

Assessment of various Biological Activities of *Hibiscus rosa-sinensis* Root Extract in Alloxan Induced Diabetic Rats

Mohan Pethe^{1*}, R.K. Gupta², Smita Manchalwar³ and Vijay Gujar¹

¹Assistant Professor, ²Ex Profesoor and Head, ³Tutor, Department of Pharmacology, Mahatma Gandhi Institute of Medical Sciences, Sewagram, Wardha, Maharashtra, India- 442102

***Correspondence Info:**

Dr. Mohan Pethe

Department of Pharmacology,

Mahatma Gandhi Institute of Medical Sciences,

Sewagram, Wardha, Maharashtra, India- 442102

E-mail: drpethemohan@gmail.com

Abstract

Aim and Objective: The present study was designed to assess antidiabetic, hypolipidemic, antioxidant and histopathological effects of hydroalcoholic extract of *Hibiscus rosa-sinensis* (EHBS) root on alloxan-induced diabetes in rats.

Materials and Methods: Study was conducted on six groups with six wistar rats in each group for the period of 4 weeks. Group I: served as normal control (NC), rats administered with gum acacia 1 ml daily, group II: consider as diabetic control (DC) treated with alloxan 150 mg/kg body wt. Whereas *Hibiscus rosa-sinensis* roots extract was given orally in group III (DE1), group IV (DE2), group V (DE3) at doses of 50, 100 and 200 mg/kg body weight dissolved in distilled water respectively. Group VI (DG) was given glibenclamide (5 mg/kg) as a standard drug and results were compared in reference to it.

Results: Administration of root extract for 28 days resulted in significant decreased in the levels of blood glucose, plasma lipids but increases in HDL levels, also significant decrease in plasma MDA level as well as increased in SOD level in a dose dependent manner. Histological examination of pancreas has shown increase in diameter as well as number of beta cells which indicates the proliferation with regeneration of beta cells after 28 day treatment with root extract of HRS in rats. Root extract of hibiscus is more efficacious in antidiabetic, hypolipidemic and antioxidant activity than glibenclamide.

Conclusion: Root extract of HRS may be a safe and better alternative available over the antidiabetic agents with minimal adverse effects. Before establishing it as an antidiabetic agent for human use, clinical trial will be required.

Keywords: Alloxan induced diabetes; Wistar rats; *Hibiscus rosa sinensis*; Glibenclamide.

1. Introduction

Diabetes mellitus (DM), a global public health problem, is now emerging as an epidemic world over. According to a widely accepted estimation, the number of diabetic patients would reach 366 million by the year 2030. India now has the world's largest diabetic population, encompassing an estimated 35 million people out of an overall population of 1 billion. In just over 20 years (i.e. 2025) the country will have almost 200 million people (approximately 15% of the population) affected by diabetes or its precursor [1]. Diabetes mellitus is an endocrinal disorder with depleted insulin secretions, damaged pancreatic β -cells with altered carbohydrate, lipid and protein metabolism and additionally increased risk of complications of various vascular diseases [2]. The aging populations, consumption of calorie-rich diets,

obesity and sedentary lifestyles have led to a tremendous increase in the number of individuals with diabetes worldwide. Hyperlipidemia associated atherosclerosis is the most common causes of death in diabetes [3]. Long standing DM is associated with complications like neuropathy, nephropathy and retinopathy which have significant economical impact on individual, family, health system and country.

Though various treatment options are available in the form of insulin, sulphonylurea, biguanides, thiazolidinediones and meglitinides, none of the groups of drugs provide the permanent cure for diabetes [4]. At the same time these drugs may be associated with undesirable adverse effects, patient non-compliance, drugs resistance and disease relapse. Some drugs may be expensive and

troublesome to take like daily injection of insulin. Hence, the need to search newer and better drugs continues. People all over the world are looking towards alternative systems of medicine among which herbal medicines are used which are non toxic, cost effective and equi-effective in comparison to allopathic drugs.

Considering the above fact, the plants HRS was selected for the present study. HRS is commonly known as Jaswand in Marathi, gudhal in Hindi, belong to the family malvaceae. HRS has been claimed to be useful in many common ailments in traditional medicine. It has been investigated for various biological activities like antifertility, anti-implantation, antiestrogenic, abortifacients, analgesic, antipyretic, antiinflammatory, anticonvulsive, antifungal, antiviral and hair growth activity [5]. Though it has been investigated for its hypoglycemic effect but very few studies have been reported.

