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## ANTIDIABETIC ACTIVITY OF HYDROETHANOLIC LEAF EXTRACT OF *GYMNEMA HIRSUTUM* WIGHT & ARN IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

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### Keywords:

*Gymnema hirsutum* (Gh), STZ-induced diabetic rats, Glibenclamide (GB), Diabetic rats, Ethanolic extract and Hypoglycaemic activity

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**ABSTRACT:** The present study has investigated the hypoglycaemic effect of oral administration of 50% ethanolic leaf extract of *G. hirsutum* Wight & Arn in normal and Streptozotocin-induced diabetic rats. Albino Wistar rats (190 to 220 gm) were induced diabetic condition by administration of Streptozotocin (STZ) (55 mg/kg bw) intraperitoneally. 50% ethanolic extract of *G. hirsutum* Wight & Arn leaves (300 mg/kg bw /day) was orally administered to normal and STZ-induced diabetic rats (150, 300 and 450 mg/kg bw /day) for 30 days to determine hypoglycaemic and antihyperglycaemic activity. Serum and tissue biochemical analysis including fasting blood sugar level in normal and STZ-induced diabetic rats were investigated. Oral administration of 50% ethanolic extract of *G. hirsutum* Wight & Arn leaves (300 and 450 mg/kg bw/day) for 30 days exhibited a significant ( $p < 0.05$ ) reduction in serum Glucose (88%), Total Cholesterol (80%), Triglycerides (76%), HbA<sub>1c</sub> (75%) and in an oxidative stress. The findings of investigation showed similar to those produced by Glibenclamide (GB) at dose of 600 mg/kg bw /day for 30 days (positive control). Histopathological observation showed significant improvement in the rats treated with *G. hirsutum* Wight & Arn leaf extract. The results demonstrate that 50% ethanolic extract of *G. hirsutum* leaves possess potent antidiabetic activity in STZ-induced diabetic rats. Alkaloids, Flavanoids, Phenols and Tannins present in this plant might be responsible for the effect.

**INTRODUCTION:** Diabetes mellitus is often termed as diabetes. It is a metabolic disorder characterized by hyperglycaemia and with imbalance in serum lipids and proteins<sup>1</sup>. The number of people suffering from the diabetes globally will be increasing to 366 million by the year 2030<sup>2</sup>.

Diabetes associated with Cardiovascular, Neurological, Nephrological and increasing rate of childhood and adult obesity gives economic burden to the individual and their family.

Diabetes is likely to become even more prevalent over the forthcoming years<sup>3</sup>.

In modern medicine (Insulin, Sulphonylurea, Biguanides and Thiazolidinediones) side effect like hypoglycaemia is seen. Hence, many studies are required to investigate the plant principles to treat hyperglycaemia with better effect and safety<sup>4</sup>.

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*G. hirsutum* Wight & Arn a member of Asclepiadaceae family is distributed in southern part of Uttar Pradesh adjoining Madhya Pradesh, Bihar and Western Ghats of Nilgris in Tamil Nadu. Sathyavathi, (2007) <sup>5</sup> conducted a study in which folklore based village herbal healers were interviewed to know plants or plant products that are used for curing various diseases. Leaves of *G. hirsutum* Wight & Arn, which is one among such plants is said to be used to treat diabetes, infertility, inflammation and jaundice by both external and internal administration.

The present study was conducted to evaluate the effect of 50% ethanolic extract of *G. hirsutum* Wight & Arn leaves on hypoglycaemic and anti-hyperglycaemic activity in normal and STZ-induced diabetic rats and its effect on the vital organ.

## MATERIALS AND METHODS:

**Plant collection:** *G. hirsutum* Wight & Arn was collected from Keelkothagiri (Western Ghats) of Nilgiris in Tamil Nadu with the help of a Badaga family. Fresh leaves were washed with water and allowed to dry under shade.

