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ANTI DIABETIC ACTIVITY OF THE ETHANOL AND AQUEOUS EXTRACT OF THE LEAVES OF *SEARSIA MYSORENSIS* ON ALLOXAN INDUCED DIABETIC ALBINO RATS

***P. Prasanna, N. Danamuthy and D. Dhachinamoorthi**

Department of Pharmacology, QIS College of Pharmacy, Ongole-523272.

***Corresponding Author: Dr. P. Prasanna**
Department of Pharmacology, QIS College of Pharmacy, Ongole-523272.

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ABSTRACT
The present investigation reports the anti-diabetic activity of *Searsia mysorensis* on alloxan induced diabetic albino rats. Evaluation of antidiabetic activity using Alloxan induced hyperglycemia model The observed hypolipidemic effect may be because of decreased cholesterogenesis and fatty acids. Significantly lowering the total cholesterol and raise in HDL Cholesterol is a very desirable biochemical status for prevention of atherosclerosis and ischemic conditions. the results shows that administration of *Searsia mysorensis* extract increases the HDL level in treated group significantly in dose deponent manner.

KEYWORDS: Searsia mysorensis, alloxan induced, anti diabetic activity.

INTRODUCTION
Diabetes mellitus is a chronic disorder characterized by impaired metabolism of glucose and other energy-yielding fuels, as well as the late development of vascular (involving small and large blood vessels) and neuropathic complications. Diabetes mellitus consists of group of disorders involving distinct pathogenic mechanisms in which hyperglycemia is the denominator. Regardless of the cause, the disease is associated with a common hormonal defect, namely, insulin deficiency, which may be total, partial, or relative when viewed in the context of coexisting insulin resistance. Lack of insulin plays a primary role in the metabolic derangements linked to diabetes, and hyperglycemia in turn plays a key role in the complications of the diabetes.[1,2]

Searsia mysorensis (Anacardiacae) is a small aromatic, often gregarious shrub with a thin brown bark and very spinous branches. Commonly distributed in forest of the Chittoor district, A.P., India.

Fig: 1.Searsia mysorensis leaves.

Principle constituents[44]
The following chemical constituents were revealed from ethanol extract of *Rhus mysorensis* are Carbohydrates Alkaloids, Steroids and Sterols, Glycosides, Saponins, Flavonoids, Tannins and Phenolic compound, Proteins and Amino acid.

TRADITIONAL USES
The Sushruta describes *Rhus mysorensis*, as a destroyer of madhumeha (glycosuria) and other urinary disorders. might neutralize the excess of sugar present in the body in Diabetes mellitus. The drug is also used in the composition of Ayurvedic preparations like Ayaskri; it's reported to be anti-inflammatory, hepatoprotective activity, antimicrobial activity.

4. MATERIALS AND METHODS

4.1 Materials

Anesthetic ether, Absolute alcohol, Formalin, Ethanol, Chloroform, Kcl, Nacl, EDTA, KH_2PO_4, H_2O_2, Alloxan (Coral clinical systems, Verna Goa, India), Serum glucose diagnostic kit, Serum ALP diagnostic, Serum ALA T diagnostic, Serum ASA T diagnostic kit, Chem. Kit for Cholesterol estimation, Chem. Kit for Triglycerides estimation

EQUIPMENTS

Shimadzu UV Spectrophotometer (model 1800), Incubator, Digital balance, Rotary flash evaporator (SUPERFIT, Rotary "Vaccum Digital Bath"), Deep freezer, Albino rats (Wistar strain)

4.2 Collection of plant material

Plant material (*Searsia mysorensis*)

The fresh plant leaves of *Searsia mysorensis* were collected from Sri Venkateshwara University, Tirupati, and Chitoor District of Andhra Pradesh and authenticated by Botanist.

Preparation of plant material

4.3 Preparation of ethanol extract[41]

The *Searsia mysorensis leaves* was shade dried at room temperature about 200 g pulverized in a 500 ml of 95% ethanol at temperature 30-50^{0}C, in a Soxhlet extractor for 24 h. The combined extracts were concentrated at 45^{0}C to obtain dark brownish green residue. The selection of the solvent was based on their extractive values of the plant. The extract was concentrated in a rotary flask evaporator and dried in a desicator over the sodium sulphide and preserved in a refrigerator.

4.4 Preparation of Aqueous Extract

The aqueous extract was prepared by maceration; 500 g of plant powder were soaked in about 1 L of distilled water for 24 h. The extract was decanted; remaining material was re soaked in the distilled water twice. The companied extract was dried completely by rota evaporator at 37^{0}C.

4.5 Qualitative chemical tests[45]

Preliminary phytochemical studies

Experimental animals

Albino-Wister rats of either sex, weighing 150-200 g were procured. The animals were acclimatized for seven days under laboratory conditions, all rats were kept at room temperature of 37^{0}C in the animal house. The animals were fed with commercially available rat pelleted diet. Water was allowed *ad libitum* under strict hygienic conditions. Prior to the experiments, rats were fed with standard food for 1 week in order to adapt to the laboratory conditions.