The literature is scanty on antidiabetic aspect of roots of *Hibiscus rosa-sinensis* and moreover the mechanism of antidiabetic activity has not been explored. Therefore, it is found of interest to study the antidiabetic effect, as well as effect on the lipid profile, oxidative stress and histopathology of beta cell of pancreas by using rats as experimental animal.

2. Materials and Methods

After obtaining Institutional Animal Ethics Committee (IAEC) approval, this study was conducted on six groups with six wistar rats in each group for the period of 4 weeks. For the study purpose fresh *Hibiscus rosa-sinensis* roots were collected from the central garden of medical college, in the month of December to February. The botanical identity of plant was confirmed and authenticated by taxonomist. Animals used in this study were albino rats of wistar strain, weighing between 150-250 g of either sex. Wistar rats were procured from local breeder and kept in animal house of the department for 7 days for adaptation in the new environment before subjecting them to the experiment. Animals were fed on pellet diet. Rats were maintained under standard laboratory conditions of temperature 25-26°C, relative humidity 50-70% and light and dark cycles of 12 hours each. Food and water was provided *ad-libitum*.

The collected roots were cleaned, dried under shade, powdered and stored in a separate airtight container until it was used for the preparation of the extract. Forty gram of dried roots was macerated separately in 95 % of ethanol overnight. Then it was packed in the tumbler of Soxhlet and was extracted using 95 % ethanol refluxing at 60-80°C yielded an extract which was reddish brown semi solid, from 40 g of dried powder 8 g extract was yielded. The stock of 30 gm of root extract was preserved in

airtight container separately and kept inside the refrigerator.

The diabetes was induced by intraperitoneal injection of alloxan (150 mg/kg) in rats. Since alloxan is capable of producing fatal hypoglycemia as a result of massive pancreatic insulin release, animals were treated with 30% glucose solution orally at different time intervals after six hours of alloxan induction, and 5% glucose solution was kept in bottles in their cages for the next 24 hr to prevent hypoglycemia. Fasting blood glucose (FBS) was recorded daily morning at 9.00 am for one week. Animals developed stable hyperglycemia after 4-5 days. Only those animals with blood glucose >250 mg/dl were selected for the study. Doses (50, 100, 200 mg/kg orally) for the study were selected on trial and error basis. Food and water were provided *ad libitum* to the animals.

Overnight fasted rats were divided into six groups of six rats in each. Group I: served as normal control (NC), administered with distilled water 1ml daily, Group II: consider as diabetic control (DC) treated with alloxan 150 mg/kg body weight whereas *Hibiscus rosa-sinensis* root extract was given orally in group III (DE1), group IV (DE2), group V (DE3) at doses of 50,100 and 200 mg/kg body weight dissolved in distilled water respectively. Group VI (DG) was given glibenclamide at a dose of 5 mg/kg orally. Study was conducted for the total 28 day (4 week).

2.1 Collection of the blood and estimation of serum glucose

Blood was withdrawn from the retro orbital sinus under ether inhalation anesthesia and glucose levels were estimated at interval of 0th, 1st, 3rd, 7th, 14th, and 28th day, using a glucose oxidase-peroxidase reactive strips and a prestige IQ blood glucometer (manufactured by Home diagnostics inc USA)

2.2 Estimation of lipid profile

On day 28, estimation of lipid profile was done by using autoanalyzers (Random Access Analyzer model no: Erba XL 300 provided by Trans Asia Ltd Mumbai) and low density lipoprotein (LDL), very low density lipoprotein (VLDL) values were calculated by Friedewalds formula [6].

2.3 Estimation of antioxidant activity

On day 28th estimation of superoxide dismutase (SOD) and plasma malondialdehyde (MDA) was done [7,8].

2.4 Histopathological studies

Pancreatic tissues from all groups were subjected to histopathological studies. The whole pancreas from each animal was removed on 28 day after sacrificing the animal under anaesthesia and was collected in 10% formalin solution and immediately processed by the paraffin technique. Sections of 5 µm thickness were cut

and stained by hematoxylin and eosin (H and E) for histological examination [9].

2.5 Acute Toxicity study

Acute oral toxicity study for the test extract of the plant was carried out using OECD/OCED guideline 425. The test procedure minimizes the number of animals required to estimate the oral acute toxicity. The test also allows the observation of signs of toxicity and can also be used to identify chemicals that are likely to have low toxicity [7]. All groups were given hydroalcoholic extract of roots of *Hibiscus rosa-sinensis* (HRS) in graded doses of 100, 200, 400, 800, 1000 and 2000 mg/kg body wt. The single dose of extract of roots of HRS was administered orally after overnight fasting. The animals were observed continuously for 30 min, 1 hrs, 24 hrs and then occasionally for further 14th day for any toxic effect of extract. No animal died. Therefore, the LD₅₀ is greater than 2000 mg/kg [7].