**Preparation of extracts:** Dried leaves were made to coarse powder and used for preparing extracts. Benzene, acetone, ethanol, 50% hydroethanolic and water extracts were used for qualitative analysis. 50% hydroethanolic extract was chosen to study the activity. 200 gm leaf powder was mixed with 500 ml of 50% ethanol and allowed to 72 hrs with occasional shaking. The extract was filtered and the filtrate was evaporated. The residue was stored for further uses. Percentage yield of *G. hirsutum* Wight & Arn dry leaf extract was found to be 18.7%.

**Drug:** Glibenclamide (GB), Sulphonylurea group, an oral hypoglycaemic drug was used.

**Animal used:** Albino Wister rats, weighing 190-220gm were maintained in standard environmental condition (20 - 25<sup>0</sup>C) using acrylic cage. Fed them with standard rodent diet and water *ad libitum*. The experiments on animal were conducted in accordance with the IAEC and CPCSEA and our protocols were duly approved by the Institutional Ethical Committee (Reg No. 158/1999/CPCSEA).

## Experimental procedure:

**Induction of diabetes:** The animals were grouped and left for fasting overnight. Diabetes was induced by a single intraperitoneal injection of a freshly prepared streptozotocin solution (55 mg/kg bw) in 0.1M citrate buffer (pH 4.5) <sup>6</sup>. Animals with their Blood Glucose level (BGL) above 200 mg/dl were considered as diabetic rats <sup>7</sup>. The treatment was started on 4<sup>th</sup> day onwards, after STZ injection. The treatment was continued for 28 days.

## Experimental Design:

- (a) **Determination of hypoglycaemic effect in normal healthy rats:** Eighteen normal male rats were left for fasting overnight. These fasting animals were divided into 3 groups of six rats each. Fasting Blood Glucose (FBG) were evaluated in each group before starting experiment. Group -1 served as untreated normal control and group 2 and 3 were given 50% hydroethanolic leaf extract of *G. hirsutum* Wight & Arn (300 mg/kg bw/day) and Glibenclamide (600 µg/kg bw/day) respectively. Blood samples were collected from retroorbital bleeding and BGLs were tested in a week intervals for 30 days.
- (b) **Determination of hypoglycaemic effect in diabetic rats:** The overnight fasting rats were divided into five groups (Group 4,5,6,7 & 8) of six rats each. Pretreated – FBG levels of each group was evaluated. Group -1 and 4 served as normal and diabetic control respectively and received the vehicle only. Group 5, 6, 7 and 8 received 150, 300 and 450 mg/ kg bw/day of 50% hydroethanolic leaf extract of *G. hirsutum* Wight & Arn and Glibenclamide (600µg/kg bw/day) respectively. BGL of each group was evaluated in a weekly interval for 30 days.

All the rats were sacrificed and their serum, key tissues biochemical parameters and tissue architecture were examined.

**Statistical Analysis:** The data were expressed as mean ±SD. Statistical comparisons were performed by one – way analysis of variance (ANOVA), followed by Duncan multiple range test.

The results were considered statistically significant if the p – values are 0.05 or less.

## RESULTS AND DISCUSSION:

**Contents of Plant leaf extract:** Phytochemical constituents in the various solvent extracts of *G. hirsutum* Wight & Arn leaves are given in the **table 1**.

**TABLE 1: PHYTOCHEMICAL CONSTITUENTS OF *G. HIRSUTUM* WIGHT & ARN LEAF EXTRACT**

Sl. No.	Plant Constituents	Solvent Extracts			
		Water	50% ethanol	Benzene	Chloroform
1	Alkaloid	+	++	-	+
2	Flavonoid	-	+	+	-
3	Saponin	++	++	-	-
4	Phenolics	+	+	-	-
5	Tannins	++	++	-	-
6	Carbohydrates	+	+	-	-
7	Glycosides	++	++	-	-
8	Proteins	+	+	-	-
9	Steroids	-	-	+	+

+ = Presence; - = Absence

**Effect of *G. hirsutum* Wight & Arn leaf extract on FBG in normal rats:** Effect of *G. hirsutum* Wight & Arn leaf extract on BGL of normal rats is presented in **Table 2**. Oral administration of an optimum dose of the extract (300 mg/kg bw/day) for 28 days did not produce any significant hypoglycaemic effect on normal rats.