Pharmacological evaluation

Alloxan induced diabetic model

Hyperglycemia and glucosuria after administration of alloxan has been described in several species, such as in dogs, in rabbits, in rats and in other species.[46]

Alloxan (2, 4, 5, 6-tetraoxypyrimidine; 2, 4, 5, 6-pyrimidinetetrone) is an oxygenated pyrimidine derivative and was originally isolated in 1818 by Brugnatelli and got its name in 1838 by Friedrich Wohler and Justus von Liebig. Alloxan is a toxic glucose analogue, which selectively destroys insulin-producing cells in the pancreas when administered to rodents and many other animal species.

Experimental design

Experimental rats were divided into seven groups of six animals in each and treated for 21 days as shown in table.

Anti-diabetic activity

Alloxan induced diabetic model in wistar strain rats

Table Experimental design.

Group	Treatment
Group I	Normal control (0.3% CMC.,0.5ml by oral route)
Group II	Diabetes induced control (Alloxan 120 mg/kg., i.p.)
Group III	Diabetes induced + Standard drug treated.
	(Standard drug : Glibenclamide 1mg/kg by oral route)
Group IV	Diabetes induced + Test drug treated Dose 250 mg/kg.
	(*Searsia mysorensis* ethanol extract), orally.
Groups V	Diabetes induced +Test drug treated Dose 500 mg/kg.
	(*Searsia mysorensis* ethanol leaf extract), orally.
Group VI	Diabetes induced +Test drug treated Dose 250 mg/kg.
	(*Searsia mysorensis* aqueous leaf extract), orally.
Group V II	Diabetes induced +Test drug treated Dose 500 mg/kg.
	(*Searsia mysorensis* aqueous leaf extract), orally.

Preparation of suspension

Plant extracts and standard drugs were suspended in distilled water using sodium carboxy methyl cellulose (sodium CMC, 0.3%) and administered orally to the animals with the help of an intragastric catheter.

Experimental procedure

Wistar rats of either sex weighing between 150-200 g

were divided into seven groups of six rats in each.

Hyperglycemia / diabetes were induced by single Intraperitonial injection of freshly prepared aqueous solution of alloxan monohydrate 120 mg/kg, to overnight fasted rats. After 72 h of alloxan injection, the animals which did not developed hyperglycemia i.e. glucose level >200mg/dl, were rejected/replaced with new animals. Immediately after confirmation of diabetes (glucose level > 250 mg/dl), rats were classified into seven groups of six rats each.

Group I was maintained as normal control, which was given normal saline. Group II received alloxan 120 mg/kg of body wt by i.p. at every 24 h for 3 Days. Group III animals were treated with Glibenclamide 1mg/kg kg in 0.3% Carboxy Methyl Cellulose (CMC) by oral route which served as standard. Groups IV and V animals were treated with two different doses of ethanol test extract were prepared, 250 mg/kg and 500 mg/kg in 0.3% Carboxy Methyl Cellulose (CMC) and were given orally and Groups VI and VII animals were treated with two different doses of aqueous test extract were prepared, 250 mg/kg and 500 mg/kg in 0.3% Carboxy Methyl Cellulose (CMC) and were given orally.

All the groups except the control were pre treated with alloxan monohydrate to produce diabetic activity to the rats. The treatment (p.o.) was started from the same day except normal control and diabetic control groups for a period of 21 days during this period, animals in all groups had free access to standard diet and water.

Blood sampling

The blood samples were collected by the retro orbital plexus puncture method under mild ether anesthesia for the estimation of blood glucose level and body weight. Approximately 1 ml of blood sample obtained from each animal was placed into an Eppendorf tube, centrifuged at 3000 rpm. Blood glucose levels and body weight were estimated on 1st, 7th, 14th and 21st day of the treatment. On the 21st day, blood samples were collected from overnight fasted rats by cardiac puncture under mild ether anesthesia for biochemical estimations. The blood samples were subjected to centrifugation to obtain serum. Serum was analyzed for serum triglycerides, serum total cholesterol, serum HDL - C, serum LDL – C and serum VLDL – C were estimated. The animals were sacrificed by overdose of ether and autopsied.

Procedures for tested parameters

1. Determination of blood glucose

Blood glucose (BG) level of all rats was determined before the start of the experiment. Blood samples were collected for the measurement of blood glucose by retro-orbital plexus puncture method under mild ether anesthesia on 1st day, 7th, 14th and 21st day. The blood glucose level was determined by accu check glucometer (one touch). The values of sample treated were compared with that of the standard group which was treated with Glibenclamide. The animals were sacrificed by overdose of ether and autopsied.

2. Body weight

Body weight of all the groups of animals in each group was noted on before the treatment and after the treatment.

Procedure for Biochemical parameters

3. Estimation of serum total cholesterol: Span diagnostic kit was used for the estimation of total cholesterol, which followed cholesterol oxidase/peroxidase (CHOD-POD) method.

Principle: The enzyme, cholesterol esterase catalyzed hydrolysis of cholesterol esters to free cholesterol and fatty acid molecules. Then free cholesterol gets oxidized in the presence of cholesterol to form cholesten-3-one and H_2O_2. Liberated H_2O_2 reacts with phenol and 4 AAP in presence of peroxidase to form red colored quinoneinine complex the intensity of which was measured at 505 nm.

Cholesteryl ester hydrolase

$$\text{cholesterol ester} + H_2O \xrightarrow{\text{CHE}} \text{cholesterol} + \text{fatty acid}$$

The 3-OH group of cholesterol is then oxidized to ketone in oxygen requiring reaction catalyzed by cholesterol oxidase.

