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RESEARCH ARTICLE

COMPARISON BETWEEN ANTI-DIABETIC EFFECT OF ETHANOLIC EXTRACT OF *HIBISCUS ROSA SINENSIS* LEAVES AND FLOWERS IN DIABETIC RATS

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ABSTRACT

Diabetes continues to cause a great economic loss every year. With commercially available synthetic drugs having serious adverse events, traditional source of drugs like plants continue to play a significant role in search for a safer and effective treatment. In this study, the antidiabetic effect of ethanolic extract of leaves and flowers of *Hibiscus Rosa Sinensis* (HRS) are compared along with a standard drug, metformin. Diabetes was induced using Alloxan in Wistar Albino Male rats and were divided into 4 group respectively. Rats were treated separately with the extracts and standard drug with blood glucose monitoring at regular intervals for 21 days. Ethanolic extract of flowers is more effective then ethanolic extract of leaves with neither of them developing hypoglycemia which was observed with metformin treatment.

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INTRODUCTION

With an estimation of 415 million people to suffer from diabetes by 2040, diabetes a multi-headed monstrous disease is nothing but the backlash of the sedentary lifestyle homo sapiens have got accustomed due to advancements in day to day technology and services. With symptoms ranging from as rudimentary as a mood swing to lethal as keto acidosis, diabetes is an endocrinal disorder primarily due to either depleted insulin secretion or insulin dysfunction. This eventually leads to impaired metabolism of lipids, proteins and glucose. Diabetes is commonly and popularly classified mainly into two types; Type 1diabetes which is an autoimmune disease and Type 2 Diabetes which is a complex endocrine and metabolic disorder. However gestational diabetes and other specific types of diabetes namely genetic defect of the beta cells or insulin action, endocrinopathies, drug induced are other accepted classifications (American Diabetes Association, 2015; Thomas and Philipson, 2015; Alam *et al*., 2014). India is home to 69.1 million people with Diabetes Mellitus (DM), second only to China in 2015 (https://www.idf.org/e-library/epidemiology-research/diabetes-atlas). With an estimated 12% of total health spending, diabetes is a colossal global burden (https://www.idf.org/e-library/epidemiology-research/diabetes-atlas). With the prevalence rate almost doubling from the data recorded in the late 90's, diabetes is no longer a rich man's disease as recorded by rise in DM cases in urban as well as rural regions in India. Synthetic drugs available commercially are effective in the treatment of diabetes although most of them have serious adverse events. Search for effective, economical and safe treatment for diabetes is therefore an utmost priority in modern research. Before the discovery and invention of modern day drugs, natural cures were used to treat diseases such as diabetes (Patel *et al*., 2012; Pk *et al*., 2006; Alam *et al*., 1990; Medicinal Plants and Natural Products with Demonstrated Wound Healing Properties, 2017; Traditional medicinal uses of Hibiscus rosa-sinensis [Internet]. ResearchGate, 2017). Traditionally, plants have been an important source of drug discovery, however, with newer and superior research methods available, plants continue to play an important role in modern day drug discovery in search for a safer alternative to synthetic drug. World Health Organization has emphasized evaluation of traditional plant treatments for diabetes apart from creating awareness, public education on prevention and essential treatment for diabetes. Several medicinal plants have been identified to have anti-diabetic effect and have been used long before modern medicine took the front stage. Over 800 plants species have been identified to have antidiabetic effect. India being a tropical country has a vast pool of biodiversity for plants with medicinal use. Hibiscus Rosa Sinensis (HRS) commonly known as Jasum/Jasund in Hindi, is a popularly cultivated shrub in India with well supported ethnobotanical data suggestive of its numerous medicinal use (https://www.researchgate.net/publication/288970136_Review_on_Hibiscus_rosa_sinensis; https://www.researchgate.net/publication/312148872_therapeutic_potential_of_hibiscus_rosa_sinensis_a_review). In 1753,

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Linnaeus established genus Hibiscus in Malvaceae family. Amongst the 250 species widely scattered in the tropical and subtropical regions of the world, in medicine, the red 5 petal flower variety is preferred (Pethe *et al*., 2017).

