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EVALUATION OF ANTIDIABETIC ACTIVITY OF GLUCOVA - A HERBAL FORMULATION

BINA AGHERA, N. M. PATEL, S. B. BHATT, HARDIK SONI,*
HELENA MAKWANA AND BHAVNA SOLANKI

Vasu Research Centre, G.I.D.C., Makarpura, Vadodara – 390010 Gujarat (India)

Abstract: There are more than 125 million people with diabetes in the world. This study was carried out on a herbal formulation Glucova for its antidiabetic activity in alloxan induced diabetic rats. Glucova tablet and capsule had shown significant antidiabetic activity in comparison to the standard reference drug marketed with the name Glibenclamide on various parameters such as body weight, blood glucose, serum cholesterol, serum triglyceride, serum HDL, serum VLDL, serum LDL, serum urea, serum creatinine, serum ALPH, serum ALT, serum AST, serum bilirubin, serum total protein and albumin. It was observed to have almost parallel values for all the biochemical parameters estimated in comparison to reference standard drug - Glibenclamide. Furthermore, the histopathological analysis of pancreas showed that the herbal formulations Glucova tablet and Glucova capsule (GT and GC) retained the tissue degeneration in comparison to control group.

Keywords: Diabetes, Glucova, Glibenclamide, Histopathological study, Herbal formulation.

Introduction

Diabetes is a disorder of metabolism, the way our body use digested food for growth and energy. Most of the food, we eat is broken down by the digestive juice into a simple sugar called glucose. Glucose is the main source of fuel for the body. After digestion, the glucose passes into blood stream where it is available for body cells to use it for growth and energy. Glucose level in the body at particular level is very essential for good health. The glucose level is regulated by the regulatory hormone insulin produced by pancreas. If body does not make enough insulin or insulin secretion is very less, the glucose cannot be metabolized and stay in blood stream. The condition is called Hyperglycemia.[1,2]

There are more than 125 million people with diabetes in the world and by 2010 this number is expected to approach 220 million. It is also estimated that there are 30 to 33 million diabetics in India and every fourth diabetics in the world, is an Indian. Indians are genetically more susceptible to diabetes and the World Health Organization (WHO) predicts the number of diabetes in India would go up to 50 million by 2010 and 80 million by 2030.[3] Thus the need of antidiabetic herbal drugs are the need of the time. Herbal formulation i.e. Glucova tablet /capsule was evaluated for antidiabetic activity in this study.

Materials and Methods

Preparation of drug sample

Glucova tablets were crushed with motar pastle and made into fine powder. Cap and body of glucova capsule were removed and powder was used. Powder of both GT and GC which was weighing 900 mg individually made into fine powder with motar pastle and to it 20 ml of water were added and triturated to make the fine solution. Dose of glucova tablet and capsule was calculated on the basis of human dose.[4,5]

Contents and pharmacological review

The herbal contents, parts used and quantity of GT and GC are detailed in **Table A1** and **Table A2.**

Preparation of standard sample

Glibenclamide tablet was used as a standard drug. It was purchased from local market

* Corresponding author

weighing (5 mg each tablet) 150 mg and made into fine powder with mortar pestle and to it 30 ml of water was added and triturated to make a fine solution.[6]

Animals

Healthy albino rats of Wistar strain, weighing 150-250 gm of either sex were used for the study. The animals were housed in a two rat per polypropylene cages, maintained under controlled condition of temperature (25±1ºC), humidity (55±5%) and 12-hr/12-hr light/dark cycle. Animals had free access to standard pellet diet and purified drinking water *ad libitum*. All experiments and protocols described in present study were approved by the Institutional Animal Ethics Committee (IAEC) of B.M.C.P.E.R., Modasa and with permission from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

Experimental design

The experimental animals were divided into five groups, six animals each and drugs were given in following order:

Group A: Healthy normal animals received only the vehicle (Tap water) served as Normal Control (NC).

Group B: Untreated diabetes induced animals (Alloxan monohydrate 120 mg/kg b.w, i.p) served as a Diabetic Control (DC).

Group C: Diabeties induced animals and treated with standard drug (Glibenclamide 5 mg/kg b.w, p.o/day) served as Reference Standard (RS).