An investigation with 1/40th, 1/20th, 1/10th, and 1/5th of 2000 mg/kg, i.e. 50, 100, 200, and 400 mg was done in pre-screening, hence this dose was used in final screening.

2.6 Statistical Analysis

The statistical analysis was done using one way analysis of variance (ANOVA) using student's t test. P value < 0.05 is statistically significant.

3. Observations and Results

3.1 Preliminary normoglycemic study

In non-diabetic rats, root extract administered in daily doses 100 and 200 mg/kg for 28 day, did not show any change in the baseline blood glucose level and normal lipid level. However, MDA level was decreased and SOD levels was increased significantly after 28 day of drug treatment (Table 1).

Table 1: Effect of hydroalcoholic roots extract of *Hibiscus rosa-sinensis* (REHRS) on blood sugar, Lipid profile, MDA & SOD level in normal rats

Groups N=6	Drugs	Blood sugar (mg/dl) mean±SD		Lipid profile mean±SD					MDA nmol/ml mean±SD	SOD Units/dl mean±SD
		On day 0	On day28	TC (mg/dl)	TG (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	HDL (mg/dl)		
GR I normal	DW 10 ml/kg	63.2 ±9.16	61.3 ±5.23	86.16 ±25.22	105.13 ±21.22	65.33 ±22.35	23.19 ±8.15	11.33 ±6.21	3.44 ±0.069	3.44 ±0.089
GR II NDC	REHRS 100 mg/kg	65.8 ±7.16	67.7 ±9.26	83.26 ±25.12	104.21 ±33.24	61.23 ±15.25	22.14 ±7.33	11.61 ±7.12	3.24 ±0.151	4.02 ±0.069
GR III NDC	REHRS 200 mg/kg	63.3 ±6.11	62.3 ±5.33	84.26 ±25.42	101.13 ±33.24	59.33 ±15.35	19.44 ±9.76	11.21 ±8.41	2.984 ±0.029*	5.01 ±0.094*

* p<0.05 as compared to normal GRI, NDC-non diabetic control, DW-distill water, TC-total cholesterol, TG- triglycerides, LDL-low density lipoprotein, HDL-high density lipoprotein, VLDL-very low density lipoprotein.

3.2 Effect of root extract (HEFHR) on Body weight

In normal control group (I) there was progressive increase in the weight over the 28 day period. In Alloxan treated group (II) there was progressive fall in weight over the 28 day period, weight loss was statistical significant in this group from 3rd day of treatment onwards. In group III there was decrease in weight initially for first 14 days. However on day 28 slightly increase in weight was seen (not significant). In group IV initial weight loss was seen until day 7, followed by increase in weight. Weight in this

group was significantly high compared to group II (Diabetic control) at 28th day. In group V initial weight loss was seen up to day 3 followed by weight gain. This weight gain was significant on day 14 and highly significant on day 28 compared to group II (Diabetic group). In group VI effect on weight was comparable to group V. Weight loss in this group was seen for first 3 day followed by weight gain, this weight gain was significant on day 14 and highly significant on day 28 (Table 2).

Table 2: Effect of hydroalcoholic roots extract of *Hibiscus rosa-sinensis* (REHRS) on weight in rats

GR n=6	Drugs & Dose	Weight of rats (gm) Mean ± SD					
		Before T/t	After drug administration (ADA)				28 day
			24 hr	72 hr	7 day	14 day	
I	DW 10 ²	176.6±10.32	178.33±7.52	181.6± 7.52	186.6±7.52	195±8.36	205±8.36
II	Alloxan 150 ¹	178.3±13.29	175.83±10.20 [#]	166.6±10.80 ^{##}	159.1±11.58 ^{##}	154.1±11.58 ^{##}	143.3±10.32 ^{##}
III	Alloxan 150 ¹ +REHRS 50 ²	169.1±8.010	169.16±8.61	160±7.74	150 ± 4.91	148.6± 5.16	150.6± 4.08
IV	Alloxan 150 ¹ +REHRS 100 ²	183.3±8.75	180.66±10.80	178.3±8.16	171.8± 9.70	180.8± 9.70 ^{**}	190.8± 8.01 ^{**}
V	Alloxan 150 ¹ +REHRS 200 ²	180±8.23	163.33±5.16	160±5.47	160.8± 2.04	169.1 ± 2.04 [*]	180 ±3.16 ^{***}
VI	Alloxan 150 ¹ +gliben 5 ²	185±10.48	183.5±12.14	180.5±12.14	182.5 ± 9.87	185.3± 6.83 [*]	190.8 ±7.35 ^{***}

