Hypoglycemic and hypocholesterolemic activity of leave of few medicinal plants against steptozotocin induced hyperglycemia

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Abstract: Diabetes mellitus (DM) is universal of the hormonal problem and Type II diabetes is foremost obstacle. Accessible management in medicine has numerous contrary paraphernalia. Medical flora shows an essential part in managing diabetes specifically in unindustrialized nations. The present study was done on leaves of *Rhazya stricta* Decane, *Adhatoda zeylanica*, *Berberis lycium* Royle and *Olea furrrignea*, whose methanolic extracts were used to check their hypoglycemic and hypolipidemic activity by using glucometer and kit method respectively in blood of male and female albino mice Balb C. Results showed that leaves of *R. stricta* were best for hypoglycemia (125.34±63.79mg/dl, 107.34±18.00mg/dl, 146.00±40.36mg/dl and 178.34±17.03mg/dl), hypocholesterolemia (147.88±21.83mg/dl and 125.89±14.03mg/dl) and triglycerides (103±8.88mg/dl and 89.±43.4mg/dl) in random and fasting conditions, in male and female mice respectively. All plant extract were most effective for hypoglycemia and hypocholesterolemia in female mice as compared to male mice. Moreover statistical analysis revealed that leaves of other plants were also effective but less than leaves of *R. stricta.* So this plant part and a combination of presently used plants can be used for further studies particularly among females by the purification of active compounds against hyperglycemia and hypercholesterolemia.

Keywords: Methanolic extract, leaves, hyperglycemia, hypercholesterolemia, *Rhazya stricta*, Albino mice,

INTRODUCTION

Diabetes is one of the prominent origins of bereavement in humans and animals. In animals it take place most habitually in the dog with an incidence of approximately 0.2%. According to National Institute of Diabetes and Endocrinology, Pakistan has an average of 7.6% diabetic population in it while in 2030, it will be 4th principal diabetic populace in the domain with about 13.8 million diabetic people. Presently among 88,000 diabetic people in Pakistan, 35,615 are men while 52,397 are women. In the indigenous Indian system of medicine, good number of plants were cited for the cure of diabetes and some of them have been experimentally assessed and dynamic principle were quarantined (Grover *et al*., 2002 and WHO, 1980). The ethanobotanical evidence informations state that about 800 plants may possess antidiabetic potential (Al-yahya *et al*. 1990). Among all type of diabetes, type 2 diabetes is main impediment due to adverse effects of treatment options in modern medicine. Many developing countries are curing diabetes mellitus by using medicinal plants because they have very low income to treat it with allopathic medicines (Jaya, 2013).

As many diabetic patients in the UAE use medicinal plants to treat diabetes with insulin or oral hypoglycaemic medicines, so effect of *Rhazya stricta* extract and glibenclamide, on the concentrations of glucose, insulin and glucagon in plasma and blood respectively, has been examined by simultaneous treatment of streptozotocin-diabetic rats. Amounts of $C_6H_{12}O_6$, insulin or glucagon in Control rats were not affected significantly with the extract at oral doses of 0.5, 20 and 4.0g kg for up to 4 hours extract post- administration while in diabetic rats it reduced the concentration of glucose after 1h (2 and 4g kg^{-}) and 2h (4g kg) which was supplemented by maximum increases in the level of insulin after 1, 2 and 4 hours of extract directions at doses of 2 and 4gkg^{-}. When the plant extract at doses of 0.5, 2 and 4g/kg/ day for six consecutive days, the glucose level was reduced by 6%, 8 % and 30%, one-to-one. These results may suggest that administration of the extract and glibenclamide simultaneously might badly interfere with glucose control in diabetic patients (Ali, 1997).

Oleuropeoside showed maximum hypoglycaemic activity of winter Olive leaf at 16mg/kg in Alloxan induced diabetetic rats due to potentiation of glucose-induced insulin release and increased peripheral uptake of glucose (Gonzalez *et al*., 1992).

