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Research Article

Evaluation of Antidiabetic Activity of *Gymnema sylvestre* and *Andrographis paniculata* in Streptozotocin Induced Diabetic Rats

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ABSTRACT

Diabetes mellitus is a difficult metabolic disorder that has seriously impact the human health and quality of life. Medicinal plants are being used to control diabetes However, they are not entirely effective and no one has ever been reported to have fully recovered from diabetes. Many plants have been used for the management of diabetes mellitus in various traditional systems of medicine worldwide as they are a great source of biological constituents and many of them are known to be effective against diabetes. Medicinal plants with antihyperglycemic activities are being more desired, owing to lesser side-effects and low cost. Streptozotocin was induced to all groups of rats at dosage of 35 -55mg/kg except for the normal. Streptozotocin induced diabetes in sprague dawly rats were used to study antidiabetic activity of methonolic extract of two medicinal plants *Gymnema sylvestre,Andrographis paniculata* methanolic leaf extract was administered orally in graded doses of 30 mg/kg,50mg /kg sprague dawly rats *Gymnema sylvestre* at a dose of 30mg/kg and *Andrographis paniculata* at a dose of 50mg/kg showed significant anti-hyperglycemic and anti-oxidative effect which was evident from the 1st week of treatment.

Keywords: Diabetes, Sprague Dawly, Streptozotocin, *Gymnema Sylvestre, Andro Graphis Paniculata.*

INTRODUCTION

Diabetes mellitus (pronounced /or—often simply referred to as diabetes—is a condition in which a person has a high blood sugar (glucose) level as a result of the body either not producing enough insulin, or because body cells do not properly respond to the insulin that is produced. Insulin is a hormone produced in the pancreas, which enables body cells to absorb glucose, to turn into energy. If the body cells do not absorb the glucose, the glucose accumulates in the blood (hyperglycemia), leading to various potential medical complications.

There are many types of diabetes, the most common of which are:

Type 1 Diabetes: results from the body's failure to produce insulin and presently requires the person to inject insulin.

Type 2 Diabetes: results from insulin resistance, a condition in which cells fail to use insulin properly, sometimes combined with an absolute insulin deficiency.

Gestational Diabetes: is when pregnant women, who have never had diabetes before, have a high blood glucose level during pregnancy. It may precede of type 2 DM.

Other forms of diabetes mellitus include congenital diabetes, which is due to genetic defects of insulin secretion, cystic fibrosis-related diabetes, steroid diabetes induced by high doses of glucocorticoids, and several forms of monogenic diabetes.

All forms of diabetes have been treatable since insulin became medically available in 1921, but a cure is difficult. Pancreas transplants have been tried with limited success in type 1 DM; gastric bypass surgery has been successful in many with morbid obesity and type 2 DM; and gestational diabetes usually resolves after delivery. Diabetes without proper treatments can cause many complications. Serious long-term complications include cardiovascular disease, failure, and retinal. Adequate treatment of diabetes is thus important, as well as blood pressure control and lifestyle factors such as smoking and maintaining a healthy body weight.

MATERIALS AND METHODS

500g of the coarsely powdered leaves of *Gymnema sylvestre and Andro graphis paniculata* was packed in cheese cloth pouches so as to be inserted into the assembly of soxhlet apparatus the individual weight of each cheese cloth pouch was noted. The extraction process was continued using hexane and methanol as solvents successively for 48 hours. The temperature was strictly maintained at 30±0.5°C to prevent the evaporation of volatile components present, if any. The resultant extracts were further concentrated using Rotary Vacuum Flash Evaporator at 30°C to get a constant volume.

Anti-diabetic Activity

Animals

Twenty two male mouse of SD strain weighing 125-190gms were housed individually in plastic cages with free access to water and food throughout the experimental period. The standard laboratory conditions of light and temperature at 25-27ºC and 55% relative humidity are maintained (*Grijesh Kumar Mall, Pankaj et al.,* 2009)[1].

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Table 1: Assay procedure of glucose estimation.

Pipette into test tube labeled as	Blank	Standard	Test sample
Sample	-	-	10 micro lit
Standard	-	10 micro lit	-
Enzyme reagent	1.0 ml	1.0 ml	1.0 ml

The animals were randomly divided into control and diabetic groups.

Experimental Groups

22 mouse selected from an inbred colony were divided into 4 groups. Streptozotocin was induced to all groups of rats at dosage of 35 -55mg/kg except for the normal (A. Akbarzadeh, D. Norouzian et al., 2007).[2]

Group I (Normal): Mouse of this group did not receive any induction and treatment. (4 mouse in this group)

Group II (Control): Mouse was induced diabetes with STZ. (6 mouse in this group)

Group III (STZ +*Gym*): Mouse was induced diabetes with STZ and then treated with *Gymnema sylvestre* leaf extract. (6 mouse in this group)

Group IV (STZ + *Andro*): Mouse was induced diabetes with STZ and then treated with *Andrographis paniculata* leaf extract. (6 mouse in this group)

Induction material

Streptozotocin is available as a dry-frozen, pale yellow, sterilized product. Streptozotocin (111 mg) is mixed with sodium citrate buffer (6 ml) and is adjusted for a p^H 4.5. The STZ – Na Citrate buffer solution should only be prepared immediately before injection as the drug degrades after 15 – 20 min in Na – Citrate buffer. Mouse should be kept for fasting four hours prior to STZ induction.

