutes at room temperature. The clear serum was separated by centrifugation at 2500 rpm for 10 minutes. The serum triglycerides (STG) and serum cholesterol (SC) levels, were determined by standard kits using Autoanalyzer and very low density lipoprotein cholesterol (VLDL-c) was calculated using Friedwald formula VLDL-c= TG/5. And HDL and LDL were also determined. Then the rats were sacrificed after 48 hours of last dose by cervical decapitation for histopathological analysis.

RESULTS AND DISCUSSION

The extractive values are preliminary useful for the determination of exhausted or adulterated drugs. The Acetone, ethanol plus water, ether soluble extractive values have been tabulated table 1.

Table. 1: Extractive values of leaf powder of *bougainvilla glabra "snow white".*

Sl. No	Extracts	Values % (w/w)
1	Acetone soluble extracts	6.2
2	Ethanol+water (50:50)soluble extracts	6.9
3	Ether soluble extracts	2.0

According to this study we can say that among of these extracts the hydroalcoholic extract (50:50) is best and have good extractive value.

Preliminary Phytochemical Screening: Preliminary phytochemical screening of extracts revealed the presence of alkaloid, glycosides (minute amount), flavonoids, tannins, steroids, protein and saponins (Table 2). Hence these extracts were investigated for potential anti-hyperlipidemic action.

Table. 2: Preliminary phytochemical screening of extracts of *Bougainvilla glabra* 'snow white'.

Sl no	Tests	Petrolium. Ether	Acetone	Ethanol+water (50:50)
1	Alkaloids	-	+	+
2	Carbohydrate	-	-	-
3	Flavanoids	-	+	+
4	Phenolic compounds and tannins	-	+	+
5	Proteins	-	+	+
6	Steroids	+	-	-
7	Glycosides	-	-	+
8	Fat and oils	+	-	-

+ Denotes presence of respective class of compounds, -Denotes absence of compounds.

Antihyperlipidemic Activity

Triton-100X Induced Hyperlipidemia in Rats

The hydro alcoholic extract (50: 50) of *Bougainvilla glabra* plant have significant ($p<0.05$) anti hyperlipidemic activity on triton-100X induced hyperlipidemia in rats. (Table 3).

Table. 3: Anti hyperlipidemic activity of hydro alcoholic extract (50: 50) of *Bougainvilla glabra* plant on triton-100X induced hyperlipidemia in rats.

Groups	Treatment (dose mg/kg)	Cholesterol and Triglyceride level (mg/dl) After 7 days	
		Cholesterol	Triglyceride
Normal	Normal saline	64.44±2.564	74.91±3.590
Triton control	Triton treated only	188.43±2.567	121.45±3.178
Test 1 (200mg/kg)	B. glabra	77.44±2.476	79.40±3.444
Test 2(400mg/kg)	B. glabra	68.54±2.312	75.71±3.415
Std (65mg/kg)	Fenofibrate	64.55±2.120	71.33±3.109

Values are Mean ± S.E.M; n=6; $p< 0.05$, significant as compared to triton control of respective group.

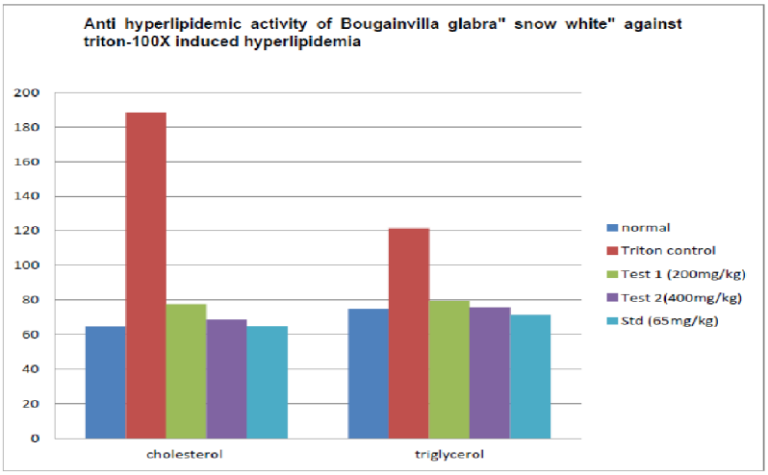

Fig. 1: Anti hyperlipidemic activity(cholesterol and triglyceride levels) of hydro alcoholic extract (50: 50) of *Bougainvilla glabra "snow white"* on triton-100X induced hyperlipidemia in rats.

Table. 4: Antihyperlipidemic activity of hydroalcoholic extract (50:50) of *Bougainvilla glabra "snow white"* on triton 100X induced hyperlipidemia in rats (HDL, LDL, VLDL).