GR-groups, ¹-mg/kg body wt i.p; ²-mg/kg body wt orally, DW-distil water; [#] P<0.05 compared to DW group. ^{*}p<0.05 compared to Alloxan group; ^{##} p<0.001 compared to DW; ^{**}p<0.001 compared to Alloxan group

3.4 Effect of root extract (REHRS) on blood sugar level

There was no significant difference in the baseline blood sugar level in all six groups. In group II, III, IV, V, and VI after administration of alloxan, there was significant increase in blood sugar level after 48 hr. In group II (Diabetic control) there was significant increase in blood sugar level throughout 28 days. In group III, IV and V treated with REHRS in graded doses (50,100,200 mg/kg) there was decrease in blood sugar level. This

decrease was significant in group III on 28 day, in group IV from day 14 onwards and group V from day 7 onward compared to group II (Diabetic control). Blood sugar level in group V almost reach to group I level on day 28. In group VI there was highly significant (sudden) fall in blood sugar level from first day of treatment compared to group II. This blood sugar level in group VI remains almost stable up to 28 day (Table 3).

Table 3: Effect of hydroalcoholic roots extract of *Hibiscus rosa-sinensis* (REHRS) on blood sugar in rats

GR n=6	Drugs & Dose	Blood sugar level (mg/dl)						
		Before		After drug administration (ADA)				
		Baseline	48 hr after alloxan	24 hr	72 hr	7 day	14 day	28 day
I	DW 10 ²	63±8.33	NA	65.66 ± 2.42	63.83 ± 4.99	62.5±4.76	62±7.771	66.83±5.26
II	Alloxan 150 ¹	64 ± 12 ^{##}	404.83±15.45 ^{##}	421.16±31.8 ^{##}	420.33±20.63 ^{##}	421±27.38 ^{##}	468.66±21.80 ^{##}	429.8±21.34 ^{##}
III	Alloxan 150 ¹ +REHRS 50 ²	66±5.32	397.66 ± 13.58	399.66±13.09	416.83±14.27	406.3±18.33	378±19.74 [*]	186.33±19.42 ^{**}
IV	Alloxan 150 ¹ +REHRS 100 ²	63.66±4.76	419.5±22.31	427.33±22.15	406.66 ± 17.22	397.83±18.25 [*]	260 ± 29.25 ^{**}	131.33±11.57 ^{**}
V	Alloxan 150 ¹ +REHRS 200 ²	64.16±4.02	436.83±46.13	431.83±49.23	404.83±48.33 [*]	288.33±21.34 ^{**}	188.16 ± 8.32 ^{**}	93 ± 6.928 ^{**}
VI	Alloxan 150 ¹ +gliben 5 ²	64.16±4.02	405.33±32.54	130.83±75.34 ^{**}	126±44.15 ^{**}	118.66±16.02 ^{**}	102.83±16.58 ^{**}	90.32 ± 8.94 ^{**}

GR-groups, 1-mg/kg body wt i.p.; 2-mg/kg body wt orally; DW-distil water; # P<0.05 compared to DW group; *p<0.05 compared to Alloxan group; ## p<0.001 compared to DW; **p<0.001 compared to Alloxan group.

3.5 Effect of root extract (REHRS) on lipid profile

Estimation of lipid profile in all groups was done on 28th day. In group II TC, TG, LDL, & VLDL were highly significantly increased and HDL was highly significantly decreased as compared to normal group I. In group III, IV, V treated with REHRS, significant decreased in TC, TG, LDL, VLDL and significant increased in HDL levels was

seen as compared to group II. In group V all changes were highly significant compared to group II and even better than group I. In group VI also shown significant decreased in TC, TG, LDL, VLDL and increase in HDL compared to group II. The lipid parameter in group IV and VI were almost the same and were almost similar to group I, (Table 4).