**Effect of *G. hirsutum* Weight & Arn leaf extract on diabetic rats:** For the selection of optimum dose for the diabetic animals, different doses of 50% ethanolic extract (150, 300 and 450 mg/kg bw/day) were used.

BGLs were evaluated in diabetic rats along with the standard drug Glibenclamide (600 µg/kg bw/day). Table 2 reveals the effects of different doses of *G. hirsutum* Wight & Arn leaf extract and GB on GBL in diabetic control and treated groups. A reduction of 94% in blood glucose level was observed at 600 µg/kg bw/day dose of Glibenclamide and 71, 90 and 95% reduction in Blood Glucose Levels were seen in doses of 150, 300 and 450mg/kg bw/day of 50% hydroethanolic leaf extract of *G. hirsutum* Wight & Arn respectively.

**TABLE 2: LEVELS OF BLOOD GLUCOSE, HbA1c AND SERUM INSULIN OF EXPERIMENTAL ANIMALS**

GROUPS	Blood Glucose mg/dL	HbA1c mg/dL	Serum Insulin µIU/L
Group 1 (NC) Normal control	109.50 ± 07.60	5.08 ± 0.24	14.50 ± 0.64
Group 2 NC – 300mg Gh	102.17 ± 4.88	4.72 ± 0.27	15.52 ± 0.38
Group 3 NC - 600µg GB	101.67 ± 11.02	4.83 ± 0.38	14.89 ± 0.42
Group 4 (DC) Diabet control	392.17 ± 18.80	9.73 ± 0.45	8.96 ± 1.00
Group 5 DC – 150mg Gh	195.67 ± 9.46	7.83 ± 0.38	12.17 ± 0.50
Group 6 DC – 300mg Gh	134.67 ± 13.79	7.98 ± 0.33	13.43 ± 0.78
Group 7 DC – 450mg Gh	122.83 ± 18.51	8.40 ± 0.42	13.59 ± 0.36
Group 8 DC - 600µg GB	128.50 ± 17.22	6.68 ± 0.52	12.88 ± 0.58

Values are mean ± SD of six samples in each group; Gh = *Gymnema hirsutum*; GB = Glibenclamide; NC = Normal Control; DC = Diabetic Control

**Levels of Serum and Tissue Biochemical:** The effect of repeated oral administration of 50% ethanolic extract of *G. hirsutum* leaves on STZ-

induced diabetic rats were shown in the following **Tables 2 & 3 and figures 1, 2 & 3**. Total Cholesterol, Triacylglycerol (TAG), LDL-

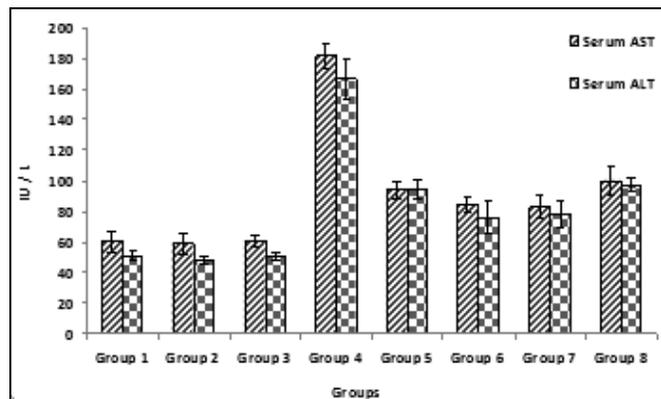
Cholesterol and VLDL-Cholesterol levels were significantly ( $p < 0.05$ ) reduced in the serum of treated groups (Table 3). Significant ( $p < 0.05$ ) increase in HDL-Cholesterol level was found in *G.*

*hirsutum* Weight & Arn leaf extract treated group. Normalisation of serum ALP, ALT, AST and LDH activity were found in *G. hirsutum* Wight & Arn leaf extract treated groups (Figure 1, 2 & 3).