Groups	Treatment (dose mg/kg)	HDL, LDL, VLDL level (mg/dl) After 7 days		
		HDL	LDL	VLDL
Normal	Normal saline	29.90±0.8838	21.56±1.35	14.98±0.8149
Triton control	Triton treated only	19.26±0.9328	143.88±0.2570	24.29±1.042
Test 1 (200mg/kg)	B. glabra	22.92±0.6948	37.64±0.79	15.88±0.874
Test 2(400mg/kg)	B. glabra	25.25±0.8607	28.15±0.6062	15.14±0.77
Std (65mg/kg)	Fenofibrate	27.24±0.7245	23.05±2.096	14.26±1.014

Values are Mean ± S.E.M; n=6; p< 0.05, significant as compared to triton control of respective group.

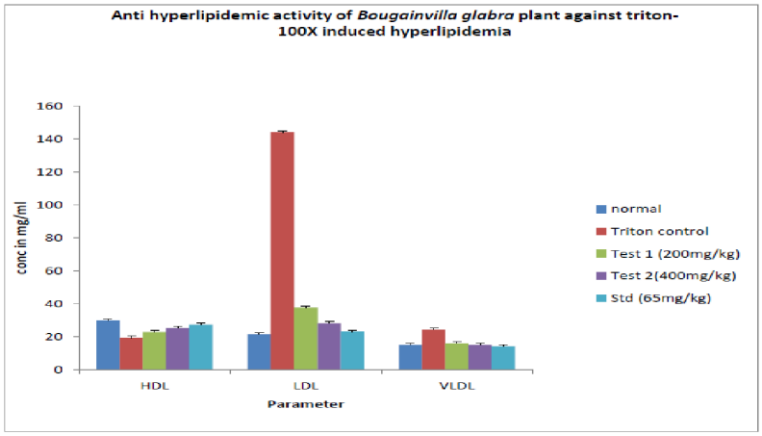

Fig. 2: Antihyperlipidemic activity of hydroalcoholic extract (50:50) of *Bougainvilla glabra* on triton 100X induced hyperlipidemia in rats (HDL, LDL, VLDL).

HISTOPATHOLOGICAL ANALYSIS

The histopathological analysis of rat's liver showed that hydroalcoholic extract (50:50) has reduced the excessive deposition of fat in the liver cells, reduced the mobilization of fats from adipose tissue on the peripheral liver cells and increased the mobilization of free fatty acids from the peripheral depots.

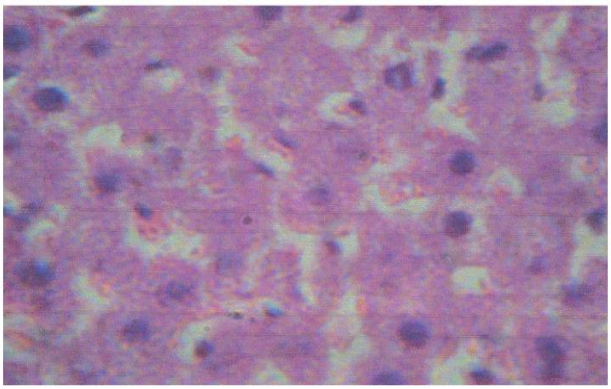

Fig. 3(A): Normal Rat.

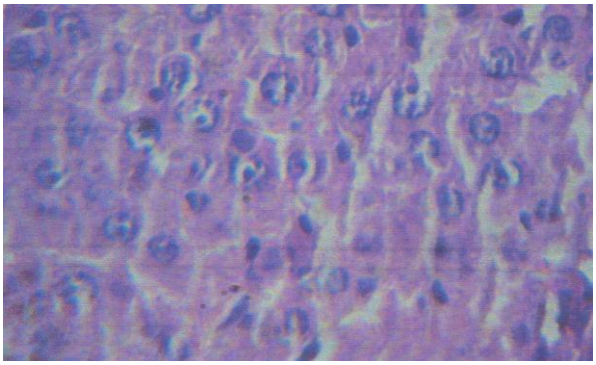

Fig. 3(B): Only Tritone-100x treated rat.

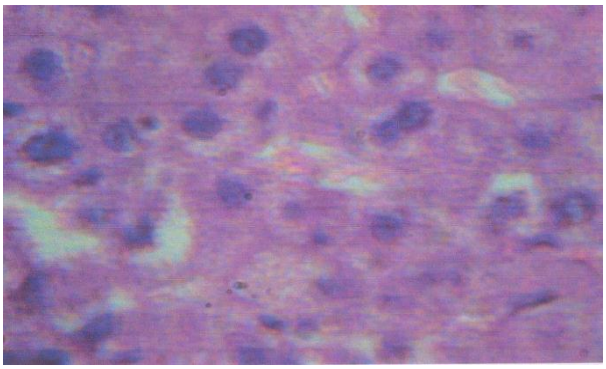

Fig. 3(B): Only Tritone-100x treated rat.