Cholesterol oxidase

$$\text{cholesterol} + O_2 \xrightarrow{\text{CHO}} \text{cholesterol-3-one} + H_2O_2$$

H_2O_2 one of the reaction products is measured in a peroxidase catalyzed reaction that forms a dye.

Peroxidase

$$2H_2O_2 + \text{4- Amino antipyrine} + \text{phenol} \xrightarrow{\text{POD}} \text{Quinonelimine} + 4\ H_2O_2$$

Reagents used

Table No: 4.2: Components and concentrations.

Sl. no.	Reagent composition	Conc. in the final test mixed
1.	Good's butter (PH 6.7)	50mmol/l
2.	Phenol	5mmol/l
3.	4 – Aminoantipyrine	0.3 mmol/l
4	Cholesterol esterase	> 200 U/l
5	Peroxidase	> 3 KU/l
6	Cholesterol Oxidase	> 100 U/l

Standard: The concentration of standard glucose used was 200mg/dl

Assay &Procedure: Fresh clear and unhaemolysed serum was used for the estimation.

Reaction parameters

Sl. no.	Reaction type	End point
1	Wavelength	505 nm
2	Optical path	1 cm
3	Temperature	37^0C
4	Measurement	Against reagent blank

The reaction mixtures were mixed well and incubated for 10 min at 37^0C. The absorbance of reaction mixtures at 505 nm against reagent blank was taken. The absorbance was measured by using a Shimadzu Spectrophotometer (model 1800).

4. Estimation of serum of triglycerides: Span diagnostic kit was used for estimation of triglycerides, which followed end point colorimetry enzymatic test using glycerol-3-phosphate oxidase.[49]

Principle: The enzyme, lipoprotein lipase catalyzes hydrolysis of TGs to glycerol and FAs. Glycerol then is phosphorylated in an ATP - requiring reaction catalyzed by glycerophosphate. The formed glycerophosphate is oxidized to dihydroxyacetone and H_2O_2 in a glycerophosphate oxidase catalyzed reaction. H_2O_2 then reacts with 4 -AAP and 4 - chlorophenol under the catalytic influence of peroxidase to form coloured quinoneimine complex, the intensity of which was measured at 505 nm.

Reagents

Components and concentrations in the test

1.	Goods buffer pH 7.2	50 mmol/ l
2.	4-chlorophenol	4 mmol/l
3.	ATP	2 mmol/l
4.	Mg2+	15 mmol/l

Standard: The concentration of standard triglyceride used was 200 mg/dl

Assay & Procedure: Fresh clear and unhaemolysed serum was used for the estimation.

Table no: 4.3 Reaction parameters.

Sl. No.	Reaction type	End point
1	Wave Length	505 nm
2	Optical length	1 cm
3	Temperature	37^0C
4	Measurement	Against reagent blank

Summary of assay details: pipette in to test tube	Blank	Standard	Test
Reagent	1000 µl	1000 µL	1000 µl
Standard	-	10 µl	250 µl
Sample	-	-	10 µl

The reaction mixtures were mixed well and incubated for 10 min at 37^0C. The absorbance of sample and standard were measured against reagent blank at 505 nm. The absorbance was measured by using a Shimadzu Spectrophotometer (model 1800).

Calculation: Serum

5. Estimation of serum high-density lipoprotein cholesterol (HDL-C): Span diagnostic kit was used for estimation of HDL cholesterol, which followed Cholesterol oxidase / peroxidase (CHOD-POD) method.

Assay & Procedure: Fresh clear and unhaemolysed serum was used for the estimation.

Standard: The concentration of standard glucose used was 50mg/dl

Reaction parameters

Sl. no.	Reaction type	End point
1	Wavelength	505 nm
2	Optical path	1 cm
3	Temperature	37^0C
4	Measurement	Against reagent blank

Standard of assay details

1. 0.5 ml of serum was taken into test tube and 0.5 ml of precipitating reagent was added, mixed well and kept at room temperature for 15 min.
2. Centrifuged for 15 min at 4000 rpm.
3. The clear supernatant was separated and immediately used to determine the cholesterol content as follows:

Pipetted in to test tube	Blank	Standard	Test
Reagent	1000 µl	1000 µl	1000 µl
Standard	-	100 µl	-
Supernatant from step3	-	-	100 µl

The reaction mixtures were mixed well and incubated for 10 min at 37^0C. The absorbance of test and standards was measured against the reagent blank at 505 nm. The absorbance was measured by using a Shimadzu Spectrophotometer (model 1800).

Calculation: Serum.

6. Estimation of serum low-density lipoprotein cholesterol (LDL-C): Using the data obtained including total cholesterol, HDL cholesterol and VLDL, the LDL cholesterol levels were calculated using the empirical equation of Friede Wald50.

Calculation: Serum LDLcholesterol = Total cholesterol - (HDLcholesterol + VLDLcholesterol)

7. Estimation of serum very low-density lipoprotein cholesterol (VLDL-C): Using the data obtained including triglycerides, the VLDL cholesterol levels were calculated Using empirical equation of Friede Wald 50.

Calculation: Serum

Statistical analysis

The data were expressed as mean ± standard error mean (SEM). The Significance of differences among the group was assessed using one way and multiple way analysis of variance (ANOVA). The test followed by Tukey-Kramer multiple comparison tests, the p values less than 0.001 were considered as significant.