According to Gulfraz *et al*., (2008), hypoglycaemic action of berberine has been publicised in quite a facsimiles in *Berberis lyceum* Royle and in old-style remedy, the complete pull out of this herb is second-hand far and wide to extravagance diseases. Ethanolic root extract from

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roots of *B. lyceum* was related with pure berberine using related dosages of apiece.

The present work done by Ilango, (2009) was aimed to discover the antidiabetic activity of leaf extracts of *Adhatoda zeylanica*, Medic (Acanthaceae). The CH_3Cl (CE), methanolic (ME) extracts and C_6H_{14} (HE) were considered for their antidiabetic activities at double unalike doses (100.00mg/kg and 200.00mg/kg B.W.).The revision of antidiabetic activity encompassed orientation of diabetes to rats of all groups by means of alloxan (100mg/kg i.p) followed by consequent handling with hexane extract, chloroform extract and Methanolic extracts, at two unalike quantities of per capita. The levels of blood glucose were then supervised by resources of GOD/POD processes. Outcomes were associated with customary remedy glibenclamide (10.00mg/kg/ day for single week).

MATERIALS AND METHODS

Materials

Collection of plant samples

The whole plant samples of *Rhazya stricta* Decane, *Adhatoda zeylanica*, *Berberis lycium* Royle and *Olea furrrignea* were collected in dully labelled (Name of plant sample, area of collection and date of collection) fine plastic bags from Khoshab (District Khoshab), Quaid-i-Azam University Islamabad, Ghorha Gali and Bahara Kahu Islamabad respectively. Plant samples were identified by expert taxonomist from the Department of Botany, Pir Mehr Ali Shah, Arid Agriculture University Rawalpindi, Pakistan and their voucher specimens were registered for future studies.

Animals

Assay was performed in the animal house of National Veterinary Laboratories, Chak Shahzad, Islamabad. Adult albino mice balb- C, weighing about 30-40g were used for assay. All mice were given a period of acclimatization for 30 days before starting the experiment. Each group had half males and half females. Mice were kept in separate cages to prevent mating of mice. All mice were fed with poultry feed No. 1 daily and free access to water. Animals labelled as abstaining were underprivileged of nutrition for minimum 8 hours before experiment but were permitted for free admittance for the consumption water. The study was approved by Ethics Committee of Pir Mehr Ali Shah, Arid Agriculture University Rawalpindi, Pakistan and National Veterinary Laboratories, Chak Shahzad, Islamabad.

Chemicals and solutions

The following equipment and reagents were used in this study: Weighing balance (Analytical Semi-Micro Balance 210g x 0.1mg, with Internal Calibration, manufacturer A and D), Blender, SHEL LAB Mini Shaking Incubator (Sheldon Manufacturing), Bench Top Lab. Centrifuge 5000 r.p.m. (Brushless Motor) Model: T-8BL, New Rotary Evaporator With 180dgree (RE-200A), Glucometer (EUSURE, SN: T044040130029). Methanol (98% Sigma), Double-distilled water and chromatographic grade methanol (Fisher Chemicals), Steptozotocin (STZ) Batch No.153378 BMAC (Merk Chemicals), 0.10M citrate buffer (pH 4.5), Glucophage (Merk Chemicals), H_2SO_4 and Standard cholesterol (Sigma Aldrich), o-phthalaldehyde reagent (Pickering's laboratories), 95.00% ethanol(Merk Chemicals), 33.00% (w/v) KOH, (Merk Chemicals)

Methods

Preparation of plant samples

The plant samples were shade dried for some days. Leaves were separated out and milled into residue with the support of a mortar and passed through 80 mesh sieve. Latter they were stored in air-tight bottles for additional use at room temperature.