Fig: 3 Histopathological analysis of rats liver for evaluation of anti-hyperlipidemic activity of hydroalcoholic extract (50:50) of "Bougainvillea glabra snow white" in triton-100x induced hyperlipidimia in rats.

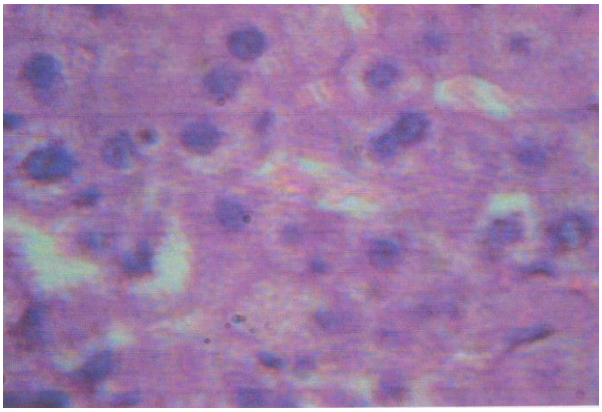

Fig: 3 (C) Triton-100x and standard drug fenofibrate treated rat.

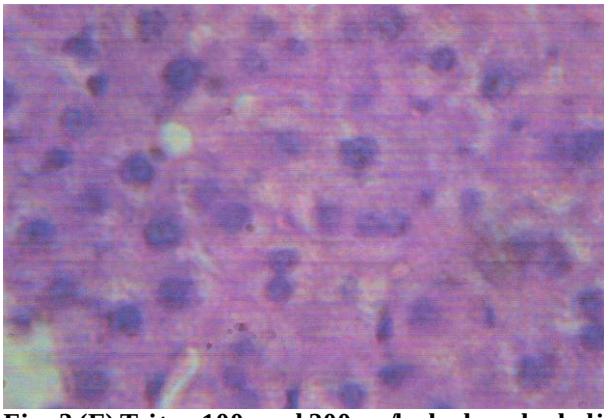

Fig: 3 (E) Triton 100x and 200mg/kg hydro-alcoholic extract (50:50) of Bougainvillea glabra 'Snow White' Carbon Tetrachloride Induced Hyperlipidemia in Rats.

The hydroalcoholic extract (50:50) of *Bougainvilla glabra* 'Snow white' have significant ($p<0.05$) anti-hyperlipidemic activity on carbon tetrachloride(CCl4) induced hyperlipidemia in rats.

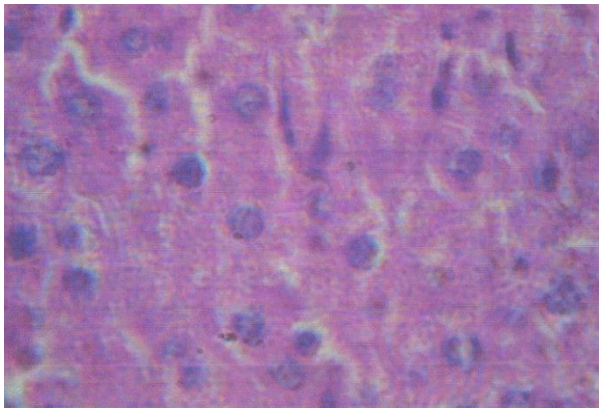

Fig: 3 (D) Triton-100x and 400mg/kg hydro-alcoholic extract(50:50) of Bougainvillea glabra "snow white treated rats.

Table. 5: Anti-hyperlipidemic activity of hydroalcoholic extracts (50:50) of *Bougainvilla glabra 'Snow white'* on carbon tetrachloride (CCl4) induced hyperlipidemia in rats.

Groups	Treatment (dose mg/kg)	Cholesterol and Triglyceride level (mg/dl) After 14 days	
		Cholesterol	Triglyceride
Normal	Normal saline	66.55±2.744	73.61±3.660
CCl4 control	CCl4 treated only	178.43±2.67	122.25±3.778
Test 1 (200mg/kg)	B. glabra	77.74±2.776	80.30±.334
Test 2 (400mg/kg)	B. glabra	69.34±2.334	74.61±3.525
Std (65mg/kg)	Fenofibrate	36.62±2.232	73.63±3.149

Values are Mean ± S.E.M; n=6; $p< 0.05$, significant as compared to CCl4 control of respective group.

