Antidiabetic effects of ethanolic flower extract of Hibiscus Rosa sinensis (L) on alloxan induced diabetes in hyperlipidaemic experimental Wister rats (WNIN)

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Abstract— Diabetes mellitus is one of the common metabolic disorders across the globe. Many lipoprotein abnormalities are seen in the untreated, hyperglycemic diabetic patients. This disease affects around 2.8% of the world's population and is likely to cross 5.4% by the year 2025. The use of herbal medicines for curing various ailments have been known since time in memorial and the herbal extracts have become a growing part of modern medicine. H. rosa sinensis has been used for the treatment of a variety of diseases. It is an easily available plant for natural remedies. The present study was undertaken to investigate the antidiabetic effects of the ethanolic Hibiscus flower extract in diabetes induced Wister rats which have been made hyperlipidaemic by feeding them with high fat diet. The serum blood glucose levels and lipid profile was found to be significantly increased in the hyperlipidaemic diabetic rat models while the level of plasma insulin was lowered. Treated groups administered with Hibiscus flower extract showed changes in the above biochemical parameters and it was found that among the three doses, 500 mg/kg showed the best result when compared to the other two doses. HRSFE exhibited potential antidiabetic against HFD fed alloxan induced diabetic Wister rats.

Keywords- Alloxan, antidiabetic, hyperlipidemia, lipoproteins, Hibiscus rosa sinensis

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1. INTRODUCTION

In the present scenario diabetes mellitus is one of the common metabolic disorders. This disease affects around 2.8% of the world's population and is likely to cross 5.4% by the year 2025) [1].

The use of herbal medicines for curing various ailments have been acknowledged since time in memorial and the herbal extracts have become a growing share of modern medicine. Increased plasma lipid levels, namely total cholesterol; triglycerides and low density lipoprotein (LDL) along with decrease in high density lipoprotein (HDL) is known to cause hyperlipidaemia, which if untreated leads to hyperlipidemia. There is an increased concentration of cholesterol, triglycerides carrying lipoproteins which is considered to be the cause of arteriosclerosis along myocardial infraction and thrombosis [2].

H. Rosa sinensis has been used for the treatment of a variety of diseases. It is an easily available plant for natural remedies. The flowers of *H. rosa sinensis* possess cardio-protective properties, this has been proved in rats [3].The ethanolic leaf extract of *H. rosa sinensis* (EHBS) exhibited cent percent post-coital antifertility activity [4]. The flower extract of *H. rosa sinensis* also possesses wound healing in rat [5]. The World Health Organization has also encouraged and recommended the evaluation of traditional plant treatments for diabetes.

1.1 General Objective

The present research was undertaken to investigate antidiabetic effects of ethanolic extracts obtained from flowers of *Hibiscus rosa sinensis* on Wister rats which were made hyperlipidemic by feeding them with high fat diet (HFD).

1.2 Specific Objectives

- To investigate and isolate the specific compound in *Hibiscus rosa sinensis* flower extract responsible for lowering the high blood sugar to near normal levels in diabetic people with hyperlipidemia.
- To find out relation of diabetic patients with hyperlipidemia in diabetic patients with concomitant diseases.

2. MATERIALS AND METHODS

2.1 Plant material

Flowers of *Hibiscus rosa-sinensis* Linn. were collected in July 2013 around local area of Hatia, Ranchi which falls in the Chotanagpur region, Jharkhand and it was authenticated by the Botanist Prof. Anjani Shrivastva, Department of Botany, Postgraduate Department of Zoology, Ranchi University, Ranchi. The collected flowers were shade dried and powdered and stored in air tight containers for extraction.

2.2 Preparation of Alcoholic *Hibiscus rosa sinensis* flower extract (HRSFE)

The flower powder obtained above (100gm) was extracted each time in a soxhlet apparatus installed at the Department of Zoology Ranchi University, Ranchi with petroleum ether (60-80º C) till complete extraction. Successively, the defatted plant material was extracted with chloroform, ethyl acetate and then with 95% ethanol. The ethanolic *Hibiscus rosa sinensis* flower extract (HRSFE) obtained was dark brown in colour which was then concentrated under reduced pressure using a concentrator to get a semisolid mass of the crude extract. The extract so obtained was kept under refrigeration below 10ºC.