Table A1. Contents of Glucova tablet - an Ayurvedic medicine.

Sr. No.	Common Name	Botanical / Latin Name	Part Used	Quantity (Each Tablet Contains)
1	Mamajjaka	*Enicostemma littorale*	Whole plant	15 mg
2	Vijayasar	*Pterocarpus marsupium*	Heartwood	30 mg
3	Jamun	*Syzygium cumini*	Seed	15 mg
4	Guduchi	*Tinospora cordifolia*	Stem	20 mg
	& powder of			
5	Pramehahar kwath	----	Formulation	40 mg
6	Chandraprabha	----	Formulation	40 mg
7	Excipient	----	----	Q.S.
8	Color:Titanium dioxide	----	----	----

Table A2. Contents of Glucova capsule - an Ayurvedic medicine.

Sr. No.	Common Name	Botanical Name / Latin Name	Part Used	Quantity (Each Capsule Contains)
1	Mamajjaka	*Enicostemma littorale*	Whole plant	40 mg
2	Vijayasar	*Pterocarpus marsupium*	Heartwood	40 mg
3	Guduchi	*Tinospora cordifolia*	Stem	40 mg
4	Karavellaka	*Momordia charantia*	Fruit	40 mg
5	Madhunashini	*Gymnema sylvestre*	Leaves	30 mg
6	Jamun	*Syzygium cumini*	Seed	15 mg
	& powder of			
7	Pramehahar kwath	----	Formulation	125 mg
8	Chandraprabha	----	Formulation	125 mg
9	Excipient	----	----	Q.S.

Group D: Diabetes induced animals and treated with Glucova tablet (45 mg/kg b.w, p.o/ day) served as Glucova tablet treated (GT).

Group E: Diabetes induced animals and treated with Glucova tablet (45 mg/kg b.w, p.o/ day) served as Glucova capsule treated (GC)**.**

To investigate anti-hyperglycemic effect of trial drugs, rats were divided into 5 groups of 6 animals each and initial blood glucose (FBG)

Table A3. Containing pharmacological review of the contents of Glucova tablet & capsule.

Sr. No.	Latin Name (Common Name)	Pharmacological review	Reference
1	*Enicostemma littorale* (Mamajjaka)	i) An Aqueous extract has hypoglycemic activity in alloxan induced diabetic rats. ii) It has potent antidiabetic agent without any toxic effect at this particular dose. (1.5 g dry plant equivalent extract/100 g body wt.)	i) Vijayvargia *et al.*, 2000[7] ii) Maroo *et al.*, 2003[8]
2	*Pterocarpus marsupium* (Vijayasar)	i) An aqueous extract of it showed statistically significant hypoglycemic activity in alloxan-induced diabetic rats. ii) It showed a novel antidiabetic mechanism on Pancreatic beta cell regeneration. iii) It showed anti-diabetic activity against streptozotocin (STZ) induced diabetic rats.	i) Mukhtar *et al.*, 2005[9] ii) Chakravarthy *et al.*, 1980[10] iii) Gayathri *et al.*, 2008[11]
3	*Tinospora cordifolia* (Guduchi)	*T. cordifolia* has significant effect on blood glucose and total lipid levels of normal and alloxan-diabetic rabbits.	Wadood *et al.*, 1992[12]
4	*Momordia charantia* (Karavellaka)	i) Orally administered *M. charantia* extract lower glucose concentrations independently of intestinal glucose absorption and involve an extra pancreatic effect. ii) A daily oral feeding *M. charantia* (MC) (200 mg/kg), for 40 days significantly reduce blood glucose concentration in streptozotocin (STZ)-diabetic rats.	i) Day *et al.*, 1990[13] ii) Grover *et al.*, 2001[14]
5	*Gymnema sylvestre* (Madhunashini)	i) Extracts of *G. sylvestre* (GS) may have therapeutic potential for the treatment of non-insulin-dependent diabetes mellitus (NIDDM), the results confirm the stimulatory effects of *G. sylvestre* on insulin release, but indicate that GS acts by increasing cell permeability, rather than by stimulating exocytosis by regulated pathways. ii) *G. sylvestre* as one of the treatment that is emerging as a potential panacea for the management of diabetes. iii) *G. sylvestre*, *Momordia charantia* and *Eugenia jambolana* have been shown to possess hypoglycemic activity of varying degree.	i) Persaud *et al.*, 1999[15] ii) Leach *et al.*, 2007[16] iii) Khan *et al.*, 2005[17]
6	*Syzygium cumini* (Jamun)	i) It seeds showed hypoglycemic activity on streptozotocin-induced diabetes. ii) the water and ethanolic extracts showed antihyperglycemic effect in alloxan-induced diabetes. iii) It has evaluated hypoglycemic activity of the antioxidant saponarin, characterized as alphaglucosidase inhibitor present in *T.cordifolia* in maltose-fed rat.	i) Ravi *et al.*, 2004[18] ii) Sharma *et al.*, 2006[19] iii) Sengupta *et al.*, 2008[20]
7	Pramehahar kwath	In all type of diabetes	Siddha Yog Sangraha, Pg. No. 96, 1995[21]
8	Chandraprabha	it is used mainly in the treatment of diabetes and for urinary tract infections	Rastantrasar and Shiddhprayog Sangrah, Vol. I[22]