RESULTS

5.1 Extraction

The plant was collected, authenticated and dried under shade and the ethanol extract was prepared by Soxhlet extraction method for 72 h and aqueous extract was prepared by maceration technique for 7 days.

5.2 Phytochemical constituents present in leaf extracts of *Searsia mysorensis*

Table 5.1: Details of qualitative phytochemical tests.

Sl. no.	Test	Ethanol extract	Aqueous extract
1	Alkaloids	+	+
2	**Glycosides**	+	+
3	**Carbohydrates**	+	+
4	**Test for Steroids and Sterols**	+	+
5	**Test for Flavanoids**	+	+
6	**Tannins**	+	+
7	**Protein and Amino acid**	+	-
8	**Saponins**	+	+

(+) Indicates present, (–) Indicates absence

5.3 Evaluation of anti diabetic activity

5.3.1 Blood glucose level: Alloxan treatment in rats resulted in raised in blood glucose levels which were evident by increase in blood glucose. The groups were treated with glibenclamide and ethanol and aqueous extracts on diabetic albino rats for 21 days showed a significant dose dependent beneficial effect when compared with the reference drug glibenclamide.

Table 5.2: Effect of *Searsia mysorensis* on blood glucose level on alloxan induced diabetic albino rats.

Group	Blood glucose levels (mg/dl) (Mean ± SEM)			
	1st day	7th day	14th day	21st day
GP I	96.66±0.33	96.33±0.49	96.66±0.21	95.83±0.30
GP II	285.66±0.42	290±0.57	291.33±0.49	294.33±0.42
GP III	234.33±1.22***	189.33±1.0***	149.66±0.76***	104.5±0.42***
GP IV	259.66±0.55**	228.83±1.53**	186.66±3.33**	138±2.082**
GP V	256.5±1.72***	207.5±2.14***	153.33±2.10***	107.16±2.10***
GP VI	267.5±2.141**	235.5±2.56**	193.83±1.83**	151.33±2.18**
GP VII	255.5±1.893***	221.66±2.47***	165.83±2.38***	116.66±2.38***

Values are expressed as mean ± SEM (n=6)

One way ANOVA followed by Tukey-Kramer mulptiple test. Where, * represents significant at p<0.05,represents moderate significant at p< 0.01 and represents highly significant at p<0.001.

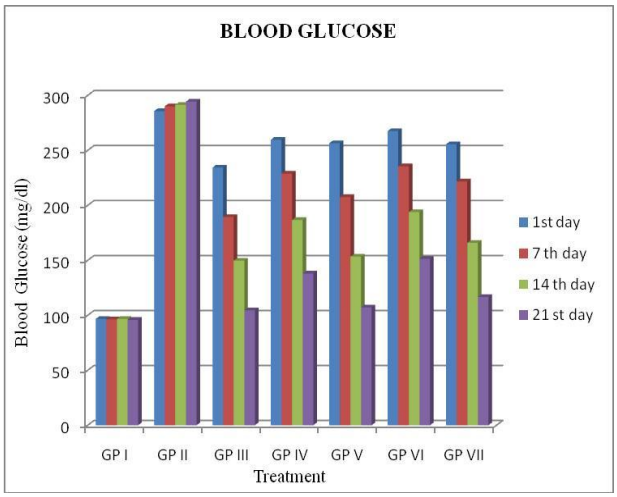

Fig. 5.1: Effect on blood glucose level on alloxan treated diabetic rats.

Body weight: The variations in body weight due to the alloxan (120 mg/kg) were observed before the treatment and after the treatment. Alloxan diabetic control significantly reduced the body weight, then treatment with standard and plant extracts which gained significant weight. The ethanol and aqueous extract treated diabetic albino rats showed a significant dose dependent beneficial effect when compared with the reference drug glibenclamide.

Table 5.3: Effect of *Searsia Mysorensis* on Body Weight (G) on Alloxan-Induced Diabetic Albino Rats.

Groups	Mean body weight (g) (mean ± SEM)	
	Before the Treatment	After the treatment
Group I	190.16±3.468	196.16±2.42
(Normal control CMC 0.5 ml)		
Group II	194.16±5.069	131.66±3.80
(Diabetic control Alloxan 120 mg/kg, i.p.)		
Group III	191.66±3.801***	188.5±6.13***
(Standard Glibenclamide 1 mg/kg, p.o.)		
Group IV	189.33±2.654**	180.83±6.88**
(RMEE-250 mg/kg., p.o.)		
Group V	190.83±4.902***	187.16±3.80***
(RMEE-500 mg/kg., p.o.)		
Group VI	190±2.887**	170.83±5.06**
(RMAE-250 mg/kg., p.o.)		
Group VI	188.33±3.33***	175.83±4.16***
(RMAE-500 mg/kg., p.o.)		
Values are expressed as mean ± SEM (n=6)		

Where, * represents significant at p<0.05,**represents moderate significant at p< 0.01 and ***represents highly significant at p<0.001.