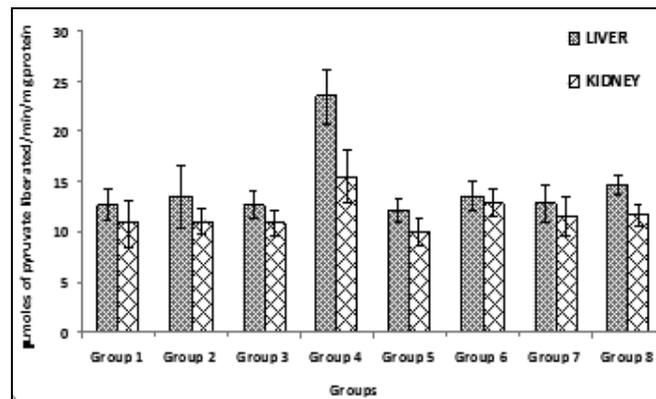
**TABLE 3: LEVELS OF SERUM TOTAL CHOLESTEROL, TAG, HDL, VLDL AND LDL-CHOLESTEROL**

Experimental Animal Groups	Serum Total Cholesterol mg/dL	Serum Triglyceride mg/dL	Serum HDL mg/dL	Serum VLDL mg/dL	Serum LDL mg/dL
Group 1 (NC) Normal control	88.83 ± 2.32	95.50 ± 12.73	38.50 ± 2.43	19.00 ± 1.16	31.33 ± 1.37
Group 2 NC - 300mg Gh	84.83 ± 5.64	92.50 ± 10.15	40.33 ± 1.75	18.50 ± 2.17	26.00 ± 7.27
Group 3 NC - 600µg GB	87.67 ± 9.79	91.33 ± 9.77	39.17 ± 1.17	18.17 ± 2.04	32.33 ± 7.89
Group 4 (DC) Diabet control	150.00 ± 11.87	159.83 ± 7.76	30.17 ± 5.04	32.00 ± 1.41	88.83 ± 6.44
Group 5 DC - 150mg Gh	114.83 ± 10.83	110.67 ± 8.05	40.33 ± 1.63	22.17 ± 1.94	55.67 ± 7.3
Group 6 DC - 300mg Gh	105.67 ± 6.98	105.50 ± 8.64	41.50 ± 2.07	21.50 ± 1.87	42.67 ± 8.8
Group 7 DC - 450mg Gh	116.00 ± 12.57	112.83 ± 5.56	39.83 ± 2.23	22.50 ± 1.05	57.17 ± 6.4
Group 8 DC - 600µg GB	108.83 ± 6.21	109.50 ± 6.16	31.33 ± 3.14	22.00 ± 1.26	77.17 ± 5.2

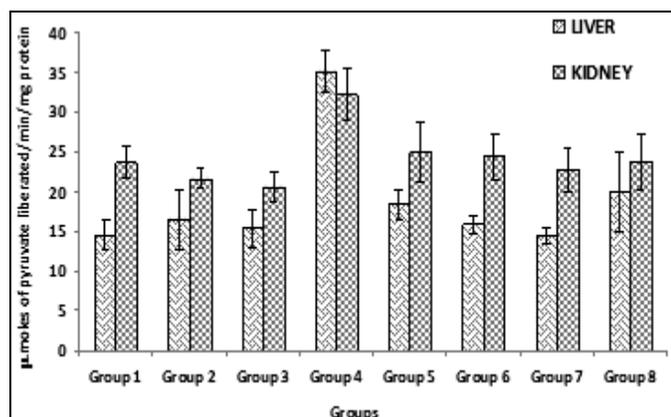
Values are mean ± SD of six samples in each group



**FIGURE 1: LEVELS OF ACTIVITIES OF SERUM AST AND ALT OF EXPERIMENTAL ANIMALS**



**FIGURE 3: LEVELS OF ACTIVITY OF ALT IN LIVER AND KIDNEY OF EXPERIMENTAL ANIMALS**



**FIGURE 2: LEVELS OF ACTIVITY OF AST IN LIVER AND KIDNEY OF EXPERIMENTAL ANIMALS**

**SUMMARY AND CONCLUSION:** Diabetes is a metabolic disorders with multiple clinical characteristics including chronic hyperglycaemia and disturbances in carbohydrate, protein and fat metabolism resulting from a decrease in circulating Insulin, a decrease in the response of peripheral tissues to Insulin or both<sup>8</sup>.