Table 4: Effect of hydroalcoholic roots extract of *Hibiscus rosa-sinensis* (REHRS) on lipid profile in rats

GR n=6	Drug	TOT CHOLE mean±SD	HDLc mean±SD	TG mean±SD	LDL mean±SD	VLDLc mean±SD
I	DW 10 ²	92.66±7.033	14.16±1.16	118.33±9.85	55.83±2.041	23±1.414
II	Alloxan 150 ¹	362.16±19.36 ^{##}	7.5±1.048 ^{##}	550.16±40.05 ^{##}	145±18.77 ^{##}	110.16±17.13 ^{##}
III	Alloxan 150 ¹ +REHRS 50 ²	117.5±11.72 ^{**}	11.66±1.75 ^{**}	130.33±11.74 ^{**}	82.66±1.21 ^{**}	26.83±1.94 ^{**}
IV	Alloxan 150 ¹ +REHRS 100 ²	97.5±12.95 ^{**}	13.33±0.516 ^{**}	127.33±9.87 ^{**}	59.16±3.92 ^{**}	25.41±2.529 ^{**}
V	Alloxan 150 ¹ +REHRS 200 ²	77.16±3.54 ^{**}	17.16±2.22 ^{**}	89.16±7.33 ^{**}	42.16±3.60 ^{**}	18.16±1.94 ^{**}
VI	Alloxan 150 ¹ +gliben 5 ²	97.66±43.36 ^{**}	13.5±1.04 ^{**}	135.5±4.13 ^{**}	57.16±1.94 ^{**}	27.1±2.07 ^{**}

GR-groups, 1-mg/kg body wt i.p.; 2-mg/kg body wt orally; DW-distil water; # P<0.05 compared to DW group; *p<0.05 compared to Alloxan group; ## p<0.001 compared to DW; **p<0.001 compared to Alloxan group

3.6 Effect of root extract (REHRS) on antioxidant activity

Estimation of MDA and SOD was done on day 28th, in group II plasma MDA level was significantly increases as compared group I. In group III, IV and V treated with REHRS (50,100,200) in graded dose, dose dependent significant decrease in plasma MDA level was seen. In group V MDA level was highly significantly decrease compared to group II as was even lower than group I, in group VI also plasma MDA level were significantly lower compared to group II. In group IV and VI plasma MDA level were almost same as group I.

Plasma SOD level was highly significant decrease in group II compared to group I. In group III, IV and V treated with REHRS (50,100,200) in graded dose, dose dependent significant increase plasma SOD level was seen. In group IV and V plasma SOD level was highly significantly increase compared to group II and was even higher than group I. In group VI also plasma SOD level were significantly higher compared to group II and were almost same as group I (Table 5).

Table 5: Effect of hydroalcoholic roots extract of *Hibiscus rosa-sinensis* (REHRS) on melanoaldehyde (MDA) and super oxides dismutase (SOD) in rats

Group (n=6)	Drug & Dose	Plasma MDA level (Mean ± SD) (nmol/ml)	Plasma SOD level Mean ± SD (Units/ml)
GR I	DW 10 ²	4.331 ± 0.069	3.22 ± 0.109
GR II	Alloxan 150 ¹	7.338 ± 0.433 ^{##}	1.66 ± 0.046 ^{##}
GR III	Alloxan 150 ¹ +REHRS 50 ²	5.301 ± 0.504 ^{**}	3.96 ± 0.114 ^{**}
GR IV	Alloxan 150 ¹ +REHRS 100 ²	4.510 ± 0.470 ^{**}	4.56 ± 0.123 ^{**}
GR V	Alloxan 150 ¹ +REHRS 200 ²	3.025 ± 0.306 ^{**}	5.07 ± 0.122 ^{**}
GR VI	Alloxan 150 ¹ +gliben 5 ²	4.708 ± 0.503 ^{**}	3.06 ± 0.078 ^{**}

GR-groups, 1-mg/kg body wt i.p., 2-mg/kg body wt orally; DW-distil water; # P<0.05 compared to DW group; *p<0.05 compared to Alloxan group; ## p<0.001 compared to DW; **p<0.001 compared to Alloxan group

3.7 Histological examination of pancreas

The histological examination of pancreas after 28 day treatment with root extract of HRS showing regular, normal appearance of nucleus, tightly arranged islet cells were observed in group I (normal control). In group II histomorphometrical analysis showed that alloxan caused significantly decreased in number and size of islets cell, there was extensive damage of the islets and they appeared to be irregular and the necrosis and atrophy were significantly seen in the diabetic group compared to the normal control group. Photomicrographs of group III, IV

and V treated with HEFHR in graded dose, (50, 100, 200 mg/kg) indicated that the size of islets was significantly increased and the necrosis and atrophy of islets were significantly improved; also increase in the number and diameter of the cell islets appeared to be regular as compared to the diabetic group, which may be a sign of regeneration. Photomicrographs of standard (glibenclamide 5mg/kg) treated group VI rat showing moderate expansion and restoration of normal cellular population size of islets with hyperplasia by glibenclamide, stained with H and E (fig 1).