2.3 Animals

Fifty non-obese male Wister rats (WNIN) (approx. 150-200g) each were purchased from Jazz Scientific store, Riada bhawan, Main road, Ranchi, Jharkhand, The animals were placed in polypropylene cages and standard environmental conditions of 12h light and 12 h dark cycle, at 23±2 ºC and 35-60% humidity were maintained. All experiments were conducted under the ethical guidelines issued by CPCSEA, Ministry of Social Justice and Empowerment, Government of India.

3. EXPERIMENTAL DESIGN

In order to monitor the antidiabetic effects of HRSFE fully acclimatized animals were distributed into four different groups with five animals in each group.

Group I: Control rats.

Group II: HFD Diabetic Control (HFDD): Rats were fed on HFD and then diabetes was induced in them by a single intra-peritoneal injection of alloxan (150 mg/kg bw)

Group III: HFD fed Diabetic rats treated with *Hibiscus rosa sinensis* alcoholic fower extract (125 mg/kg bw) daily by oral administration for a period of 4 weeks;

Group IV: HFD fed Diabetic rats treated with *Hibiscus rosa sinensis* alcoholic flower extract (250 mg/kg bw) daily by oral administration for a period of 4 weeks

Group V: HFD fed Diabetic rats treated with *Hibiscus rosa sinensis* alcoholic flower extract (500 mg/kg bw) daily by oral administration for a period of 4 weeks

4. COLLECTION OF RAT BLOOD FROM ALL THE GROUPS:

4-5mL of blood was collected by retro-orbital sinus puncture under mild anaesthesia (diethyl ether) on 14th, 21st and 28th day of the experiment respectively. The blood samples were then centrifuged at 3000 rpm for 10 minutes and the serum was then aspirated and stored in deep freezer. It was then used for various desired biochemical analyses.

Biochemical analyses

The serum was then assayed using standard test kits available from CREST (Coral Clinical Systems) and purchased from Sonal Enterprises, Ranchi for various biochemical parameters viz Total Cholesterol (TC), Triglycerides (TG), High Density Lipoproteins (HDL), Low Density Lipoproteins, (LDL), and Very Low Density Lipoprotein (VLDL**).** Blood glucose was measured using Dr. Morepen Glucose Monitoring System with biosensor technology. Similarly the assay for Plasma insulin was carried out [6].

5. STATISTICAL ANALYSES

The data recorded upon biochemical analyses of six rats each in a group were represented as mean ± standard error. It was analyzed by using ANOVA ($p < 0.05$). Statistically significant differences between the control groups, hyperlipidemia groups and the different treatment groups were compared and studied.

5.1 Body weight

Summary Statistics

	Count	*Average*	*Standard deviation*	*Coeff. of variation*
Control	6	178.167	26.3774	14.8049%
G1	6	140.333	9.7707	6.9625%
G2	6	152.5	13.4722	8.83423%
G3	6	197.167	5.77639	2.9297%
G4	6	201.833	8.01041	3.96882%

ANOVA Table

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	17546.7	4	4386.67	20.49	0.0000
Within groups	5351.33	25	214.053		
Total (Corr.)	22898.0	29			

The ANOVA table decomposes the variance of the data into two components: a between-group component and a within-group component. The F-ratio, which in this case equals 20.4933, is a ratio of the between-group estimate to the within-group estimate. Since the P-value of the F-test is less than 0.05, there is a statistically significant difference between the means of the 5 variables at the 95.0% confidence level.

5.2Blood Glucose

Summary Statistics

	Count	*Average*	*Standard deviation*	*Coeff. of variation*
Control	6	84.1667	9.76559	11.6027%
G1	6	280.167	21.1888	7.56294%
G2	6	131.0	6.9282	5.2887%
G3	6	203.167	15.3417	7.55127%
G4	6	162.0	7.45654	4.6028%

ANOVA Table

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	133002.	4	33250.5	188.22	0.0000
Within groups	4416.5	25	176.66		
Total (Corr.)	137419.	29			

The F-ratio, which in this case equals 188.218, is a ratio of the between-group estimate to the within-group estimate. Since the P-value of the F-test is less than 0.05, there is a statistically significant difference between the means of the 5 variables at the 95.0% confidence level.