level was measured. Thereafter, one group-A received water which served as the normal control (rats are not diabetic) group; second group-B also received water which is served as diabetic control, the other groups, third group received standard drug and the groups (C, D and E) received trial herbal formulation-tablet and capsule with the dose of 45 mg/kg body weight orally (p.o.).

The animals were administered with the drugs for 7 days and on the 8th day, they were sacrificed. On day zero and after 7 days blood sample was collected from the retro-orbital plexus of 8 hours fasted and anesthetized animals by slight exposure to diethyl ether. One drop of blood was poured on test strip and rest of blood was filled in vial for the estimation of the biochemical parameters. Blood glucose was measured in all groups using "CONTOUR^{TM} TS" glucometer in the unit of mg/dl manufactured by Bayer Polychem India Ltd.

The blood was collected for biochemical parameters and some organs were dissected for histopathological analysis. The biochemical parameters were done at Param Path. Lab., Himatnagar and slides for histology were prepared at Satyam Lab., Modasa (India).

Biochemical Parameters

1. SGOT
2. SGPT
3. Urea
4. Total Protein
5. Albumin
6. Bilirubin
7. Alkaline Phosphatase
8. Cholesterol
9. Triglyceride
10. HDL Precipitating Reagent with Standard

Histopathological study : Pancreas

Statistical analysis

Results are presented as mean ± SEM of 6 animals. Statistical differences between the means of the various groups were evaluated using one-way analysis of variance (ANOVA) followed by Dunnett test using graph pad prism software. The significance difference, (if any) among the groups at p value < 0.05 was considered statistically significant.

Results and Observations

In the present experimental study, the anti-diabetic effect of GT and GC was assessed by body weight, blood glucose level, different biochemical parameters and histopathological study in rats.

Alloxan induced rats treated with GT and GC (45 mg/kg b. wt. p.o.) for 7 days could prevent loss of body weight. Results indicate that glucova tablet and glucova capsule significantly prevent the loss of body weight in experimental diabetic rats. Glibenclamide also significantly prevent the loss of body weight in diebetic rats **(Table A4).**

Alloxan induced diabetic rats treated with GT and GC (45 mg/kg b. wt. p.o.) for 7 days showed decrease in the blood glucose level **(Table A5).**

Results indicate that glucova tablet showed significant decrease in blood glucose level from (345.25±4.49) to (276.80±4.76) and glucova capsule also decrease in blood glucose level from (358.50±3.75) to (256.30±4.32). Glibenclamide also decreases blood glucose level from (360.25±5.77) to (219.50±4.77). Results are

Table A4. Effect of Glucova tablet and Glucova capsule on body weight of alloxan induced diabetic rats.

