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Original Research Article

Antidiabetic, Hypolipidimic and Antioxidant Activities of *Hibiscus Rosa Sinensis* Flower Extract in Alloxan Induced Diabetes in Rabbits

Mohan Pethe[1*], Samir Yelwatkar[1], Vijay Gujar[1], Sushil Varma[2] and Smita Manchalwar[3]

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

QR Code

***Correspondence Info:**
Dr. Mohan Pethe
Department of Pharmacology,
Mahatma Gandhi Institute of Medical Sciences, Sewagram,
Wardha, Maharashtra, India- 442102

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Abstract

Aim and Objective: Aim of the present research was to evaluate various biological activities of *Hibiscus Rosa Sinensis* flower extract in alloxan induced diabetes in rabbits.
Materials and Methods: Albino rabbits of either sex weighing between 1.5 to 2.5 kg were chosen for the study and divided into 6 groups of 6 animals each. The hydroalcoholic extract of *Hibiscus Rosa Sinensis* flowers (HEFHR) was used as test extract. Glibenclamide was administered as drug for comparison. Diabetes was induced by alloxan (150 mg/kg body weight). Group I served as normal control and group II as diabetic control. Group III, IV, V received HEFHR in the doses of 50, 100, 200 mg/kg orally respectively whereas group VI received glibenclamide 5mg/kg body weight for a period of 72 hrs. **Results:** HEFHR was found to be more effective in antidiabetic activity than glibenclamide, as flower extract produced a gradual fall in blood sugar level in 72 hour whereas glibenclamide has shown sudden fall in blood sugar level within 24 hours which remains constant up to 72 hour. The oral administration of flower extract has significantly improved dyslipidemia by reducing raised level of lipids and it was more efficacious in lipid lowering effect, when compared to glibenclamide. Flower extract of hibiscus was more effective in antioxidative activity than glibenclamide.
Conclusion: Significant anitidiabetic effect of flower extract in rabbits may be due to antioxidant properties and significant antidyslipidemic effect protects the cardiac complications especially ischemic heart disease. Hence, it may be a safe and better alternative available over the antidiabetic agents.
Keywords: Alloxan induced Diabetes; Hydroalcoholic extract; *Hibiscus rosa sinensis*; Glibenclamide; Rabbits.

1. Introduction

Hyperglycemia and dyslipidemia, among other disorders, are metabolic syndromes associated with a dysfunctional endocrine system clinically referred to as diabetes mellitus (DM) [1-3]. DM is described and classified on the basis of intrinsic and extrinsic causative factors, which has been exhaustively explained elsewhere [4-6]. Although the etiology of DM is multifaceted, the prevalence of the disease worldwide is often linked to genetic/ physiologic factors, sedentary lifestyle, and obesity, [7-9] of which poor dietary habits such as high consumption of sugars and saturated fats in addition to low intake of polyunsaturated fatty acids (PUFAs) have been implicated to be major contributory factors toward the progression of the disease [10,11]. Apart from hyperglycemia, disorders of lipid metabolism following oxidative stress are the prime risk factors for initiation and progression of diabetic complications in patients and thus there is an urgent need for a simultaneous treatment [12].

Treatment of diabetes with commercially available drugs possesses some degree of adverse effects. Therefore,

research and development of a lipid lowering drug with antidiabetic and antioxidant potential altogether, from natural products are the best option and also are in great demand. There are thousands of plant species known to have medicinal values and the different parts of these medicinal plants have been used for different ailments since ancient times. *Hibiscus rosa sinensis Linn* (known as Gudhal in Hindi, Japa in Sanskrit, and Shoe Flower in English) is an Ayurvedic remedy that has been mentioned in ancient Indian medical literatures and is reported to possess anti-tumor, detoxifier, antifertility, and wound healing activities [13]. The flowers of the plant possess anti-spermatogenic, androgenic [14] and anticonvulsant activities [15]. The leaves are useful in healing of ulcers and exhibit hair growth promoting activity [16]. Recent researchers have found a variety of pharmacological effects of almost all the parts of this plant [17].