Antidiabetic activity of crude extracts

Preparation of groups and induction of diabetes

Steptozotocin (STZ) was thawed in 0.10M citrate buffer (pH 4.5) at a concentration of 7mg STZ/ml of citrate buffer. Four groups of mice (n= 6) were prepared for the assay. Group 1 was serving as negative control or vehicle and was given only 0.4ml of 0.1M citrate buffer (pH 4.5) /Kg b.w of mice. Group 2 was given only 0.4ml of freshly prepared STZ /Kg b.w of mice and this group served as positive control for diabetes. Group 3 served as positive control for antidiabetic drug Glucophage (Prepared in 0.1 Molar citrate buffer (pH 4.5) at a concentration of 1mg glucophage/ml of citrate buffer). Group 4 to 7 were experimental groups and were injected with leave extracts at a dose of 10ug/kg b.w. of animal. Group 2-5 were made diabetic prior to one hour of induction of standard antidiabetic drug (for group 3 only) and plant extracts (for group 4 to 7 only). Diabetes was confirmed by the determination of fasting (8-12 hours) and random (after 2 hrs of feed intake) blood glucose concentration for 3 consecutive days post administration of Steptozotocin (Ahmed *et al*., 2015).

Preparation of crude methanolic extracts

Crude extracts of leaves of *Berberis lycium* Royle, *Olea furrrignea*, *Rahzya stricta* Decane and *Adhatoda zeylanica* were prepared in Agriculture Biochemistry lab, UIBB, PMAS AAUR, by dissolving 5g 80 mesh plant samples in 50ml 80% methanol. Supernatant was saved while residue was again passed through 80% methanol and again supernatant was saved. Residue was discarded. Supernatant was evaporated to make concentrated extracts and were saved at 4^oC for further assay.

Evaluation of hyperglycemia

Blood samples were collected from heart and tail tip of the mice.

Table 1: Level of glucose (mg/dl) in blood of selected mice before the induction of diabetes (OGTT)

Groups	Blood glucose levels (mg/dl)				
	0 min	30min	60min	90min	120min
Group 1 (Negative control)					
(M)	110.34±56.88	136±78.9	117±60.00	130.34±54.81	91±21.74
(F)	186.67±59.09	212.34±21.36	203.67±14.46	195.67±29.02	166.67±32.25
Group 2 (Diabetic control)					
(M)	151.67±14.64	313.34±63.50	236.34±68.38	245.67±87.37	120.67±51.29
(F)	259.67±53.45	234±17.22	237.34±14.21	249±76.39	185.67±24.54
Group 3 (Antidiabetic control)					
(M)	218±40.58	183±35.38	223.34±67.30	110.34±57.735	152.67±40.69
(F)	198.67±57.07	244.67±37.93	233.34±14.20	218±44.69	233.67±84.60
Group 4 (*R. stricta* Leave)					
(M)	110.34±56.88	136±78.93	117±60.008	130.34±54.81	120.67±51.29
(F)	259.67±53.45	234±17.22	237.34±14.21	249±79.39	185.67±24.54
Group 5 (*O. ferruginea* Leave)					
(M)	195.67±84.07	348.67±2.31	195±12.58	196.34±37.311	154.34±53.07
(F)	159.67±23.45	192.34±72.45	177.34±29.56	142.67±46.45	129±39.88
Group 6 (*A. zeylanica* leave)					
(M)	195.67±84.07	348.67±2.31	195±12.58	196.34±37.311	154.34±53.07
(F)	159.67±23.45	192.34±72.45	177.34±29.56	142.67±46.45	129±39.88
Group 7 (*B. lycium* Royle Leave)					
(M)	195.67±84.07	348.67±2.31	195±12.58	196.34±37.311	154.34±53.07
(F)	159.67±23.45	192.34±72.45	177.34±29.56	142.67±46.45	129±39.88

Comparison of diabetic and non- diabetic groups of animals and various plant extracts ± = Mean of 3 replication N= 3

Blood glucose level

Blood glucose levels have been measured by pre calibrated Glucometer.