5.3 Plasma Insulin
Summary Statistics

	Count	*Average*	*Standard deviation*	*Coeff. of variation*
Control	6	15.3833	1.5237	9.90489%
G1	6	3.87	0.670999	17.3385%
G2	6	8.35	1.2872	15.4156%
G3	6	14.6883	1.18933	8.09708%
G4	6	13.8967	1.59657	11.4888%

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	592.71	4	148.178	88.28	0.0000
Within groups	41.9616	25	1.67846		
Total (Corr.)	634.672	29			

The F-ratio, which in this case equals 88.2818, is a ratio of the between-group estimate to the within-group estimate. Since the P-value of the F-test is less than 0.05, there is a statistically significant difference between the means of the 5 variables at the 95.0% confidence level.

6. RESULTS AND DISCUSSION

Since time in inception plants have been known for their effectiveness in curing and controlling many diseases with minimal side effects and low cost [6]. Therefore investigation and research on such plants have become more important. One such plant is Hibiscus rosa sinensis whose flower extract has been used in the present study. HRSFE (500mg/kg bw) was found to be an effective medicinal tool to reduce blood glucose and boost the level of insulin. HRSFE significantly decreased blood glucose level in alloxan-induced diabetic rats when compared to their respective control rats. The main characteristics of Type 1 and Type 2 diabetes is hyperglycaemia along with minimal secretion of insulin into the blood stream. However, an impaired insulin functioning can also be a probable reason for the same. There are a large number of active plant constituents which possess a marked hypoglycemic activity [6]. In the present study HRSFE has exhibited anti-hyperglycemic activity in rats with hyperlipidemia. The exact mechanism of the mode of function of HRSFE in alluring hyperglycemia and considerably increasing the plasma insulin levels is still unknown, one of the reasons may be increased insulin secretion from the existing beta cells of the pancreas [7]. In the experiment the untreated diabetic rats showed severe body weight loss. The reason behind the weight loss in diabetic rats could be the catabolism of fats and proteins [8]. The catabolic process is responsible for the muscle wasting which may be the major cause for weight loss in diabetic rats [9]. The experimental groups treated with HRSFE showed a sign of recovery in the body weight which elicits the muscle protective effect of the extract.

Besides, hypercholesterolemia and hypertriglycemia have been reported to occur in diabetic patients [10] [11]. In the present study cholesterol rich high fat diet has been used to simulate high lipid profile diabetic condition which is quite usual among people these days. Cholesterol feeding has been frequently applied to elevate serum or tissue cholesterol levels.

Table 1. Represents the changes in body weight, blood glucose and plasma insulin concentrations of rats fed with *Hibiscus rosa sinensis* flower extract (HRSE) and injected with alloxan. There was an increase in the bodyweight and plasma insulin in control rats and rats fed with the extracts a decrease in the body weight of rats treated with alloxan was also observed blood glucose level increased in diabetic rats compared with control. *Hibiscus rosa sinensis* at the doses of (125, 250 and 500 mg/kg bw) significantly decreased the alloxan-induced elevated blood glucose concentration. HRSE (500 mg/kg) showed a significant reduction in the blood sugar level compared with Group IV and Group V (125 and 250mg/kg) respectively.s

Group	**Body weight (g)**			**Blood glucose (mg/dL)**	**plasma insulin (µL/mL)**

	Initial	**Final**	**Difference**		
Control	181.67 ±11.78	178 ±13.43	3.67	84.16±6.01	15.38±0.90
HFD-Diabetic Control	177.67± 10.37	140.34 ±5.85	37.34	280.16±12.13	3.87±0.41
Diabetic ± HRSFE (125 mg/kg)	179.67±11.78	152.5 ±8.01	27.16	131±3.77*	8.35±0.74*
Diabetic ± HRSFE (250 mg/kg)	183.34 ± 11.54	197.16 ±3.18	13.83	203.16±7.46*	14.68±0.71*
Diabetic ± HRSFE (500 mg/kg)	183.5 ±9.07	201.84 ±4.59	18.34	162±4.00*	13.89±0.92*

Values are expressed as mean± SD of 6 animals. Values in parentheses represent relative change in parameter assessed ($p < 0.05$)*represent significant changes against normal control.

Lipid profile:

Table 2. Represents the changes in the various lipid profile parameters of rats fed with cholesterol rich high fat diet. There was a progressive increase in the TC, TG, LDL, and VLDL levels and a decrease in the HDL levels across various groups when compared to the control group.