However, there is no published report about the antidiabetic, hypolipidimic and antioxidant activities of hydroalcoholic extract of *Hibiscus rosa sinensis* flowers on alloxan induced diabetes in rabbits. Therefore, we designed recent study to investigate the effect of HEHRS flowers on alloxan induced diabetes with hyperlipidemia in rabbits.

2. Materials and Methods

After obtaining Institutional Animal Ethics Committee (IAEC) approval, this study was conducted on six groups with six albino rabbits in each group for the period of 72 hours. For the study purpose fresh *Hibiscus rosa-sinensis* flowers were collected from the central garden of medical college, in the month of December to February 2008-2009. The botanical identity of plant was confirmed and authenticated by taxonomist. Animals used in this study were albino rabbits of either sex weighing between 1.5 to 2.5 kg. Rabbits 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 with fresh green vegetable. Rabbits were maintained in ideal conditions of temperature 25-26^{0}C, relative humidity 50-70% and light and dark cycles of 12 hours each. Food and water was provided *ad-libitum*.

The collected flowers 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 flowers and was macerated separately in 95 % of ethanol overnight. Then it was packed in the timble of soxhelet and was extracted using 95 % ethanol refluxing at 60-80^0 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 flower 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 rabbits. 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.

Overnight fasted rabbits were divided into six groups of six in each. Group I: served as normal control (NC), administered with gum acacia 1ml daily, Group II: consider as diabetic control (DC) treated with alloxan 150 mg/kg body weight whereas *Hibiscus rosa-sinensis* flowers 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 72 hours.

2.1 Collection of blood and estimation of serum glucose

Blood samples were collected by puncturing the marginal ear vein of each rabbit of a group before and also at 1, 2, 3, 4, 5, 24, 48, and72 hr after oral administration of the drug. The samples were collected into glass vials containing a small quantity of a mixture of potassium oxalate and sodium fluoride as anti-coagulant.

2.2 Estimation of lipid profile

Blood was collected from marginal ear vein on 72 hrs and kept aside for ½hr for clotting. Serum was separated by centrifuging the sample at 6000 rpm for 20 min and analyzed for lipid profiles by using autoanalizers (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 [18].

2.3 Estimation of superoxide dismutase (SOD)

Blood samples were collected in plain vials from all the group of animals. Serum was separated and stored at –20°C to estimate SOD. The SOD activity of these supernatants was estimated by measuring the percentage of inhibition of the pyragallol auto-oxidation by SOD. The buffer was 50mM tris (pH = 8.2) containing 50 mM cocodylic acid (pH = 8.2), 1 mM ethylene diamine tetra acetic acid (EDTA) and 10mM hydrochloric acid (HCl). In a spectrophotometric cuvette, 2 mL of buffer, 100 µL of 2 mM pyragallol and 10 µL of supernatant were poured and the absorbance was noted at 420 nm for 3 min. One unit of

SOD was defined as the enzyme activity that inhibited the auto-oxidation of pyragallol by 50 % [19].

2.4 Estimation of plasma malondialdehyde (MDA)

Blood was collected in EDTA containing vials from each animal from each group. Blood samples from all groups of animals were separately centrifuged at 10,000 g at 4^{o}C for 5 min. Supernatant and plasma was used for the estimation of MDA. For the measurement of MDA, 0.5 mL homogenate and plasma were mixed separately with 0.5 mL normal saline and 2 mL of TBA-TCA mixture (0.392 g of TBA in 75 mL of 0.25 N HCl with 15 g of TCA, with the final volume of the mixture being made up to 100 mL with ethanol) and, then boiled at 100 ^{o}C for 10 min. The mixture was then cooled at room temperature and centrifuged at 4000 g for 10 min. The whole supernatant and plasma was transferred into a spectrophotometer cuvette and read at 535 nm. Calibration was performed by using the acid hydrolysis of 1, 1, 3, 3 tetra-methoxy propane, as a standard. The MDA present within the sample was calculated by using the extinction coefficient of 1.56 × 105 M/cm and expressed as the unit of nM/mg of tissue or nM/mL of plasma [20].

2.5 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

In normal rabbits, the flower extract administered in daily doses 100 and 200 mg/kg for 72 hrs, did not show any change in the baseline blood sugar level and normal lipid level. However, MDA level was decreased and SOD levels was increased significantly after 72 hrs of drug treatment. Hence there were no significant difference in baseline blood sugar level, lipid profile, MDA and SOD level, (Table 1).