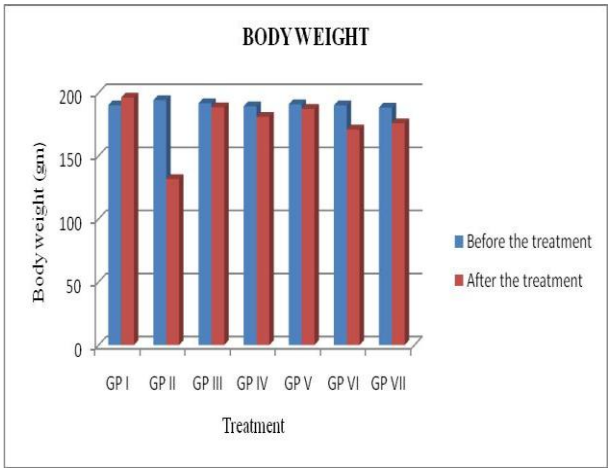

Fig. 5.2: Effect of *Searsia mysorensis* on body weight on alloxan treated diabetic rats.

5.3.3 Bio chemical Parameters

Rats treated with alloxan developed a significant diabetic stage observed as elevated serum enzymes like TC, TGs, LDL and VLDL when compared to normal control and HDL level where decreased. Treatment with Glibenclamide, ethanol and aqueous extracts showed a significant dose dependent beneficial effect on lipid profile.

Table 5.4: Effect of *Searsia mysorensis* on lipid profile on alloxan induced diabetic albino rats.

Group	Biochemical parameters(mg/dl)				
	TG	TC	HDL	LDL	VLDL
GP I	83.5±1.54	132.15±4.36	55.33±2.83	32.16±1.70	18.83±1.30
GP II	143.33±1.85	269.16±5.02	34.66±1.60	62.66±1.58	40.83±1.01
GP III	87.5±2.64***	149.83±3.85***	47.16±1.16***	34.33±1.43***	22.16±0.90***
GP IV	134.16±1.44**	253.16±2.98**	38.83±1.01**	47.83±1.01**	35.16±1.16**
GP V	97.33±2.67***	169.16±2.56***	42.83±1.37***	36.66±1.202***	26.16±1.32***
GP VI	136.83±1.53*	255.16±2.63**	32.16±1.27**	54.5±0.99**	36.83±1.30**
GP VII	102.16±2.15***	173.16±2.25***	41.66±1.08***	40.83±1.10***	28.33±0.80***

Values are expressed as mean ± SEM (n=6)

One way ANOVA followed by Tukey-Kramer mulptiple test.Where, * epresents significant at p<0.05, ** represents moderate significant at p< 0.01 and *** presents highly significant at p<0.001.

A) Effect on serum triglyceride (serum TG) level

Rats treated with Alloxan (120 mg/kg, i.p.) for 3 days had serum TG level of (143.33±1.85 mg/dl) measured on day 21. This significantly higher (P<0.001) when compared to serum TG levels in normal control rats (83.5±1.544).Alloxan rats treated with standard (Glibenclamide 1 mg/kg) had serum level of 87.5±2.643 mg/dl when measured on day 21. This was significantly

lower (P<0.001) when compared to the serum TG levels in Alloxan control rats (143.33±1.85 mg/dl).

Alloxan rats treated with Ethanol extract of R.M. (250 and 500 mg/kg, p.o., once daily) had serum level of 134.16±1.44 and 97.83±2.679 mg/dl respectively when measured on day 21.This was significantly lower (P<0.001) when compared to the serum TG levels in Alloxan control rats (143.33±1.85 mg/dl).

Alloxan rats treated with aqueous extract of R.M. (250 and 500 mg/kg, p.o, once daily) had serum TG level of 136.83±1.53 and 102.16±2.151 respectively when measured on day 21. These values were significantly (P<0.001) and (P<0.001) lower when compared to the serum TG level in Alloxan control rats (143.33±1.85 mg/dl)

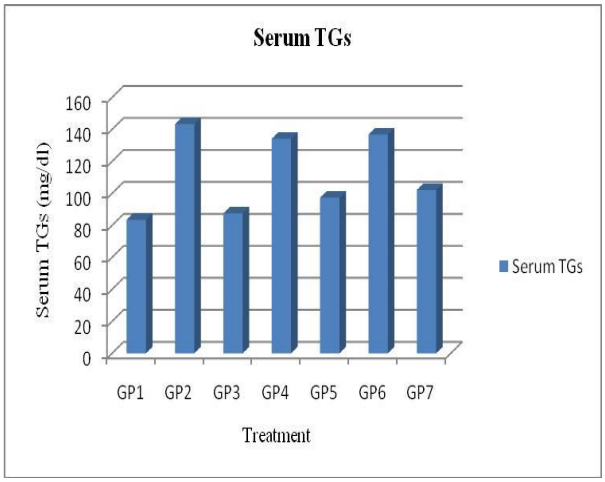

Fig. 5.3: Effect on Serum TG level on alloxan treated diabetic rats.

B) Effect on serum total cholesterol (serum TC) level

Rats treated with Alloxan (120 mg/kg, i.p., for 3 days had serum TC level of (269.16±5.029 mg/dl) measured on day 21. This significantly higher (P<0.001) when compared to serum TC levels in normal control rats (132.5±4.36).

Alloxan rats treated with standard (Glibenclamide 1 mg/kg) had serum TC level of 149.83±3.85 when measured on day 21. This was significantly lower (P<0.001) when compared to the serum TC levels in Alloxan control rats (269.16±5.029 mg/dl).