Part of the plant (extract)	Use/Treatment
Leaves	Hair growth and anti greying property, gonorrhea, piles, urinary discharge, vaginal and uterine discharge, promote growth of fetus, fatigue, skin disease, diabetes
Flower	Hair growth and anti greying property, emollient, demulcent, refrigerant drink, cystitis, genito-urinary problems, excessive menstruation, anticonvulsant, epilepsy, leprosy, bronchial catarrh, diabetes, abortifacient and anti-implantation in rodents
Root	Menorrhagia, cough, gonorrhea

With several well documented studies indicating the antidiabetic effect of leaves (Sachdewa *et al*., 2001; Moqbel *et al*., 2011; Bhaskar and Vidhya, 2012) and flower (Pethe *et al*., 2017; Venkatesh *et al*., 2008; Sachdewa and Khemani, 2003) of HRS, we considered it worthwhile to undertake a study to comparethe antidiabetic effect of ethanolic extract of flowers and leaves of HRS along with a standard drug to determine efficacious model for treatment.

MATERIALS AND METHODS

Collection of HRS flowers and leaves: Fresh HRS flower of red colorand HRS leaves were collected from area nearby JNMC, Sawangi, Wardha and authenticated by the taxonomist at Mahatma Gandhi Ayurveda College, Wardha.

Preparation of crude extract: Leaves and flowers were shade dried, powdered in a blender and stored in an air-tight container separately until further use. The powder was extracted in soxhlet apparatus using 95% of ethanol at 60-80 degree Celsius for 12 hours. The extracts were filtered and the filtrate was then concentrated with a rotary evaporator under reduced pressure to obtain crude extract. 7 grams from reddish brown semisolid extract was obtained from 50 grams of dried powder of flowers while Dark greenish brown semi solid extract was obtained when the 60 grams of dried powder of the HRS leaves was treated the same way yielding 8 grams.

Chemicals: Metformin was used as the standard drug while Alloxan was used to induce diabetes in the rats. Ethanol used was of analytical grade.

Experimental Animals: All procedures for the animal experiment were conducted after approval from the Institutional Ethics Committee, DMIMS, Sawangi and were carried out in the Animal House, DMIMS (Reg.No. 571/02/a/ CPCSEA). Male Wistar albino rats with weight of 150-250gms were used after 7-day acclimatization period (12:12 hour light dark cycle) to the laboratory environment. 16 rats were procured from the Animal House, DMIMS and were provided with pellet food and water ad libitum. Post experimental study, the rats were returned to the Animal House after rehabilitation.

Instrument: One Touch Horizone (USA) Glucometer was used to measure fasting blood (12 hour fasting) glucose levels at 0,3,7,14 and 21 days after daily administration of the extract orally. Blood samples were collected from the dorsal pedal vein.

Experimental induction of diabetes: Before inducing diabetes with single dose of Alloxan (120mg/kg body weight) intra peritoneal in rats, they were 12 hour fasted. Alloxan generates free radicals which damage the beta pancreatic cells inducing oxidative stress. Concentration of insulin decreases in the blood plasma as beta pancreatic cells store insulin. Rats were administered 30% glucose solution orally at regular intervals after 6 hours and 5% glucose solution in bottles were placed in their cage for twenty-four hours to prevent fatal hypoglycaemia. Blood glucose levels were recorded initially and 3 days after Alloxan injection to confirm evolvement of diabetes. After stabilizing the diabetic rats for 5 days, rats having blood glucose levels >250mg/dl were set apart and used for the study. Stable hyperglycaemia was induced after 4-5 days in the rats. The experiment began the next day (day 0).

Experimental design: Based on the recorded and observed experiments, the effective dose for HRS extracts was determined to be 2.0mg/kg BW whereas metformin was administered at 150 mg/70kg BW. 12 hour fasting blood sugar was taken at regular intervals. Overnight fasted diabetic rats were randomly divided into 4 groups of four rats each.

Group	No of Rats	Treatment (for 21 days)
I	4	Control, water ad libitum
II	4	Standard, oral metformin (0.002mg/gm)
III	4	oral HRS ethanolic leaves extract (0.002mg/gm)
IV	4	oral HRS ethanolic flowers extract (0.002mg/gm)

Statistical Analysis: Statistical analysis was done by using descriptive and inferential statistics using student's paired t test, one-way ANOVA and Multiple comparison: Tukey test and software used in the analysis were SPSS 22.0 version and Graphpad Prism 6.0 version and $p<0.05$ is considered as level of significance.