STZ – Na Citrate buffer solution is injected into the mouse through intraperitonial injection.

After 3- 4 days of induction, STZ induces diabetic condition by destroying β cells.

Dosage

Gymnema sylvestre – 30 mg/kg

Andrographis paniculata – 50 mg/kg

Plant extract preparation

Gymnema sylvestre and *Andrographis paniculata* are dissolved in 1 ml of distilled water.

Treatment

The treatment with *Gymnema sylvestre* and *Andrographis paniculata* leaf extract was given every day by oral feeding tubes for a period of 3- 4 weeks.

During the treatment, plasma glucose levels and lipid peroxidation levels are observed for every week.

Estimation of plasma glucose levels by GOD method (Tinder's method)

Blood samples were withdrawn from overnight fasted animals. The samples were centrifuged for 5000rpm for 5 mins at 4^o c in cooling centrifuge (Trinder P, 1969)3

Mix well after each addition and incubate at 37 0 C for 5 min. Read the absorbance of standard and test against reagent blank at 505 /670 nm by using spectrophotometer.

Table 2: Estimation of glucose levels.

Sample	Before Diabetes Induction (moles/ml)	After Induction with STZ (moles/ml)	1st week of treatment	2nd week of treatment	3rd week of treatment	4th week of treatment
C 1	96.4	104.7	100.7	98.3	96.3	95.9
C 2	74.8	105	102.6	97.8	82.3	83
C 3	82.6	89	96	86.6	99.3	97
C 4	98.2	104	98	109.9	100	99.8
DC 5	95.4	112.4	159	160	165	170
DC 6	89.8	98.8	152.3	159.3	159	162
DC 7	71.7	93.5	125	132.5	135	140
DC 8	86.2	96.2	133.2	140.5	141	145
DC 9	97.2	100.2	139.2	142.5	145	150
DC 10	98.3	110.4	138	141	145	153
G 11	86.3	100	115	101.3	100	99.6
G 12	101.4	112.3	103.8	99.8	96	94
G 13	98	108	110	96.4	95	92
G 14	100	123	120.3	93	93	91
G 15	95.92	119.8	124	107.5	105	100
G 16	95.8	110.7	121.9	105	97	95
A 17	101	118	114.3	101.2	94	89
A 18	88	126.7	120.6	106.4	104.8	102
A 19	92	113.7	101.9	96.1	95	92
A 20	97.2	110.17	111.3	104.3	103	99
A 21	95.92	119.8	124	107.5	105.6	101
A 22	96.88	121.2	122	106	107.3	102

C – Control, D C – Diabetic control, G – Treated with *Gymnema sylvestre,* A – Treated with *Andrographis paniculate.*

Table 3: Estimation of lipid peroxidation level.

Sample	Before Diabetes Induction (moles/ml)	After Induction with STZ (moles/ml)	1st week of treatment	2nd week of treatment	3rd week of treatment	4th week of treatment
C 1	2.1	3.26	4.0	4.6	5.2	5.5
C 2	3.73	3.5	4.2	5.4	5.8	6.0
C 3	3.9	5.3	5.7	4.2	5.9	6.3
C 4	2.6	4.33	4.5	4.63	5.2	5.6
D C 5	3.6	9.3	12	14.3	15.2	16.6
D C 6	2.9	10.6	14.1	16	17.2	18.1
D C 7	3.3	11	14.9	15.2	16.5	17.5
D C 8	4.2	9.33	15	16.9	17.7	18.9
D C 9	3.9	7.33	13.2	14.42	15.4	16.5
D C 10	3.7	8.1	13.6	14.2	15.6	16.8
G 11	3.9	8.2	10.2	6.5	5.8	4.8
G 12	3.5	10	9.5	5.3	4.8	4.3
G 13	4.6	9.2	15	8.9	7.5	6.5
G 14	3.2	12.2	9.82	6.7	5.3	4.8
G 15	3.3	9.5	9.5	6.1	5.7	4.9
G 16	3.6	10.6	9.2	7.5	6.8	5.4
A 17	4.4	8.2	9.65	8.7	7.2	6.5
A 18	3.8	6.3	8.9	7.6	6.8	5.4
A 19	3	9.1	7.0	5.96	5.2	4.9
A 20	3.4	7.2	9.01	7.28	6.5	5.2
A 21	4.8	6.5	7.00	6.51	5.8	4.8
A 22	4.2	7.6	8.6	7.9	6.5	5.6

C – Control, D C – Diabetic control,G – Treated with *Gymnema sylvestre,*A – Treated with *Andrographis paniculate.*

Table 4: Statistical analysis of glucose levels.