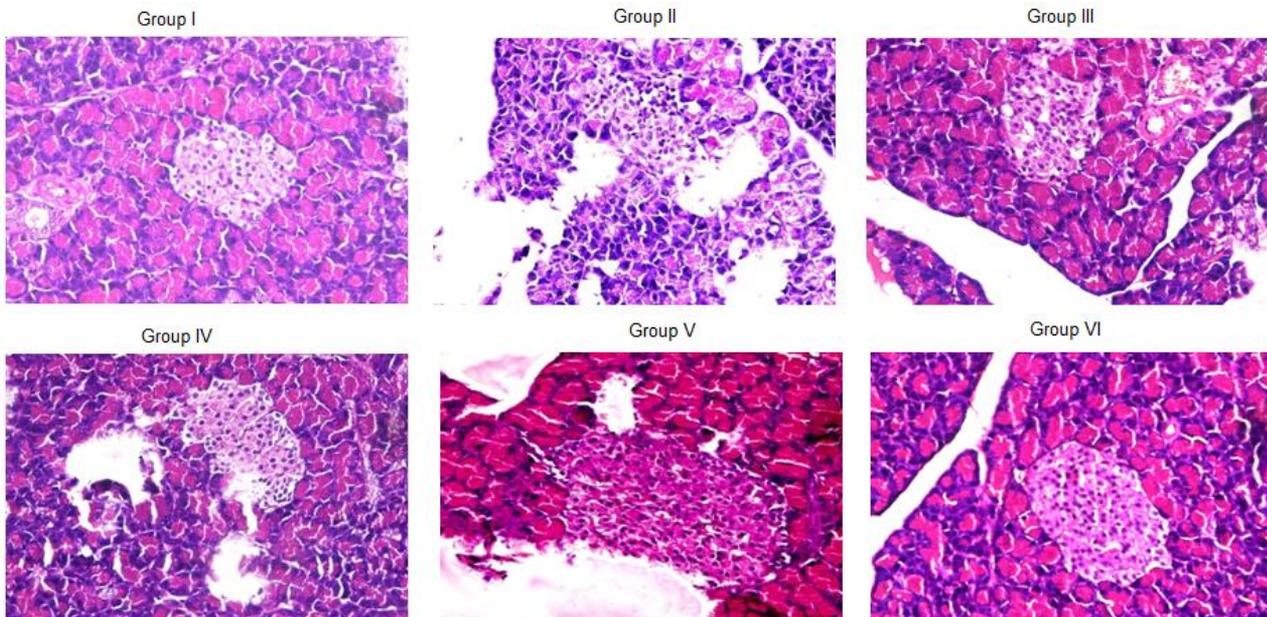


Figure 1: Photomicrograph of pancreas of rats of different groups

Group I: Photomicrograph of pancreas of normal rat; **Group II:** Photomicrograph of pancreas of rat treated with Alloxan 150 mg/kg body; **Group III:** Photomicrograph of pancreas of rat treated with hydroalcoholic extract of root of *Hibiscus rosa-sinensis* at a dose 50 mg/kg on 28 day; **Group IV:** Photomicrograph of pancreas of rat treated with hydroalcoholic root extract of *Hibiscus rosa-sinensis* at dose 100 mg/kg on day 28; **Group V:** Photomicrograph of pancreas of rat treated with hydroalcoholic root extract of *Hibiscus rosa-sinensis* 200 mg/kg on 28 day; **Group VI:** Photomicrograph of pancreas of rat treated with standard drug glibenclamide 5 mg/kg.

4. Discussion

In the present study, root of *Hibiscus rosa-sinensis* was tested for its antidiabetic, hypolipidemic and antioxidant activities in alloxan induced diabetic rats.

Alloxan causes reversible damage to insulin-producing β-cells found in the pancreas and that is why this animal model have been used for primary screening of test drugs for antidiabetic activity [3]. To explore the mechanism of

antidiabetic activity of *Hibiscus rosa-sinensis*, its effect was studied on blood glucose level, lipid profile by measuring total cholesterol, triglycerides, high density lipoprotein (HDL), very low density lipoprotein (VLDL) and Low density lipoprotein (LDL), lipid peroxidation by in term of malondialdehyde (MDA), superoxide dismutase (SOD) and histopathology of beta cell of pancreas by using rats as experimental animal.