Table 1: Effect of hydroalcoholic flower extract of *Hibiscus Rosa Sinensis* (FEHRS) on blood sugar, lipid profile, MDA and SOD level in normal rabbits

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 day 28	T C#	TG#	LDL#	VLDL#	HDL#		
GR I normal	DW 10 ml/kg	85.3 ±9.16	81.3 ±5.23	80.16 ±15.22	115.1 ±31.2	62.33 ±12.35	25.19 ±8.15	15.33 ±6.21	4.44 ±0.069	3.34 ±0.089
GR II NDC	FEHRS 100 mg/kg	86.8 ±7.16	89.7 ±9.26	86.26 ±25.12	119.2 ±33.2	60.13 ±13.25	23.14 ±7.33	15.61 ±7.12	3.44 ±0.151	4.22 ±0.069
GR III NDC	FEHRS 200 mg/kg	85.3 ±6.11	85.3 ±4.33	85.16 ±25.42	115.1 ±33.2	57.33 ±15.35	22.44 ±9.76	14.21 ±8.41	3.014 ±0.029*	5.09 ±0.09*

*p< 0.05, # - mg/kg, NDC-non diabetic control, DW-distil water TC-total cholesterol, TG- triglycerides, LDL-low density lipoprotein, HDL-high density lipoprotein, VLDL-very low density lipoprotein.

3.1 Effect of flower extract (FEHRS) 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, VI there was highly significant increase in blood sugar level, 1 week after alloxan administration compared to group I. In group II these highly significant increase in blood sugar level almost remain constant over the period of 72 hours. In group III, IV, V treated with FEHRS in graded doses (50, 100, 100mg/kg), there was progressive decreased in blood sugar level in 72 hour period and in all the three groups blood sugar level was significantly low compared to group II from 48, 24 and 5 hours onward respectively. In group VI there was sudden decrease in blood sugar level at 24 hour which remains constant up to 72 hour. This fall in blood sugar level was significant compared to Group II, (Table 2).

Table 2: Effect of hydroalcoholic flowers extract of *Hibiscus Rosa sinensis* (FEHRS) on blood sugar in rabbits

GR n=6	Drugs	Blood sugar level (mg/dl), Mean ± SD								
		Before T/t	Drugs	After drug administration (ADA)						
		Base-line	1 wk after alloxan	1 hrs	2 hrs	3 hrs	4 hrs	5 hrs	24 hr	48hr	72hr
I	DW 10^{2}	84.66 ±3.55	-NA-	84.6 ± 2.16	86.6 ± 3.16	87.4 ± 2.46	89.4 ± 5.26	90.4 ± 7.16	84.76 ±3.57	88.6 ± 7.16	90.6 ± 13.16
II	Alloxan 150^{1}	86.66 ± 5.78	376 ±17.26	379 ± 17.45##	382 ± 20.45##	380 ± 12.40##	383 ±9.30##	380 ± 6.13##	378± 14.2##	379 ± 27.45##	382 ± 10.46##
III	Alloxan 150^{1}+ FEHRS 50^{2}	85.66± 7.44	369 ± 28.90	361 ± 23.90	352 ±7.31	354 ±9.21	355 ±8.09	345 ±10.9	340 ± 28.90	301 ± 13.9s0*	200±7.3 1 **
IV	Alloxan 150^{1}+ FEHRS 100^{2}	87.50 ± 8.61	369.83± 34.4	362 ± 34.40	357 ± 24.14	355 ± 19.10	347 ± 13.10	340 ± 13.10	290 ± 34.4*	212 ± 31.20**	170 ± 14.14**
V	Alloxan 150^{1}+ FEHRS 200^{2}	87.00 ± 4.65	383 ±32.42	370 ± 15.95	360. ± 15.95	350. ± 19.05	345. ± 10.35	325 ± 11.25*	210±22.4 **	169 ± 25.95**	120. ± 15.95**
VI	Alloxan 150^{1} +gliben 5^{2}	87.16 ± 6.18	379 ± 37.69	374 ± 21.09	375 ± 11.19	371 ± 10.51	368 ± 9.31	358 ± 10.31	129 ± 33.59***	120 ± 21.03**	115 ± 14.19**

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<0.001 compared to DW, * p<0.05 compared to Alloxan group, **p<0.001 compared to Alloxan group.