Alloxan rats treated with Ethanol extract of R.M. (250 and 500 mg/kg, p.o., once daily) had serum TC level of 253.16±2.98 and 169.16±2.561 mg/dl respectively when measured on day 21. This was significantly lower (P<0.001) when compared to the serum TC levels in Alloxan control rats (269.16±5.029 mg/dl).

Alloxan rats treated with aqueous extract of R.M. (250 and 500 mg/kg, p.o, once daily) had serum TC level of 255.16±2.63 and 173.16±2.25 respectively when measured on day 21. These values were significantly (P<0.001) and (P<0.001) lower when compared to the serum TC level in Alloxan control rats (269.16±5.029 mg/dl).

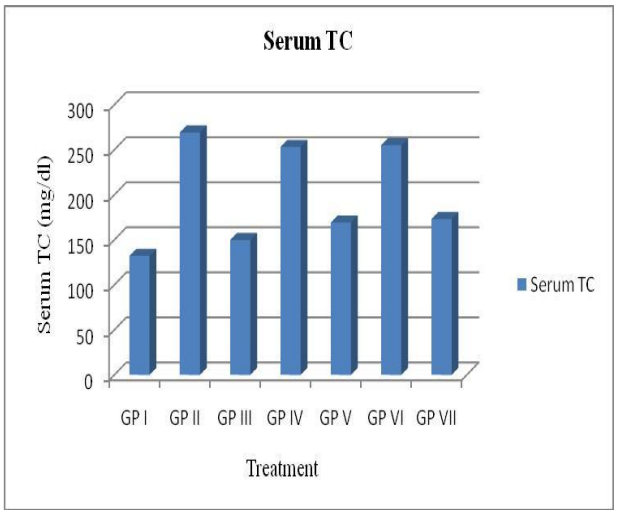

Fig. 5.4: Effect on Serum TC level on alloxan treated diabetic rats.

C) Effect on serum HDL cholesterol (serum HDL-C) level

Rats treated with Alloxan (120 mg/kg, i.p., for 3 days had serum HDL level of (34.66±1.606 mg/dl) measured on day 21. This significantly lower (P<0.001) when compared to serum HDL levels in normal control rats (55.83±2.836).

Alloxan rats treated with standard (Glibenclamide 1 mg/kg) had serum HDL level of 47.16±1.167 when measured on day 21. This was significantly higher

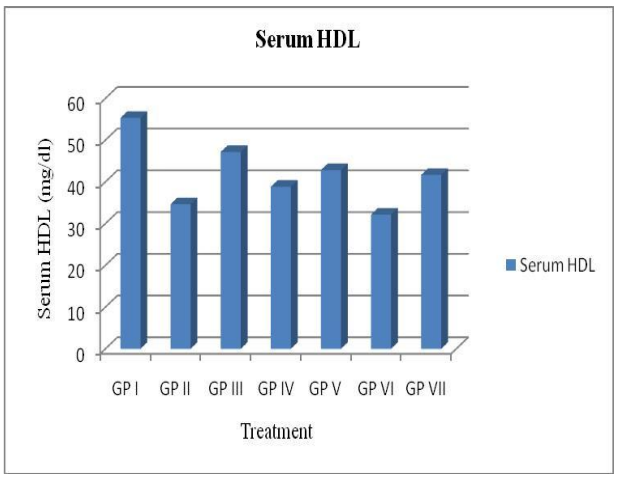

Fig. 5.5 Effect on Serum HDL level on alloxan treated diabetic rats.

(P<0.001) when compared to the serum HDL levels in Alloxan control rats (34.66±1.606 mg/dl).

Alloxan rats treated with Ethanol extract of R.M. (250 and 500 mg/kg, p.o., once daily) had serum HDL level of 38.83±1.014 mg/dl and 42.83±1.376 respectively when

measured on day 21. This was significantly higher (P<0.001) when compared to the serum HDL levels in Alloxan control rats (34.66±1.606 mg/dl).

Alloxan rats treated with aqueous extract of R.M. (250 and 500 mg/kg, p.o, once daily) had serum HDL level of 32.16±1.276 and 41.66±1.085 respectively when measured on day 21. These values were significantly (P<0.001) and (P<0.001) higher when compared to the serum TC level in Alloxan control rats (269.16±5.029 mg/dl)

D) Effect on serum LDL cholesterol (serum LDL-C) level

Rats treated with Alloxan (120 mg/kg, i.p., for 3 days had serum LDL level of (62.66±1.585 mg/dl) measured on day 21. This significantly higher (P<0.001) when compared to serum LDL levels in normal control rats (32.16±1.701).

Alloxan rats treated with standard (Glibenclamide 1 mg/kg) had serum LDL level of (34.33±1.43 mg/dl) when measured on day 21. This was significantly lower (P<0.001) when compared to the serum LDL levels in Alloxan control rats (62.66±1.585 mg/dl).

Alloxan rats treated with Ethanol extract of R.M. (250 and 500 mg/kg, p.o., once daily) had serum LDL level of 47.83±1.014 and 36.66±1.202 mg/dl respectively when measured on day 21. This was significantly lower (P<0.001) when compared to the serum LDL levels in Alloxan control rats (62.66±1.585 mg/dl).