Groups (Dose)	Body Weight (gm)		% Change
	0 day	7th day	
Normal Control	187.50 ±4.78	196.30 ±6.88	04.69 ↑
Diabetic Control	186.30 ±5.54	152.5 ±5.95	18.14 ↓
Glibenclamide (5 mg)	154.75 ±4.17	161.00 ±3.93	04.03 ↑$^{\#}$
Glucova Tablet (500 mg)	153.25 ±3.17	157.75 ±3.19	02.93 ↑$^{\#}$
Glucova Capsule (500 mg)	152.25 ±4.26	158.00 ±3.13	03.77 ↑$^{\#}$

Values are expressed as Mean ± SEM, n=6, ↑- Increase, ↓- Decrease,
#: P≤ (0.05) - significant when compared with normal control

Table A5. Effect of Glucova tablet and capsule on blood glucose levels in alloxan induced diabetic rats.

Groups (Dose)	Blood glucose (mg/dl)		% Change
	0 day	7th day	
Normal Control	078.00 ±1.95	077.00 ±3.02	01.28 ↓
Diabetic Control	352.75 ±4.26	362.0 ±2.16	02.62 ↑
Glebenclemide (5 mg)	360.25 ±5.77	219.50 ±4.77	39.90 ↓#*
Glucova Tablet (500 mg)	345.25 ±4.49	276.80 ±4.76	19.82 ↓#*
Glucova Capsule (500 mg)	358.50 ±3.75	256.30 ±4.32	28.50 ↓#*

Values are expressed as Mean ± SEM, n=6, ↑- Increase, ↓- Decrease
#: P≤ (0.05) - significant when compared with normal control
*: P≤ (0.05) - significant when compared with diabetic control

significant ($p < 0.05$) in comparison with diabetic control group.

Biochemical parameters like serum cholesterol, serum triglyceride, serum HDL, serum VLDL, serum LDL were studied and results shown in the **Table A6.**

The data obtained indicate that GT and GC significantly decrease the serum cholesterol, serum triglyceride, serum VLDL, serum LDL and increases the serum HDL level in alloxan induced diabetic rats.

Results effect of GT and GC on renal profile, total protein and albumin of alloxan induced diabetic rats are given in **Table A7.** Results indicate that there was a rise in serum urea level in alloxan induced rats. Glucova tablets,

Table A6. Effect of Glucova tablet and Glucova capsule on Lipid profile of alloxan induced diabetic rats.

Groups (Dose)	Biochemical parameters- Lipid Profile (mg/dl)									
	Serum Cholesterol	% Increase	Serum	% Increase	Serum HDL	% Decrease	Serum VLDL	% Increase	Serum LDL	% Increase
Normal Control	100.20 ±4.59	-	40.75 ±3.26	-	45.30 ±4.048	-	8.15 ±0.65	-	46.70 5±4.17	-
Diabetic Control	162.60 ±2.95	62.27	92.33 ±4.65	126.57	34.73 ±1.24	23.33	18.46 ±0.93	126.5	109.23 ±2.73	133.64
Glibenclamide (5 mg)	114.00 ±5.84	13.70*	51.97 ±3.97	27.53*	42.28± 1.55	06.66*	10.39 ±0.79	27.48*	61.40± 2.66	31.33*
Glucova Tablet (500 mg)	119.50 ±5.84	19.26#*	65.75 ±3.09	61.30#*	39.72 ±0.54	12.30*	13.15 ±0.62	61.34#*	66.68 ±5.91	42.63#*
Glucova Capsule (500 mg)	116.00 ±1.68	15.76*	59.50 ±3.01	46.00#*	41.75 ±0.86	7.83*	11.75 ±0.63	44.17#*	62.50 ±1.30	33.68#*

Values are expressed as Mean ± SEM, n=6; #: P≤ (0.05) - significant when compared with normal control; *: P≤ (0.05) - significant when compared with diabetic control

Table A7. Effect of Glucova tablets and capsule on Renal profile, Protein and Albumin of alloxan induced diabetic rats.