3.2 Effect of flower extract (FEHRS) on lipid profile

Estimation of lipid profile in all groups was done at 72 hour. In group II, TC, TG, LDL and VLDL were highly significantly increased and HDL highly significantly decreased compare to group I. In group III, IV, V treated with FEHRS in graded dose, there was highly significantly decrease in TC, TG, LDL, VLDL and highly significant increase in the HDL level compare to group II. In group V (200mg/kg), all the changes were highly significant compare to group II, moreover lipid parameter in group V was almost similar to group I. Group VI also shows significant decrease in TC, TG, LDL, VLDL and increase in HDL level compare to group II, (Table 3).

Table 3: Effect of hydroalcoholic flowers extract of *Hibiscus Rosa sinensis* (FEHRS) on lipid profile of rabbits

GR N=6	Drug	TOT CHOLE mean±SD	HDLC mean±SD	TG mean±SD	LDL mean±SD	VLDLc mean±SD
I	DW 10[2]	66.96±8.033	25.16±1.16	118.33±9.85	18.83±2.041	23±12.14
II	Alloxan 150[1]	232.16±19.36 ##	12.5±1.048 ##	330.16±40.05 ##	153±18.77 ##	66.16±17.13 ##
III	Alloxan 150[1] +FEHRS 50[2]	197.5±11.72 **	17.66±1.75 **	230.33±11.74 **	133.66±1.21 **	46.83±11.94 **
IV	Alloxan 150[1] +FEHRS 100[2]	140.5±12.95 **	19.33±0.516 **	167.33±9.87 **	87.16±3.92 **	33.41±12.52 **
V	Alloxan 150[1] +FEHRS 200[2]	68.16±3.54 **	24.16±2.22 **	100.16±7.33 **	18.16±3.60 **	20.16±09.94 **
VI	Alloxan 150[1] +gliben 5[2]	77.66±43.36 **	22.5±1.04 **	128.5±4.13 **	29.16±1.94 **	25.6±8.07 **

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<0.001 compared to DW, * p<0.05 compared to Alloxan group, **p<0.001 compared to Alloxan group

3.3 Effect of flower extract (HEFHR) on antioxidant activity

Estimation of MDA and SOD was done at 72 hrs, in group II plasma MDA level was significantly increased as compared group I. In group III, IV and V treated with HEFHR in graded dose, (50,100,200 mg/kg) dose dependent significant decreased in plasma MDA level, as compared to the group II. In group IV plasma MDA level were almost same as group I. In group V, MDA level was highly significantly decreased as compared to group II and this level were even lowered than group I. In group VI also plasma MDA level were significantly lower compared to group II. Plasma SOD was highly significantly decreased in group II as compared to group I. In group III, IV and V treated with FEHRS in graded doses, dose dependent significant increase in plasma SOD level was seen. In group IV, V and VI plasma SOD level was highly significantly increased compared to group II and was even higher than group I, (Table 4).

Table 4: Effect of hydroalcoholic flowers extract of *Hibiscus Rosa sinensis* (FEHRS) on melanoaldehyde (MDA) and super oxides dismutase (SOD) in rabbits

Groups (n=6)	Drug and Dose	Plasma MDA level (Mean ± SD) (nmol/ml)	Plasma SOD level Mean ± SD (Units/ml)
I	DW 10[2]	12.213 ± 0.768	10.92 ±1.089
II	Alloxan 150[1]	17.235 ± 0.483 ##	5.48 ±2.076 ##
III	Alloxan 150[1]+FEHRS 50[2]	15.301 ± 0.719 **	10.96 ± 1.164 **
IV	Alloxan 150[1]+FEHRS 100[2]	12.523 ± 0.521 **	13.56 ± 1.117 **
V	Alloxan 150[1]+FEHRS 200[2]	10.035 ± 0.376 **	16.07 ± 2.132 **
VI	Alloxan 150[1]+gliben 5[2]	13.708 ± 0.903 **	12.01 ± 1.098 **

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<0.001 compared to DW, * p<0.05 compared to Alloxan group, **p<0.001 compared to Alloxan group