Oral glucose tolerance test (OGTT)

For the 6 hours fast, animals were brought up to the lab between 8- 9 am. Animals were kept on the same bench where experiments were performed so that they could be accustomed to the area to reduce stress during the procedure. Weighed each mouse to determine amount of glucose to inject and their backs were marked. In the morning before the procedure, 10% glucose solution was prepared. Blood glucose level was measured before experiment. 1 CC Syringe was filled with the glucose solution in a ratio of 1 unit glucose to 1gram of animal weight. Injected the mouse with glucose. As soon as mouse was injected, timer counting up from 0:00 and recorded the time and blood glucose level. Filled the syringe with glucose solution and grabbed second mouse to pick the tail tip with needle and blood glucose was checked by glucometer. Then injected the second mouse with glucose solution and recorded the time of injection and the original blood glucose level. Continued these steps until blood glucose was checked for all mice. Exactly after 30 minutes form the time of injection, the second blood glucose level was noted down by collecting down the blood from the first mouse injected when timer measured 30 minutes, and those mouse who were injected at 1 minute, their blood was collected from second mouse when timer measured 31 minutes and so on for the rest of mice. Waited for 60 minutes and blood of those mouse who were injected at 1 minute was collected form second mouse when timer measured 61 minutes and so on for the rest of mice. Waited for 120 minutes from the time of injection to take third blood glucose level and then collected blood from the first mouse injected when timer measured 2 hours and if mouse was injected at 1 minute, blood was collected from the second mouse when timer measured 2 hours and one minute and so on for the rest of mice.

Serum lipid profile

Seven test tubes was categorized, alpha, beta for test 1, chalie, delta for test 2, epsilon, gamma for the standard, and gega for the blank and 1.0ml of sample was added in tube A and B, followed by the addition of samples in tubes C and D. Standard cholesterol was added in tube E and F while distilled water was added in tube G. Then cholesterol reagent was added in all tubes and tubes were gestated at 25^{o}C for twenty minutes. Then 1.0ml of H_2SO_4 was supplemented to each tube. All tubes were nurtured in a water bath at 25^{o}C for 15.00 minutes. They were disinterested from the water bath and shake forcefully. Absorbance was dignified after 10 minutes for the samples in contradiction of the blank at 610nm. Calculation was done by using following formulas:

Table 2: Level of glucose (mg/dl) in blood of mice after the induction of Streptozotocin (STZ)

Groups		Normal	STZ induced	Glucophage induced	Plant extract induced			
					R. stricta Leaves	*O. ferruginea* Leave	*A. zeylanica* leave	*B. lycium* Royle Leave
R	M	156.34±10.16	322±42.15	104.67±36.29	125.34±63.79	135.34±27.30	198.67±57.97	193±35.79
	F	117.67±18.23	210.34±86.75	92.95±9.5	107.34±18.00	211.34±28.02	194.67±12.42	162.34±41.78
F	M	181.33±24.66	358.33±43.34	84.67±21.78	146.00±40.36	171.34±38.08	232±76.97	160.34±53.16
	F	148.66±19.29	238.34±68.88	85±25.06	178.34±17.03	267.67±10.41	200±48.14	202±4.35
DMRT Resuls		151.0^{D}	307.3^{A}	91.58^{E}	159.8^{CD}	193.9^{BCD}	206.3^{BC}	209.9^{B}

Comparison of diabetic and non- diabetic groups of animals and various plant extracts ± =Mean of 3 replications N= 3, R= Random, F= Fasting, M= Male, F= Female, N= 3, Data obtained after triplicate analysis, DMRT= Duncan's Multiple Range Test

Table 3: Level of total Cholesterol (mg/dl) after the induction of diabetes

Groups		Level of total Cholesterol (mg/dl)	DMRT Results
Group 1 (Negative control)	M	147.88±21.83	136.9^{CDE}
	F	125.89±14.03	
Group 2 (Diabetic control)	M	261.34±47.74	276.1^{A}
	F	290.89±34.25	
Group 3 (Antidiabetic control)	M	107.13±20.00	119.1^{E}
	F	134.31±31.01	
R. Stricta	M	147.88±21.83	136.9^{DE}
	F	125.89±14.03	
O. ferruginea	M	157.59±27.62	206.8^{B}
	F	189.13±72.83	
A. *Zeylanica*	M	161.34±18.34	168.3^{BCD}
	F	202.67±47.72	
B. lyceum	M	211.34±28.02	182.3^{BC}