Groups (Dose)	Biochemical parameters							
	Renal profile				Serum Total protein (g/dl)	% Decrease	Serum Albumin (g/dl)	% Decrease
	Serum Urea (mg/dl)	% Increase	Serum Creatinine (mg/dl)	% Increase				
Normal Control	046.50 ±4.29	-	0.63 ±0.083	-	06.58 ±0.045	-	04.55 ±0.14	-
Diabetic Control	110.25 ±6.57	137.09	1.07 ±0.083	69.84	04.23 ±0.025	35.98	02.44 ±0.12	46.37
Glebenclemide (5 mg)	054.00 ±5.87	016.12*	0.64 ±0.026	01.58*	05.47 ±0.076	16.86#*	03.43 ±0.09	24.61#*
Glucova Tablet (500 mg)	068.75 ±7.68	047.84#*	0.69 ±0.046	09.52*	04.53 ±0.026	31.39#*	02.80 ±0.08	38.46#*
Glucova Capsule (500 mg)	063.50 ±3.22	036.55*	0.66 ±0.072	04.76*	04.61 ±0.021	30.16#*	03.06 ±0.06	32.74#*

Values are expressed as Mean ± SEM, n=6 #: P≤ (0.05) - significant when compared with normal control; *: P≤ (0.05) - significant when compared with diabetic control

Table A8. Effect of Glucova tablet and Glucova capsule on Hepatic profile of alloxan induced diabetic rats.

Groups (Dose)	Biochemical Parameters-Hepatic Profile (mg/dl)							
	Serum ALPH	% Increase	Serum ALT	% Increase	Serum AST	% Increase	Serum Bilirubin	% Increase
Normal Control	047.00 ±1.65	-	056.75 ±5.96	-	063.40 ±2.49	-	0.17 ±0.012	-
Diabetic Control	156.25 ±6.10	232.44	149.75 ±4.09	163.87	107.35 ±3.04	069.32	0.47 ±0.040	176.47
Glibenclamide (5 mg)	052.00 ±6.79	010.63*	068.00 ±2.97	019.82*	070.00 ±4.65	010.41*	0.29 ±0.010	070.58#*
Glucova Tablet (500 mg)	077.64 ±6.67	065.19#*	089.93 ±4.34	058.46#*	083.00 ±4.41	030.91#*	0.37 ±0.020	117.64#*
Glucova Capsule (500 mg)	068.55 ±5.68	045.85#*	087.50 ±5.80	054.18#*	079.75 ±3.83	025.78#*	0.33 ±0.020	094.11#*

Values are expressed as Mean ± SEM, n=6 #: P≤ (0.05) - significant when compared with normal control; *: P≤ (0.05) - significant when compared with diabetic control
ALPH: Alkaline Phosphatase, ALT: Alanine Transaminase, AST: Aspartate Transaminase

Glucova capsule and Glibenclamide significantly reduced the elevated serum urea level. There was also rise in serum creatinine level in alloxan induced rats. Glucova tablets, Glucova capsule and Glibenclamide significantly reduced the elevated serum creatinine level. Results indicate that glucova tablet and capsule significantly increase the serum total protein and albumin in alloxan induced diabetic rats **(Table A7).**

Results of effect of GT and GC on hepatic profile include serum alkaline phosphate (ALPH), serum alanine transaminase (ALT), serum aspartate transaminase, serum bilirubin level of alloxan induced diabetic rats is given in **Table A8.**

Data indicates that GT and GC decrease the serum alkaline phosphate (ALPH), serum alanine transaminase (ALT), serum aspartate transaminase, serum bilirubin level in alloxan induced diabetic rats.

Histopathological studies of pancreas showed marked cytoarchitectural changes in diabetic control group which is reversed by GT, GC and Glibenclamide.

Conclusion

The present study data suggest that, GT and GC had shown significant antidiabetic activity in alloxan induced diabetic rats with comparison to the standard reference drug marketed with the brand name Glibenclamide for various parameters such as body weight, blood glucose, serum cholesterol, serum triglyceride, serum HDL, serum VLDL, serum LDL, serum urea, serum creatinine, serum ALPH, serum ALT, serum AST, serum bilirubin, serum total protein and albumin.

GT and GC was observed to have almost parallel values for all the biochemical parameters estimated in comparison to Reference Standard drug- Glibenclamide. Furthermore, the histopathological analysis of pancreas showed that the trial herbal formulations in capsule and tablet retained the tissue degeneration in comparison to the Diabetic Control group.

The herbal formulations GT and GC showed remarkable results in terms of quality and efficacy. GC was found to be more efficacious in comparison to GT. The observed change may be due to the addition of two more herbs *viz. Momordia charantia* (Karela) and *Gymnema sylvestre* (Madhunashini) in Glucova capsule, which are reported to have good antidiabetic potential.