All types of diabetes increase the risk of long-term complications. The major long-term complications relate to damage to blood vessels and double the risk of cardiovascular diseases<sup>9</sup>.

The findings of this study revealed that the 50% hydroethanolic leaf extract of *G. hirsutum* Wight & Arn has no significant hypoglycaemic effect in normal rats. Good normalization in BGL was observed in 50% hydroethanolic leaf extract (300 and 450 mg/kg bw/day) of *G. hirsutum* treated groups. A dose of 600 µg/kg bw/day of GB for 28 days reduced BGL by 94% in STZ-induced diabetic rats.

The levels of serum lipids like Triacylglycerol (TAG), total Cholesterol and LDL-Cholesterol were significantly reduced in 50% hydroethanolic leaf extract of *G. hirsutum* treated groups. Phytochemicals like Alkaloids, Phenolics, Tannins and Glycosides are known to be bioactive principles for the management of diabetes<sup>10</sup>.

Thus, the significant antidiabetic effect of 50% hydroethanolic leaf extract of *G. hirsutum* may be due to the presence of more than one antihyperglycaemic principle and their synergetic properties. From this study we may conclusively state that *G. hirsutum* Wight & Arn leaves extract has remarkable effect on blood glucose level, and marked improvement on hyperlipidemia due to diabetes. Further pharmacological and biochemical investigations are underway to elucidate the mechanism of antidiabetic effect of *G. hirsutum* Wight & Arn leaves extract.

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#### REFERENCES:

1. Sheen J A: Drug treatment of non-insulin dependent diabetes mellitus in the 1990s. Achievement and future development. *Drugs* 1997; 54: 355-368.
2. Wild S G, Roglic R, Green H and King: Global prevalence of diabetes. Estimated for the year 200 and projection for 2030. *Diabetes Care* 2004; 27:1047 – 1054.
3. Shoback, David G, Gardner and Dolores: Greenspan's basic & clinical endocrinology. McGraw-Hill Medical, New York, Edition 9, Chapter 17: ISBN 0-07-162243-8. 2011.
4. Bailey C J and Day C: *Diabetes Care* 12, 1989; 553, doi: 10.2337/diacare.12.8.553.
5. Sathyavathi R: Folklore medicinal practices of Badaga community in Nilgiris biosphere reserve, Tamil Nadu, India. *Indian J Pha Res and Dev online (IJPRD)* 2007; 3: 50 -55.
6. Chen V and Ianuzzo C D: Dosage effect of streptozotocin on rat tissue enzyme activities and glycogen concentration. *Can. J. Physiol. Pharmacol* 1982; 60: 1251 – 1256.
7. Nyunai N, Manguelle-Dicoum B, Njikam and Abdennebi EI H: Antioxidant potential of aqueous leaf extract of *Ageratum conyzoides* Linn. In diabetic rats. *J. Pharmacog. And Phytotherapy* 2009; 1(4): 41-46.
8. Ali M A, Sultana M C, Rahman B M, Khatune N A and Wahed M I I: Antidiabetic activity of ethanolic extract of *Semecarpus anacardium* Linn stem barks in normal and alloxan induced diabetic rats. *IJPSR* 2012; 3(8): 2680-2685.
9. Hu E A, Pan A, Malik V and Sun Q: White rice consumption and risk of type 2 diabetes: Meta-analysis and systematic review. *BMJ (Clinical research ed.)* 2012; 344: e1454. PMID 22422870.
10. Ashwini C S, Pramod H J, Abhishekumar R, Geet P, Asnani M B and Patil: Antidiabetic activity of aqueous Extract of *Rotula aquatic* Lour Roots in Streptozotocin – induced diabetic rats. *JPSJ* 2012; (3): 14 – 17.