Table 4: Level of Triglycerides (mg/dl) of selected diabetic mice

Groups		Level of total Cholesterol (mg/dl)	DMRT Results
Group 1 (Negative control)	M	135.31±19.33	130.3^{BC}
	F	146.44±38.49	
Group 2 (Diabetic control)	M	181.85±21.96	130.3^{BC}
	F	234.04±56.42	
Group 3 (Antidiabetic control)	M	143.15±24.39	130.3^{BC}
	F	128.93±14.65	
R. Stricta	M	103±8.88	130.3^{BC}
	F	89.±43.4	
O. ferruginea	M	105±20.27	130.3^{BC}
	F	92±15.39	
A. *Zeylanica*	M	106.67±11.54	130.3^{BC}
	F	89.34±53.59	
B. lyceum	M	133.28±21.86	130.3^{BC}
	F	102.69±9.99	

N= 3 ± = Data obtained after triplicate analysis DMRT= Duncan's Multiple Range Test

(Absorbance of sample/ Absorbance of standard) × 300
Alpha+ Beta/2, 0.222+0.189/2=0.411/ 2=0.2O55 SAMPLE 01.
Charlie+ Delta/2, 0.322+0.386/2=0.708/2= 0.354 SAMPLE 02.
Epsilon+ gamma/2,0.237+0.439/ 2=0.676/ 2=0.338 Standard.
The ordinary amount of cholesterol was 150.00 -250.00 mg/dl.

Blood cholesterol level

In a representative inspect, 0.1ml of plasma or serum, 0.3 ml of 33.00% (w/v) KOH and 3ml of 95.00% ethanol were positioned and assorted meticulously. The tube was then capped and engaged in a 60°C cooking wedge for 15 minutes. After the assortment has been chilled, 10.00ml of hexane was compellingly auxiliary to the tube to mix with the junior deposit and 3.00ml of distilled water was additional, capped and shake for 1 minute to confirm far-

reaching mingling. A blank, a standard and a sample of united plasma were saponified and haul out side by side. Apposite aliquots (generally 1ml) of the hexane layer were pipetted in replica into colorimeter tubes, and solvent was faded under nitrogen. 2.00ml of the o-phthalaldehyde reagent was supplementary to per capita and the way out was systematically diversified to thaw the entire sample. About 10.00 min after the totaling of the o-phthalaldehyde reagent, 1.00ml of concentrated H_2SO_4 was cautiously added by permitting it to run down the inside of the tube; the solutions were instantaneously varied on a tube vibrator. Then absorbance was recorded at 550nm amongst 10.00 and 90.00minutes after the accumulation of the conc. H_2SO_4.

STATISTICAL ANALYSIS

Results obtained were analyzed by applying analysis of variance (ANOVA) in which Completely Randomized Design (CRD) was used to compare the results between all plants used and then between males and female mcie. Moreover obtained data was further checked by applying Duncan's Multiple Range Test (DMRT) in MSTAT- C software.

RESULTS

Hypoglycemic activity of plant samples

Oral glucose Tolerance test (OGTT) was performed in animals to progress diabetic conditions. Among tested animals, those male mice were tested to develop diabetes whose glucose level of blood was more than 130 mg/dl while those female mice were used to develop diabetes whose blood glucose level was more than 220mg/dl in OGTT (table 1).

Results of Blood glucose concentration of tested animals has shown in fig. 1 that shows that in males during fasting condition, leaves of *R. stricta* reduced blood glucose level more rapidly than other plants because blood glucose concentration was found to be 146.00±40.36mg/dl while it was found to be 125.34±63.79mg/dl in fasting and random conditions respectively. These blood glucose levels were lowered almost as much as the Glucophage has done in which blood glucose was lowered up to 84.67±21.78mg/dl while it was found to be 104.67±36.29 mg/dl in fasting and random conditions respectively (table 2). Among female mice, concentration of blood glucose was reduced up to 162.32±41.078 in fasting while it was 107.34±18.00mg/dl in random conditions and these results are compatible to hyperglycemic activity of Glucophage (85.00±25.06mg/dl and 92. 95±9.5mg/dl in fasting and random conditions respectively) in female mice.