Alloxan rats treated with aqueous extract of R.M. (250 and 500 mg/kg, p.o, once daily) had serum LDL level of 54.5±0.99 and 40.83±1.108 respectively when measured on day 21. These values were significantly (P<0.001) and (P<0.001) lower when compared to the serum LDL level in Alloxan control rats (62.66±1.585 mg/dl)

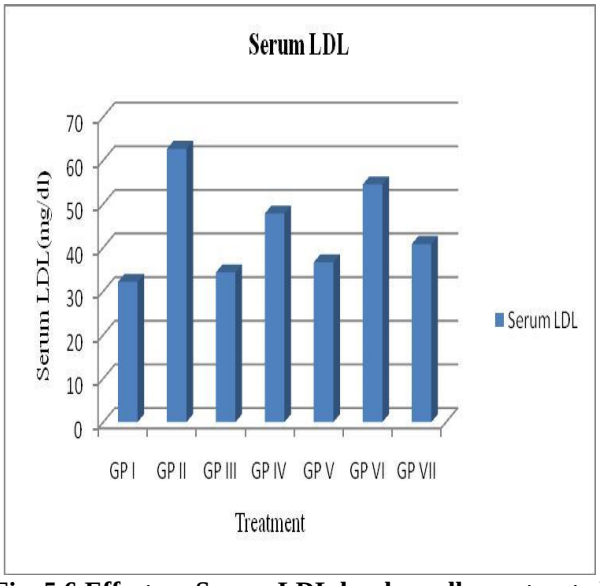

Fig. 5.6 Effect on Serum LDL level on alloxan treated diabetic rats.

E) Effect on serum VLDL cholesterol (serum VLDL-C) level

Rats treated with Alloxan (120 mg/kg, i.p., for 3 days had serum VLDL level of (40.83±1.014 mg/dl) measured on day 21. This significantly higher (P<0.001) when compared to serum VLDL levels in normal control rats (18.83±1.302).

Alloxan rats treated with standard (Glibenclamide 1mg/kg) had serum VLDL level of (22.16±0.90 mg/dl) when measured on day 21. This was significantly lower (P<0.001) when compared to the serum VLDL levels in Alloxan control rats (40.83±1.014 mg/dl) Alloxan rats treated with Ethanol extract of R.M. (250 and 500 mg/kg, p.o., once daily) had serum VLDL level of 35.16±1.167 and 26.16±1.327 mg/dl respectively when measured on day 21. This was significantly lower (P<0.001) when compared to the serum VLDL levels in Alloxan control rats (40.83±1.014 mg/dl) Alloxan rats treated with aqueous extract of R.M. (250 and 500 mg/kg, p.o, once daily) had serum VLDL level of 36.83±1.302 and 28.33±0.802 respectively when measured on day 21. These values were significantly (P<0.001) and (P<0.001) lower when compared to the serum VLDL level in Alloxan control rats (40.83±1.014 mg/dl).

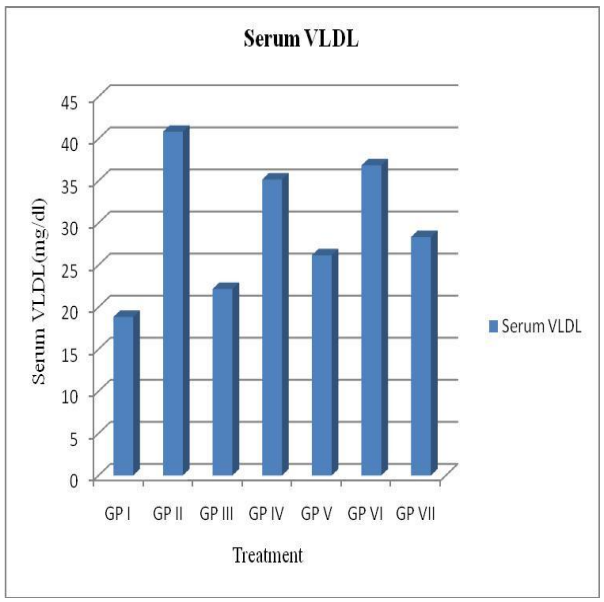

Fig. 5.7 Effect on Serum VLDL level on Alloxan treated diabetic rats.

DISCUSSION

The present investigation reports the anti-diabetic activity of *Searsia mysorensis* on alloxan induced diabetic albino rats. Evaluation of antidiabetic activity using Alloxan induced hyperglycemia model has been described by Dash et al. Intraperitoneal administration of alloxan (120 mg/kg of body weight) effectively induced diabetes mellitus in normal rats as reflected by glycosuria, hyperglycaemia, polyphagia, polydipsia and body weight loss compared with normal rats.

The aim for the present work is to explore the scientific basis of the utility of the ethanol and aqueous extract of *Searsia mysorensis* for correction of hyperglycemia and hyperlipidemia in diabetes mellitus.

The ethanol and aqueous extract of *Searsia mysorensis* was screened to explore the scientific basis of its utility for correction of biochemical changes in Alloxan induced diabetic rats. Despite the folk medicine use, so far there have been no studies on its anti-diabetic effect. However, presence of triterpenoids[51] which possess hypoglycemic and anti-hyperglycemic properties, has been reported in literature.[52] These compounds has been implicated in the anti diabetics activities of many plants.[53] Models of experimental diabetes that utilizes diabetogenic agent Alloxan induced blood glucose levels higher than 250 mg/dl[54] which have been considered as severe diabetes.Glibenclamide is a standard anti-diabetic drug used. It has been involved in stimulating insulin secretion from pancreatic β-cells principally by inhibiting the ATP sensitive K_{ATP} channels.