In non diabetic rats, root extract administered in daily doses 100 and 200 mg/kg for 28 day, did not show any change in the blood glucose level. Therefore it does not have hypoglycemic activity which is usually the important and harmful side effect of insulin and sulphonylurea. The root extracts did not have any effect on normal lipid level. However, MDA level was decreased and SOD levels was increased significantly after 28 day of drug treatment. Root extract of *Hibiscus rosa-sinensis* showed the significant reduction in blood sugar level in the doses of 50, 100, 200 mg/kg. In doses root extract were administered at hourly interval up to 5 hr, then next three doses were given at the 24, 48 and 72 hrs interval (1, 2, 3, 4, 5, 24, 48 and 72 hr). the present study is in the favor of study of Venketesh *et al.*, [10]. They have also shown the reduction of blood sugar in both acute (1, 3, 5 hrs) and sub acute (1, 3, 5, 7 days) treatment in a dose of 250, 500mg/kg p.o.

Root extracts of *Hibiscus rosa-sinensis* have been found to possess hypolipidemic activity. Hypolipidemic activity was observed, in dose dependent manner, as evidence by reduction in the level of total cholesterol, triglycerides, VLDL, LDL and increases in HDL levels significantly. The most common lipid abnormalities in diabetes are hypertriglyceridemia and hypercholesterolemia. It is well established fact that increase level of triglyceride is a risk factor for atherosclerotic coronary disease. Repeated administration of roots extract of *Hibiscus rosa-sinensis* for 28 days significantly improved hypertriglyceridemia and hypercholesterolemia, bringing their levels in groups IV (100mg/kg) and V (200mg/kg) to that of the control group. LDL and VLDL carry cholesterol to the peripheral tissues where it is deposited; hence, high levels of LDL and VLDL are atherogenic. HDL transports cholesterol from peripheral tissues to the liver and thus helps in its excretion. HDL, therefore, has a protective effect. In the present investigation, all these parameters improved significantly in the extract treated diabetic rats. Our study is in favor of previous studies [11-14].

Root extracts of *Hibiscus rosa-sinensis* in rats showed significant antioxidant activity in response to oxidative stress due to alloxan induced DM. The antioxidant activity was determined by measuring the level of plasma malondialdehyde (MDA) and superoxide

dismutase (SOD). The extracts showed a decrease in plasma MDA level as well as increased in SOD level in a dose dependent manner in rats. Malondialdehyde (MDA) is a stable secondary aldehyde degeneration product of lipid peroxidation and it is used as a biological marker for the assessment of lipid peroxidation [15]. Superoxide dismutase (SOD) is an enzymatic antioxidant [16] which protect organ from reactive oxygen species mediated damage to the cell component. The decrease in plasma MDA level by *Hibiscus rosa-sinensis*, in response to oxidative stress induced by alloxan in rats could be due to its antioxidant activity and thus direct damage to the mucus membrane of the beta cell by alloxan is prevented.

Histological examination of pancreas has shown increase in diameter as well as number of beta cells which indicates the proliferation with regeneration of beta cells after 28 days treatment with root extract of HRS in rats. In alloxan induced diabetic rats, the reduction in beta cell number and islets diameter was noticed which indicates the loss of integrity between the cells in the islets. It suggests the regeneration effect of the root extract. This might contribute as an additional mechanism of antidiabetic activity of root extract of HRS. No such changes were seen in the normal rats which further support the activity of *Hibiscus rosa-sinensis*.

Glibenclamide, a standard drug for the comparison also showed the similar effect though it was less significant. A possible explanation could be that glibenclamide may be producing its blood sugar lowering effect by increasing the release of insulin from beta cells. Hibiscus root extract increase the serum SOD level in rats which indicates its antioxidants activity and it was more significant as compared to glibenclamide.

Thus, in our study the roots extract of *Hibiscus rosa-sinensis* might be producing antidiabetic effect by preventing mucosal damage of beta cell as an additional mechanism. However root extract of HRS has shown significant antidiabetic, hypolipidemic and antioxidant activity. The antidiabetic activity of extract of HRS in present study appeared to be due to prevention of oxidative stress and regeneration of beta cell of islets of langerhens. This in turn might improve the increased secretion of insulin from beta cell and finally increase the peripheral utilization of glucose. The lipid lowering effect of extract of Hibiscus in our study is a useful effect which avoids the complication of diabetes.