RESULTS

Table 1. Comparison of Fasting Blood Sugar in control group as compared to pre alloxan Student's paired t test

	Mean	N	Std. Deviation	Std. Error Mean	t-value	p-value
Pre Alloxan	78.70	4	0.95	0.47		
Post alloxan 72 hrs	314.50	4	9.98	4.99	47.091	0.0001
Day 0	306.25	4	15.41	7.70	29.514	0.0001
Day 3	304.00	4	13.85	6.92	32.661	0.0001
Day 7	300.50	4	14.05	7.02	31.605	0.0001
Day 14	301.75	4	16.74	8.37	26.590	0.0001
Day 21	301.00	4	14.44	7.22	30.655	0.0001

Table 2. Comparison of Fasting Blood Sugar in standard group as compared to pre alloxan Student's paired t test

	Mean	N	Std. Deviation	Std. Error Mean	t-value	p-value
Pre Alloxan	87.00	4	7.39	3.69		
Post alloxan 72 hrs	312.25	4	11.55	5.77	27.137	0.0001
Day 0	307.25	4	8.61	4.30	37.924	0.0001
Day 3	259.00	4	18.77	9.38	23.088	0.0001
Day 7	187.00	4	15.01	7.50	18.464	0.0001
Day 14	121.00	4	19.20	9.60	5.040	0.015
Day 21	66.50	4	11.81	5.90	7.137	0.006

Table 3. Comparison of Fasting Blood Sugar in leaves group as compared to pre alloxan Student's paired t test

	Mean	N	Std. Deviation	Std. Error Mean	t-value	p-value
Pre Alloxan	92.75	4	7.27	3.63		
Post alloxan 72 hrs	309.50	4	14.707	7.35	32.530	0.0001
Day 0	303.75	4	18.397	9.19	25.810	0.0001
Day 3	272.25	4	18.788	9.39	23.287	0.0001
Day 7	236.00	4	21.711	10.85	16.534	0.0001
Day 14	201.00	4	26.742	13.37	10.070	0.0002
Day 21	155.75	4	21.23	10.61	6.908	0.006

Table 4. Comparison of Fasting Blood Sugar in flowers group as compared to pre alloxan Student's paired t test

	Mean	N	Std. Deviation	Std. Error Mean	t-value	p-value
Pre Alloxan	88.00	4	7.39	3.69		
Post alloxan 72 hrs	309.75	4	12.44	6.22	29.749	0.0001
Day 0	306.75	4	11.64	5.82	25.027	0.0001
Day 3	268.25	4	13.86	6.93	17.273	0.0001
Day 7	226.50	4	24.61	12.30	8.672	0.003
Day 14	178.75	4	21.96	10.98	6.252	0.008
Day 21	140.50	4	10.21	5.10	5.980	0.009

Table 4. Comparison of Fasting Blood Sugar in four groups at day 21 Descriptive Statistics

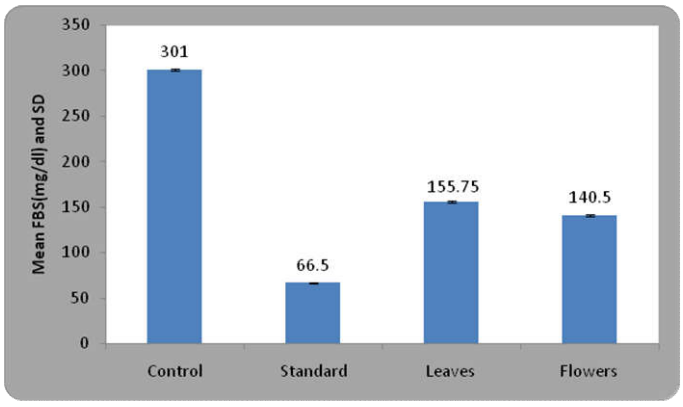
Group	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Control	4	301.00	14.44	7.22	278.01	323.98	280.00	311.00
Standard	4	66.50	11.81	5.90	47.69	85.30	50.00	78.00
Leaves	4	155.75	21.23	10.61	121.96	189.53	136.00	180.00
Flowers	4	140.50	10.21	5.10	124.24	156.75	128.00	153.00
Total	16	165.93	88.78	22.19	118.62	213.24	50.00	311.00