In this study effect of *Searsia mysorensis* on hyperglycemia is evaluated in alloxan-induced diabetic rats. It was found that the blood glucose levels (BG) of the animals which are treated with *Searsia mysorensis* (Groups IV, V, VII and VII) and the standard drug glibenclamide (Group-III), significantly reduces when compared with diabetic control (untreated) group. The BG of all groups was observed on 1st, 7th, 14th, and 21st day. The diabetic rats which treated with *Searsia mysorensis* and glibenclamide showed a significant decrease in blood glucose level on 1st, 7th, 14th, and 21st day. On 21st day BG of Group-III decreases nearly to normal range. In the case of Group-IV, V, VI and VII animals BG significantly reduces, but it was some less when compared to Group-III. When compared with untreated group, the *Searsia mysorensis* at the dose of 250 & 500 mg/kg significantly reduces the hyperglycemia. These results give us the suggestion that *Searsia mysorensis* having significant hypoglycemic effect.

The possible mechanism includes the stimulation of β-cells and subsequent release of insulin and activation of the insulin receptors. The plant's antihyperglyacemic action may be by potentiation of pancreatic secretion of insulin, which was clearly evidenced by the increased level of insulin in diabetic rats treated with *Searsia mysorensis*.

Dehydration and loss of body weight have been associated with diabetes mellitus.[55] In diabetic rats, increased food consumption and decreased body weight were observed. This indicates the polyphagic condition and loss of weight due to excessive breakdown of tissue proteins. The decrease in body weight in diabetic rats could be due to dehydration and catabolism of fats and proteins.[56] Increased catabolic reactions leading to muscle wasting might also be the cause for the reduced weight gain by diabetic rats.[57] Oral administration of *Searsia mysorensis* extract for 21 consecutive days to diabetic rats decreased their food consumption and improved body weight. This could be due to a better control of the hyperglycaemic state in the diabetic rats. Decreased BG could improve body weight in alloxan-induced diabetic rats.[58,59]

Diabetes mellitus is also associated with hyperlipidemia with profound alteration in the concentration and composition of lipid.[60] Changes in the concentrations of the lipid with diabetes mellitus contribute to the development of vascular disease.[61,62] Fatty acids, an important component of cell membranes, are eicosanoid precursors and are therefore required for both the structure and function of every cell in the body.[63] Alloxan significantly increased TC, TG, LDL, VLDL and decreases the HDL levels. The abnormally high concentration of serum lipids in diabetes mellitus is mainly due to an increase in the mobilization of free fatty acids from the peripheral fat depots, since insulin inhibits the hormone sensitive lipase. The marked hyperlipidaemia that characterises the diabetic state may therefore be regarded as a consequence of the uninhibited actions of lipolytic hormones on the fat depots.[64] Excess of fatty acids in plasma produced by alloxan promotes the liver conversion of some fatty acids to phospholipids and cholesterol. These two substances, along with excess of TG formed in the liver, may be discharged into lipoproteins in the blood.[65] As a result, serum phospholipids is elevated. Administration of *Searsia mysorensis* to diabetic rats reversed all the above mentioned changes and improved the HDL levels. The results of the present investigation clearly indicate that the *Rhus mysorensis* has a glucose lowering effect on alloxan-induced diabetic rats. It was also found to be highly effective in managing the complications associated with diabetes mellitus, such as body weight maintenance and hyperlipidaemia and prevents the defects in lipid metabolism. Therefore *Searsia mysorensis* showed therapeutic promise as a protective agent against the development and progression of atherosclerosis and possible related cardiovascular complications in diabetes mellitus.

Hypertriglyceridemia is a common finding in patients with diabetes mellitus and is responsible for vascular Complications. The deficiency of lipoprotein lipase (LPL) activity may contribute significantly to the elevation of triglycerides in diabetes. In study administration of *Searsia mysorensis* to diabetic rats significantly improved these parameters. Table: 5.4 shows that decrease in the TG, Total cholesterol level in dose dependent in manner.

The observed hypolipidemic effect may be because of decreased cholesterogenesis and fatty acids. Significantly lowering the total cholesterol and raise in HDL Cholesterol is a very desirable biochemical status for prevention of atherosclerosis and ischemic conditions.

Table: 5.4 shows that administration of *Searsia mysorensis* extract increases the HDL level in treated group significantly in dose deponent manner.

The ability of LDL-cholesterol to form lipid peroxides was found specifically responsible for the atherogenesis in diabetic patients. Oral administration of *Rhus mysorensis* extract normalized these effects its shows the Table: 5.4 as compared to control Thus, we could say that *Rhus mysorensis* had a beneficial effect on carbohydrate metabolism in diabetic rats. The hypoglycemic effect of *Rhus mysorensis* may be due to potentiating the insulin activity either by increasing the pancreatic secretion of insulin from cells of islets of langerhans or its release from bound insulin.[66] Treatment of diabetic rats with *Searsia mysorensis* had shown lipid profile values and activities of marker enzymes near to normal level. So, oral administration *Searsia mysorensis* (250 and 500 mg/kg. p.o) once daily showed significant reduction of blood glucose.

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