Subject	Before Diabetes Induction (moles/ml)	After STZ Induction	1st week of treatment	2nd week of treatment	3rd week of treatment	4th week of treatment
Control (normal healthy)	86.8± 10.8	100.02± 6.90	97.6± 5.65	98.04± 8.24	98.9± 8.3	99.7 ± 7.5
Gymnema	95.9± 6.58	111.6± 8.51	114.4± 7.5	98.9± 4.2	99 ± 11.4	100 ± 11.0
Andrographis	94.824± 4.988	117.67± 6.28	114.4± 8.6	103.1± 4.5	116 ± 4.3	127.6± 3.7
Diabetic Control	88.06± 10.14	100.24± 7.31	141.74± 13.8	146.9± 12.2	169.7± 5.66	185± 5.61

Calculations:

Glucose (mg/dL) =

Absorbance of test

---------- X Conc. Of standard (100 mg/dL)

Absorbance of standard

Estimation of per oxidation levels by using MDA (melondialdehyde) as a marker.

The amount of plasma MDA levels (Vadde Ramakrishna, Rama Jailkhani 2007)[4] 0.5 ml of plasma was made upto 1 ml with 0.9% saline and an equal volume of TCA (20%) was added and incubated at 37^0C for 20 min and centrifuged at 3000 rpm/10 min.1ml of protein free supernatant 250 µl of TBA was added and heated in water bath at 95 0 C for 1 hr till a faint color appears after cooling the intensity was read at 532 nm against water with colorimeter .

RESULTS AND DISCUSSION

Anti-diabetic activity

Twenty two male SD rats were selected and divided into four groups. Group I, II, III, IV constituting 4,6,6,6 rats respectively and the body weight, blood glucose levels were checked at regular time intervals. STZ is toxic to β cells and is widely used to induce diabetes in the animals. Administration of STZ induced, diabetes within a span of 4-5 days, the animals became progressively hyperglycemic. There was significant elevation of glucose and lipid peroxidation levels in the blood plasma of STZ induced rats when compared to that of normal ones (shown in tables 3 and 4) and due to the elevation of peroxidation levels there occurs a significant DNA damage in diabetic rats. In order to minimize all these effects of STZ, animals are treated with crude leaf extract of *Gymnema* and *Andrographis*. The administration of *Gymnema sylvestre* at a dose of 30mg/kg and *Andrographis paniculata* at a dose of 50mg/kg showed significant anti-hyperglycemic and anti-oxidative effect which was evident from the 1st week of treatment. The decrease in plasma glucose and MDA levels was significant on the 2nd week in the group

Table 5: Statistical analysis of MDA levels.

Subject	Before Diabetes Induction (moles/ml)	After STZ Induction	1st week of treatment	2nd week of treatment	3rd week of treatment	4th week of treatment
Control (normal healthy)	2.986±0.786	4.318±0.804	4.38±0.822	4.546±0.564	6.231±0.3	8.154± 0.3
Gymnema	3.7±0.57	9.8±1.483	10.764±2.377	6.7±1.34	7.612±1.0	10.41±0.9
Andrographis	3.88±0.729	7.46±1.18	8.312±1.231	7.386±1.034	10.9±0.9	9.715±0.7
Diabetic Control	3.58±0.506	9.512±1.432	13.84±1.2	15.3±1.9	17.51±0.7	20.4±0.6

treated with *Gymnema* when compared to that of *Andrographis.*

The blood glucose level of STZ-induced diabetic rat was significantly high compared with normal control (NC) group ($p< 0.01$), respectively. There was no significant differences in initial body weight of rats among groups. 30mg, *Gymnema sylvestre*, and 50mg *Andrographis paniculata* leaves methanolic extracts were given to rats glucose levels started decrease from the 2nd week and normal level glucose was observed after 4th week , *Gymnema sylvestre*, *Andrographis paniculata* highly effective against diabetic rats. MDA levels of diabetic rats decrease 1st week to 4th week as the blood glucose level was decrease in induced diabetic rats of all group. At the beginning, the body weight of rat in normal group increased regularly during the experiment. Compared to diabetic control rat, Diabetic Control group rats exhibited a significant loss in body weight to normal control mice.

CONCLUSION

Our investigation clearly demonstrates that methanolic extract of Gymnema and Andrographis leaves possess significant anti inflammatory and antioxidant properties. Methanolic extract was found to be more potent.

In conclusion, it is quite evident from our results that methanolic extract of *Gymnema* and *Andrographis* can be considered as a promising natural remedy for antidiabetic. Thus, the anti-inflammatory activity may be attributed to the presence of β-sitosterol and antioxidant activity can be attributed to the presence of phenolic compounds. However further studies are recommended to trace the active principle responsible and its possible mechanism of action.

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