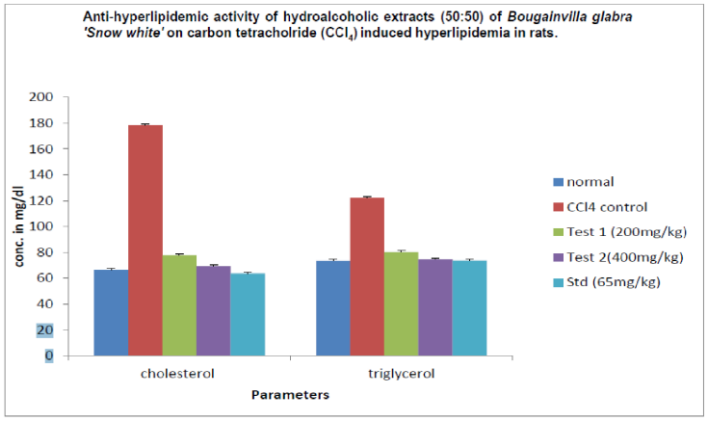

Fig. 4: Anti-hyperlipidemic activity of hydroalcoholic extracts (50:50) of *Bougainvilla glabra 'Snow white'* on carbon tetrachloride (CCl4) induced hyperlipidemia in rat.

Table. 6: Anti-hyperlipidemic activity of hydroalcoholic extracts (50:50) of *Bougainvilla glabra 'Snow white'* on carbontetrachloride(CCl4)induced hyperlipidemia in rat.

Groups	Treatment (dose mg/kg)	HDL, LDL, VLDL level (mg/dl) After 7 days		
Normal	**Normal saline**	**HDL**	**LDL**	**VLDL**
Triton control	Triton treated only	18.26±0.83	135.29±1.237	24.45±1.052
Test 1 (200mg/kg)	B. glabra	21.82±0.8948	39.86±0.89	16.06±0.7748
Test 2(400mg/kg)	B. glabra	24.45±0.7607	29.9±0.676	14.92±0.8708

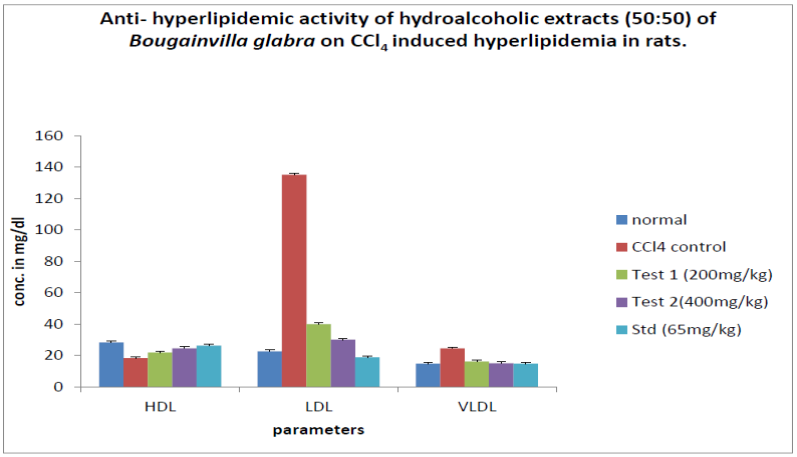

Fig. 5: Anti-hyperlipidemic activity of hydroalcoholic extracts (50:50) of *Bougainvilla glabra 'Snow white'* on carbon tetrachloride(CCl4) induced hyperlipidemia in rat.

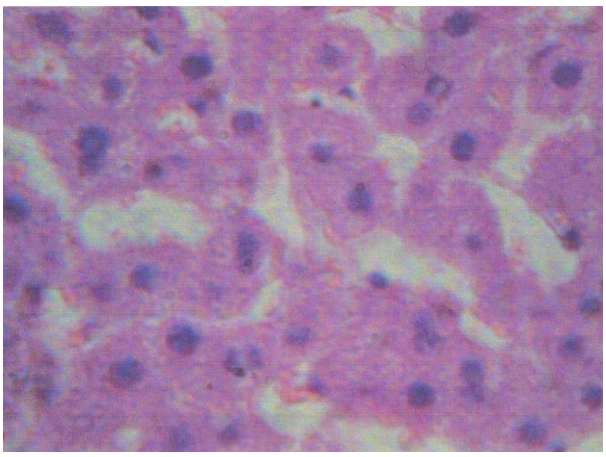

Fig. 6 (A): Normal rat Fig.