One-way ANOVA

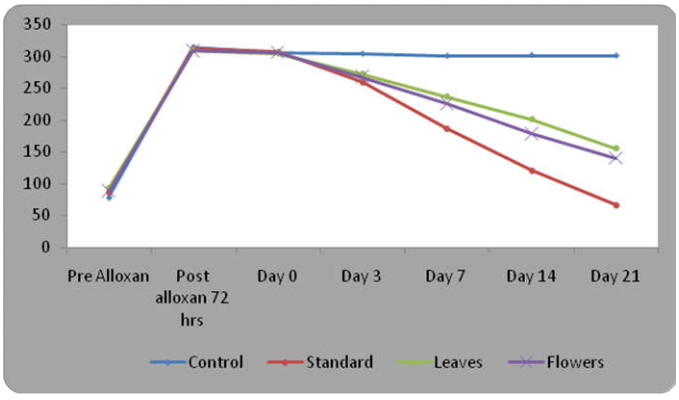
Source of variation	Sum of Squares	df	Mean Square	F	p-value
Between Groups	115522.18	3	38507.39	170.4	0.0001
Within Groups	2710.75	12	225.89	6	
Total	118232.93	15			

Multiple Comparison: Tukey Test

Group		Mean Difference (I-J)	Std. Error	p-value	95% Confidence Interval	
					Lower Bound	Upper Bound
Control	Standard	234.50	10.62	0.0001	202.94	266.05
	Leaves	145.25	10.62	0.0001	113.69	176.80
	Flowers	160.50	10.62	0.0001	128.94	192.05
Standard	Leaves	-89.25	10.62	0.0001	-120.80	-57.69
	Flowers	-74.00	10.62	0.0001	-105.55	-42.44
Leaves	Flowers	15.25	10.62	0.503	-16.30	46.80



Graph 1. Mean fasting blood sugar at Day 21



Graph 2. Mean fasting blood sugar over a period of 21 days

DISCUSSION

In this study, the anti-diabetic effects of ethanolic extract of HRS flowers and leaves were compared along with metformin in Alloxan induced diabetic rats for a period of 21 days. It is indisputable from the data collected that both the extracts and metformin produced consistent fall in blood glucose levels after continuous treatment but no such effect was observed in the control rats. Rats with continuous treatment of metformin for 21 days were at a higher risk of developing severe hypoglycemia as compared to the rats treated with the extracts highlighting the dangers of overdose of such drugs and the need to find a safe and efficient treatment. While both the extracts produced comparable suppression in blood glucose levels with the flower extract bring down the blood glucose levels closer to the pre-Alloxan levels then the leaves extract, the difference between them were insignificant to determine the most efficacious extract. The control and standard groups of rats consumed large amount of water as compared to the extracts treated groups. Alloxan damages the beta cells of pancreatic islets and produces triphasic blood glucose response (https://www.researchgate.net/publication/287289833_Different_models_used_to_induce_diabetes_A_comprehensive_review) . HRS has a chemical composition of anthocyanins, ascorbic acid, tannins and phenolic acids (Falade *et al*., 2009). These chemical components most probably produce the observed effects by preventing mucosal damage of beta cells, prevention of oxidative stress and regeneration of beta cell of Islets of Langerhans (Pethe *et al*., 2017; Bhaskar and Vidhya, 2012). This leads to increased insulin secretion and peripheral utilization of glucose eventually leading to decrease in blood glucose levels. Thus, the antidiabetic activity of HRS extract might be due to the presence of secondary metabolites having one or more of the anti-hyperglycemic properties and their harmonious effects (Bhaskar and Vidhya, 2012).

Conclusion

Thus from the above findings, it is crystal clear that HRS extracts have anti-diabetic property without displaying any risk of serious side effect such as severe hypoglycemia as recorded in metformin treatment. Further studies involving larger sample size and clinical studies are required to consolidate the claim.

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Conflict of interest: Declared none

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