	NC			HFDD		
	14 days	**21days**	**28 days**	**14 days**	**21days**	**28 days**
TC(mg/dl)	61.29±2.14	63.42±1.82	60.44±2.9	138.78±1.03	139.08±0.83	136.95±2.43
TG(mg/dl)	59.76±1.8	61.49±1.84	60.3±1.82	135.92±1.30	137.1±0.86	133.93±1.30
HDL(mg/dl)	25.5±0.69	21.01±0.82	26.65±0.10	17.47±0.72	16.72±0.72	17.15±0.79
LDL(mg/dl)	23.83±1.59	30.11±1.85	24.09±9.20	94.12±1.98	94.94±0.84	93.01±6.54
VLDL(mg/dl)	11.95±0.36	12.29±0.36	12.06±7.96	27.18±0.26	27.42±1.57	26.78±2.97

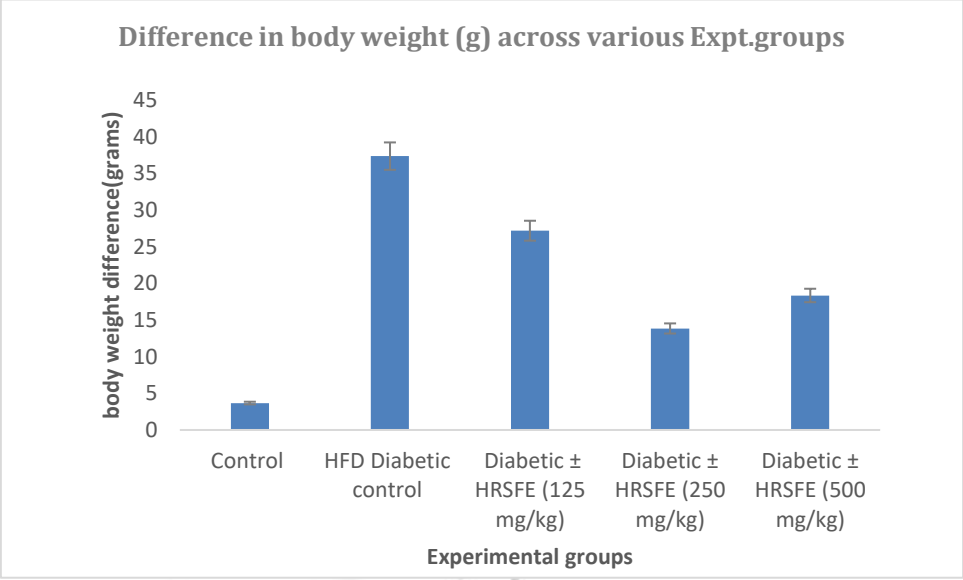

Figure.1 Graphical representation of difference in body weight in the beginning and end of the experiment across various experimental groups.

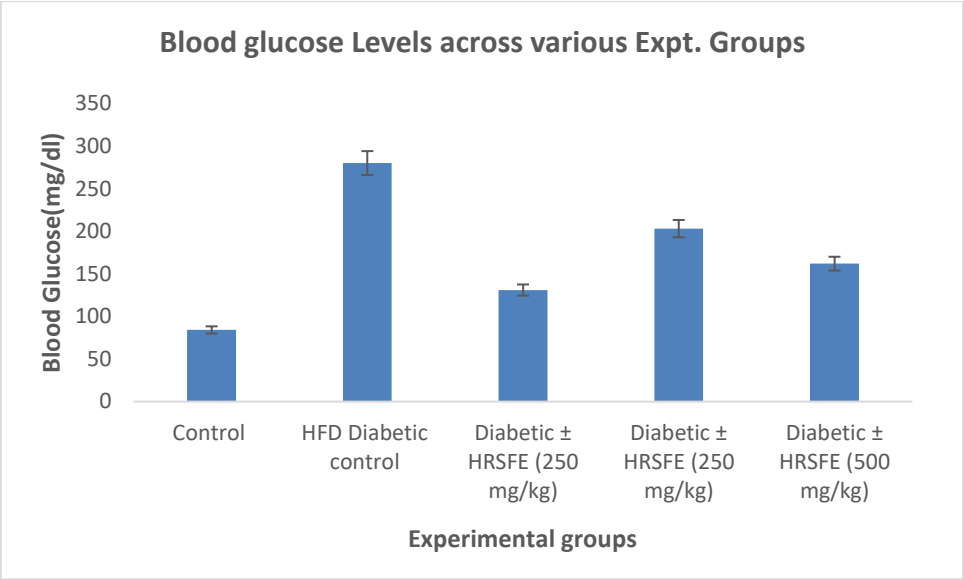

Figure.2 Graphical representation of blood glucose levels across various experimental groups.