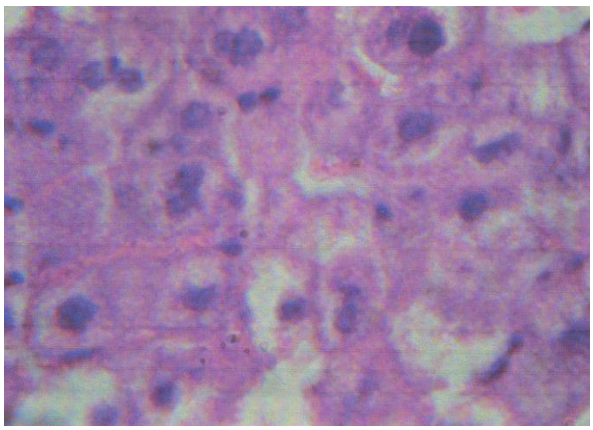

Fig. 6 (C): carbontetrachloride and standard drug fenofibrate treated rat.

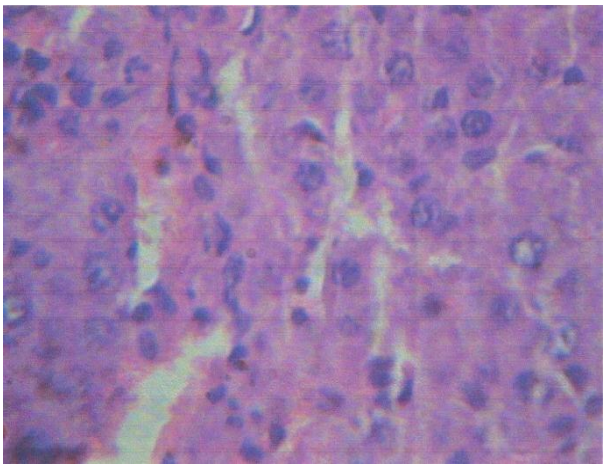

Fig. 6 (B): only carbon tetrachloride treated rat.

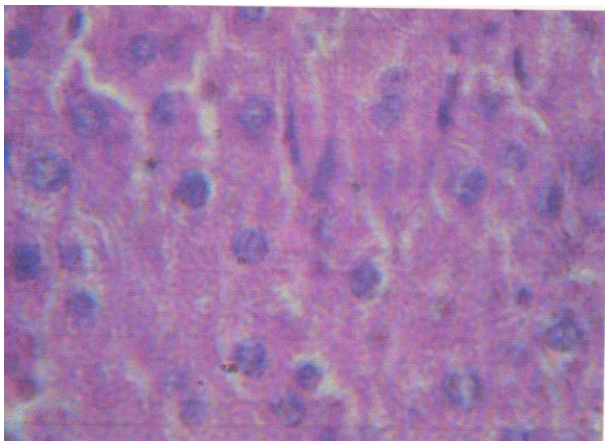

Fig. 6 (D) Carbon tetrachloride and 400mg/kg hydro-alcoholic extract (50:50) of Bougainvillea glabra 'Snow white' treated rats.

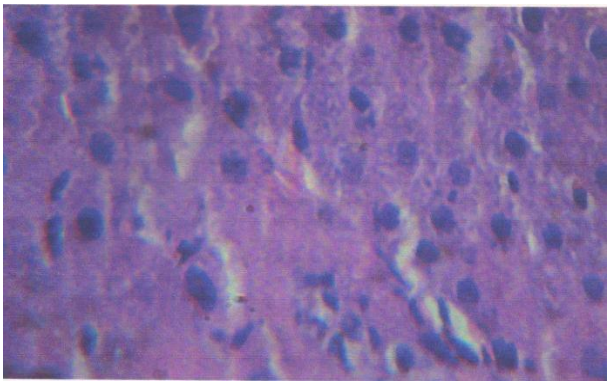

Fig. 6 (E) Carbon tetrachloride and 200mg/kg hydro-alcoholic extract (50:50) of Bougainvillea glabra 'Snow white'.

Fig. 6: Histopathological analysis of rats liver for evaluation of anti-hyperlipidemic activity of hydroalcoholic extract (50:50) of Bougainvillea glabra 'Snow white' in carbon tetrachloride induced hyperlipidemia in rats.

CONCLUSION

The current research concludes that this plant have flavonoids, alkaloids, phenols, glycosides, protein and saponins. According to the phytochemical study the hydroalcoholic extract of Bougainvillae glabra Snow White based on acute toxicity studies we can say that it is safe at the decided dose level of 200 and 400mg/kg of body weight and reduced elevated blood cholesterol and triglycerides and also shows significant effect on rats without harm.

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