It is, therefore, inferred that *Hibiscus rosa-sinensis* may be useful and in true sense antidiabetic agent as shown by our experimental work. Since it has not decreased the normoglycemic level, it will definitely be avoid the major side effect as hypoglycemia, which generally occurs with insulin and oral hypoglycemic agents. In view of its significant antidiabetic activity

against alloxan induced diabetes mellitus as well as hypoglycemic activity, clinical trial will be required before establishing it as an antidiabetic agent for human use. Furthermore, it will be a safe alternative.

5. Conclusion

The observations of the present study suggested that *Hibiscus rosa-sinensis* is a useful and safe antidiabetic agent with minimal adverse effects. Before establishing it as an antidiabetic agent for human use, clinical trial will be required.

Acknowledgement

The authors would like to thank the department of pharmacology and administration of Mahatma Gandhi Institute of Medical Sciences, Sewagram, Wardha, Maharashtra, India for permission to study and providing necessary facility to carry out the research work.

References

- [1] Geetanjali K et al. Commonly consumed Indian plant food materials in the management of diabetes mellitus, Diabetes & Metabolic Syndrome: *Clinical Research & Reviews* 2010; 4: 21–40.
- [2] Goodman and Gilman's, 'The Pharmacological Basis of Therapeutics', McGraw-Hill, 5th Edition, New York, 1996, 1286-89.
- [3] Trivedi, N.A, Majumdar, B., Bhatt, J.D. and Hemavathy, K.G. 2004. Effects of shilajit on blood glucose and lipid profile in alloxan induced diabetic rats. *Ind. J. Pharmacol.* 36 (6), 373-386.
- [4] Chattopadhyaya R, Pathak D, Jindal DP. Antihyperlipidemic agents. A review. *Indian Drugs.* 1996; 33:85–97.
- [5] Paranjpe P. Indian medicinal plants, forgotten healers: a guide to Ayurvedic herbal medicine. Delhi, India: Chaukhamba Sanskrit Pratishthan; 2001. p. 89–90, 102–3.
- [6] Badole S, Patel N, Bodhankar S, Jain B, Bhardwaj S. Antihyperglycemic activity of aqueous extract of leaves of *Cocculus hirsutus* (L.) Diels in alloxan-induced diabetic mice. *Indian J Pharmacol* 2006; 38:49-53.
- [7] Grover J.K., Yadav S., Vats V. Medicinal plants of India with antidiabetic potential. *J. Ethnopharmacol.* 2002; 81:81–100.
- [8] Scartezzini P., Sproni E. Review on some plants of Indian traditional medicine with antioxidant activity. *J. Ethnopharmacol.* 2000; 71:23–43.
- [9] Seth S.D., Sharma B. Medicinal plants of India. *Indian J. Med. Res.* 2004; 120:9–11.
- [10] Venkatesh S, Thilagavathi J and Shyam Sundar D, Anti-diabetic activity of flowers of *Hibiscus rosa-sinensis*, *Fitoterapia*, 2008; 79 (2): 79-81.
- [11] Sachdewa A, Khemani LD. Effect of *Hibiscus rosa-sinensis* Linn. ethanol flower extract on blood glucose and lipid profile in streptozotocin induced diabetes in rats. *J Ethnopharmacol.* 2003; 89: 61–6.
- [12] Vishnu Kumar, p Shingh, ramesh chander. Hypolipidemic activity of *Hibiscus rosa-sinensis* root in rats. *Indian J Biochemistry & Biophysics* 2009; 46:507-10.
- [13] Gosain S, Ircchiaya R, Sharma PC. Hypolipidemic effect of ethanolic extract from the leaves of *Hibiscus sabdariffa* L. in hyperlipidemic rats. *Acta Pol Pharm* 2010 Mar-Apr; 67(2):179-84.
- [14] Lee WC, Wang CJ, Chen YH. Polyphenol extracts from *Hibiscus sabdariffa* Linnaeus attenuate nephropathy in experimental type 1 diabetes. *J Agric Food Chem.* 2009 Mar 25; 57(6): 2206-10.
- [15] Bird RP, Draper HH. Effect of malondialdehyde and acetaldehyde on synthesis of macromolecules. *J Toxicol Environ Health* 1980;6:811-815
- [16] Halliwell B, Gutteridge JMC. The antioxidant of human extracellular fluid. *Arch Biochem and Biophys* 1999; 280(1): 1-8.