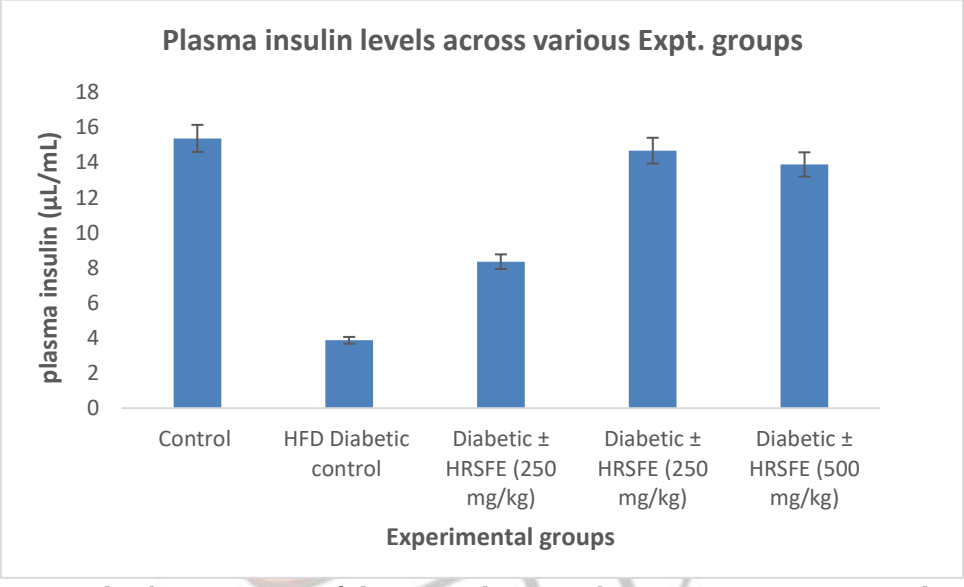

Figure.3 Graphical representation of changes in plasma Insulin across various experimental groups.

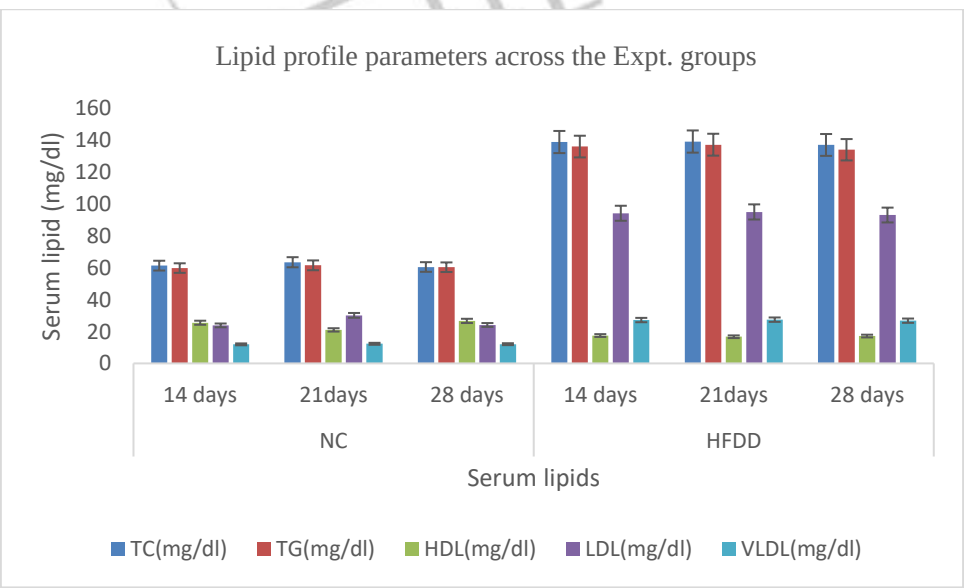

Figure.4 Graphical representation of changes in various lipid profile parameters across the experimental groups.

7. ACKNOWLEDGMENT

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8. CONCLUSION

The ethanolic extract of *Hibiscus rosa-sinensis* (L) flowers has significant anti-diabetic activity in hyperlipidemic rat models. Hence, it can be used as a healing factor or in the supportive treatment to existing therapy for the treatment of diabetes in hyperlipidemic conditions. Further research on isolation and characterization of fractioned compound is the future scope of this investigation.

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