Moreover lowest blood glucose level effects were shown by *O. ferruginea* leaves in which blood glucose in male and female mice was found to be 135.34±27.30mg/dl and 211.34±28.02mg/dl respectively in random condition while it was 171.34±38.08mg/ dl and 267.67±10.41mg/dl in males and females respectively in fasting condition. These results showed that leaves of *R. stricta* are more effective to be used against hyperglycemia as compared to the rest of the plant samples.

Hypolipidimic activity of plant samples

Effects on Cholesterol

Concentration of cholesterol was most strongly reduced by *R. stricta* leaves (147.88±21.83mg/dl and 125.89± 14.03mg/dl) in both male and female mice respectively while leaves of *B. lycium* Royle were least effective (211.34±28.02mg/dl and 203.34±32.93mg/dl) in both male and female mice respectively. Statistical analysis of obtained results indicated that *R. stricta* leaves were reducing blood cholesterol level much more while *A. zeylanica* leaves were least in both male and female mice (table 3).

Effect on triglycerides

Similarly amount of triglycerides was most strongly reduced by *R. stricta* leaves in random conditions in blood of both male and female mice (103.00±08.88mg/dl and 89.00±43.4mg/dl respectively) while *B. lycium* leaves were least effective in reducing blood triglyceride levels in males and females respectively (table 4).

DISCUSSION

Results obtained in this study are compatible according to the work done by (Mohamed *et al*., (2006) who observed that extract of *R. stricta* Decne with oral doses of 0.50, 2.00 and 4.00g/kg abridged glycaemia after 1h (2 and 4 g/kg) and after 2h (4g/kg) when directed to Steptozotocin-diabetic rats. The insulin concentration amplified 1, 2 and 4 hours, subsequently induction of the extract of 2 and 4 g/kg. Handling of control creatures with the extract did not disturb insulin, glycaemia or glucagon levels for up to 4 hours, after the introduction of the extract. Concurrent handling of fit and diabetic rats with the extract (0.5, 2.0 and 5.0g/kg) and glibenclamide (5.0mg/kg) exaggerated the effects on insulin, glucose and glucagon. At prescribed amount of 0.5 to 2.0 and 4.0g/kg/day for 6 uninterrupted days showed reduction of glycaemia by roughly 6, 8 and 30%, in turn.

The blood cholesterol lowering behavior of *R. stricta* leaves was found to be parallel to the results indicated by Baeshin *et al*. (2010) who exhibited that, aqueous extract of the *R. stricta* leaves pointedly lessened concentrations of LDL-c, TGs, uric acid, cholesterol and creatinine, but amplified concentration of HDL-c.

These results about triglycerides were similar to the findings done by Ali *et al*. (2000b). Tanira *et al*. (1996b)

described that the leaves of *R. stricta* have been cast-off, between extra ailments, for the dealing of diabetes. Diabetic ills in the Arabian Gulf section frequently use water extracts of verdures for critical uttered management. While the extract at dose of 4g/kg fashioned a noteworthy and transitory proliferation in plasma insulin amounts, go together with a substantial drop in plasma glucose concentration.

CONCLUSION

In the present study focus was on Methanolic extracts of Leaves of four medicinal plants, *Rhazya stricta* Decane, *Adhatoda zeylanica*, *Berberis lycium* Royle and *Olea furrrignea.* for their hypoglycemic and hypolipidemic activities.. Results obtained revealed that leaves of *R. stricta* have shown best antidiabetic activity by reducing cholesterol, glucose and triglycerides in the blood samples of albino mice balb- C. Moreover this plant extract was most effective to reduce all these factors in female mice as compared to male mice. Whereas statistical analysis revealed that other plants were also effective to reduce blood glucose, cholesterol and triglycerides but their activities were lower than leaves of *Rhazya stricta.* So this plant part can be used for further studies by the purification of active compounds against hyperglycaemia, particularly among females.

Although all plants showed were more or less effective to reduce blood glucose, blood cholesterol, urea and glycosylated hemoglobin but leaves of *R. stricta* were most effective in this process. That's why *R. stricta* leaves were used to check their blood glucose lowering activity in *n- Hexane*, ethyl acetate, chloroform and water extracts.

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