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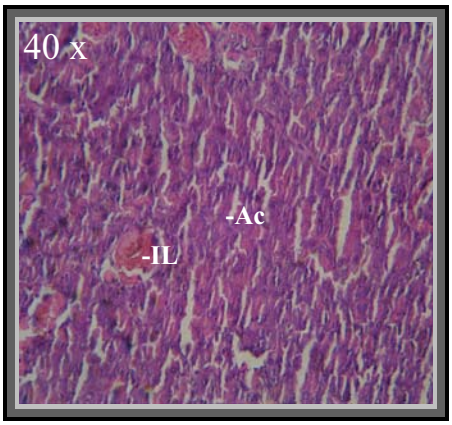
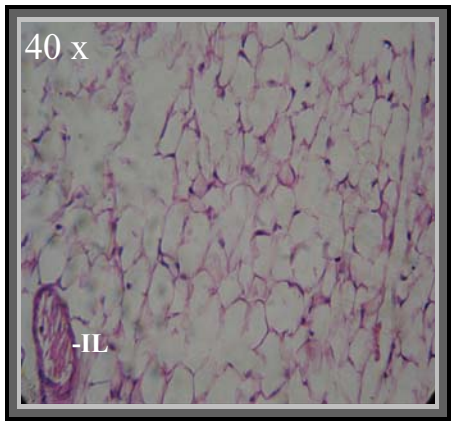
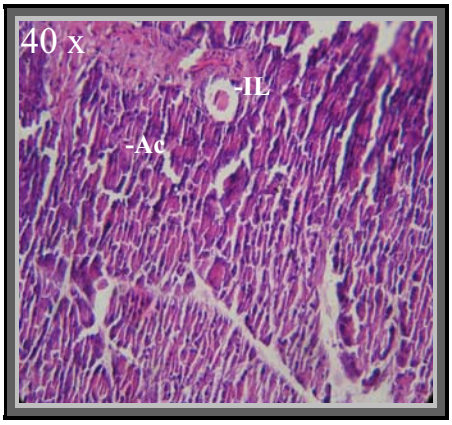


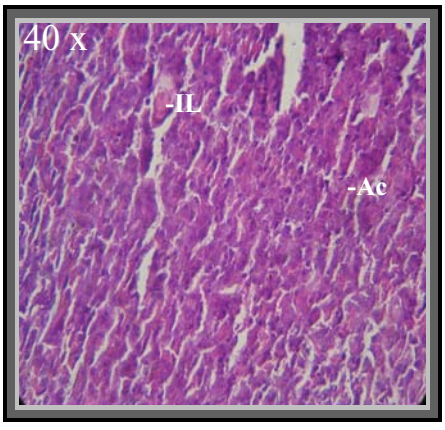
[A] Normal Control
Note: Normal Cytoarchitecture



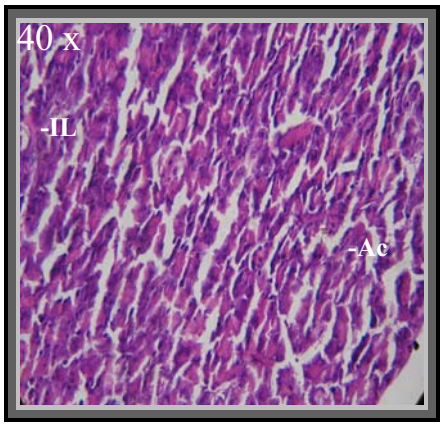
[B] Diabetic Control
Note: Severe islet cell degranulation



[C] Glibenclamide
Note: Almost Normal Cytoarchitecture with Mild degranulation



[D] Glucova Tablet
Note: Moderate-Severe degranulation



[E] Glucova Capsule
Note: Mild-Moderate degranulation

IL: Islet of Langerhans; **Ac:** Acini cells

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Address for correspondence: Mr. Hardik K. Soni, Sr. Executive, R & D, Vasu Research Centre, A Division of Vasu Health Care Pvt. Ltd., 896/A, G.I.D.C., Makarpura, Vadodara - 390010 Gujarat (India).
E-mail: hsoni@vasuhealthcare.com

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