4. Discussion

Since flower extract in present study did not showed any change in the normal blood glucose level and did not have any effect on normal lipid level even on treatment for a period of 72 hours which suggest that flower extract of *Hibiscus Rosa Sinensis* (HRS) do not show decreased the normal blood glucose level and therefore it does not have hypoglycemic activity which is usually the important and harmful side effect of insulin and sulphonylurea. In the normoglycemic rabbits, extract of flower did not decrease the blood sugar level even at the dose of 2000 mg/kg p.o. This suggests that its use is safe in diabetes as it does not produce hypoglycemia. The three doses (50,100 & 200 mg/kg) were selected by trial and error to conduct the present study. Also the HRS extract do not have lipid lowering effect on normal lipid profile. However, MDA level was decreased and SOD levels was increased significantly after 72 hours of drug treatment.

The flower extracts of HRS showed significant antidiabetic activity against alloxan induced diabetes mellitus in rabbits. This was evident from a decrease in blood sugar level. In doses flower 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). This decrease in blood sugar level could reach to normal level over a period of 72 hours. When comparing antidiabetic effect of flower extract with glibenclamide, it was found to be more effective than glibenclamide in dose 200 mg/kg p.o. Glibenclamide in our study has shown sudden fall in blood sugar level within 24 hr, whereas an extract of hibiscus produced a gradual fall in blood sugar level which is desirable in clinical situation. Our study was in the favor of study of Venketesh *et al* [21] and Sachdewa *et al* [22], in both the studies dose of flower extract used was 250mg/kg and 250, 500 mg/kg respectively. However, in present study blood glucose lowering effect was observed in the doses of 50, 100, 200 mg/kg orally in rabbits. This discrepancy might be due to different experimental situation.

The dyslipidemia is commonly associated with diabetes mellitus; therefore hypolipidemic activity was also evaluated. In current study, the alloxan treated rabbits have shown significant dyslipidemia as indicated by increase in TC, TG, LDL, and VLDL and decrease in HDL level. The oral administration of flower extract of hibiscus has significantly improved dyslipidemia in dose dependent manner. Though glibenclamide has reduced raised level of lipids, but it was less efficacious in lipid lowering effect, when compared to flower extract of HRS. The better control of the lipid profile by HRS will able to protect diabetes related cardiac complications. The present study was supported by the studies of Sachdewa *et al* [23], Vishnu Kumar *et al* [24], Gossain *et al* [25] and Lee *et al* [26], were they have shown lipid lowering activity of *Hibiscus rosa sinensis* flower extract in rats. However, they have used higher doses.

In present experimental work, *Hibiscus rosa sinensis* flower extracts in rabbits 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 flower extracts showed a decrease in plasma MDA level as well as increased in SOD level in a dose dependent manner in rabbits, thus it was more efficacious in antioxidative activity than glibenclamide. The antioxidant action of hibiscus extract may be responsible for antidiabetic effect by protecting beta cells and therefore increasing the insulin secretion. The regeneration of beta cells in terms of increased number and diameter, in our study has further supported the improvements in secretion of insulin. The increased secretion of insulin enhances the peripheral utilization of glucose and corrects the metabolisms of carbohydrate, fat and protein, hence producing the antidiabetic effect. Thus, in present study significant anitidiabetic effect of Hibiscus extract in rabbits may be due to antioxidant properties as well as regeneration of beta cells. Our study on oxidative stress was supported by Nade *et al* [27] were they have shown antioxidant properties of flower extracts of *hibiscus rosa sinensis* in reserpine induced oxidative stress. The antioxidant properties of *hibiscus rosa sinensis* can be attributed to polyphenols and flavonoid present in the flower. Thus, *hibiscus rosa sinensis* flower extract help in the restoration of antioxidant enzymes, together with inhibition of lipid peroxidation.

5. Conclusion

In conclusion, flower extract of *hibiscus rosa sinensis* (FEHRS) showed hypoglycaemic, hypolipidemic and antioxidant activities in diabetic rabbits, which suggested that FEHRS might be used as therapeutic alternative in the treatment of diabetes associated dyslipidemia. Before establishing it as an antidiabetic agent for human use, clinical trial will be required.

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