

Effect Of Aqueous Extracts Of Leaves Of Globimetula Cupulata (Dc) Van Tieghem In Normoglycemic Rats

D Edem

Citation

D Edem. *Effect Of Aqueous Extracts Of Leaves Of Globimetula Cupulata (Dc) Van Tieghem In Normoglycemic Rats*. The Internet Journal of Alternative Medicine. 2008 Volume 8 Number 1.

Abstract

Globimetula cupulata leaves were evaluated for their hypoglycemic activity in rats. The animals were divided into 4 groups (n=6). Water extract of G. cupulata leaves was administered in graded doses of 0, 300, 600 and 900 mg kg⁻¹ body weight (wt) of experimental animals for 14 days. Blood samples were collected by cardiac puncture. The levels of blood glucose were significantly reduced (p < 0.05) by 35.79 – 43.48% on consumption of the extracts, with greater effect exhibited by the 900mg/kg body wt extract. Histological studies showed increases in the number and density of the pancreatic islet cells. The result suggested restorative (protective) effect of the extract on pancreatic islet cells. Administration of aqueous extract of Globimetula cupulata leaves may contribute significantly to the reduction of blood glucose levels by a pancreatotrophic action and possible potentiation of insulin secretion, which may be useful in the treatment of diabetes.

INTRODUCTION

Globimetula cupulata (a mistletoe) is a member of the family Loranthaceae. It is a parasitic shrub which grows on some dicotyledonous trees and attaches itself to the host by modified roots. The plant is found in African countries like Togo, Senegal, Benin Republic, Cameroon, Gabon, Congo Democratic Republic, Angola, Tanzania, Uganda, Southern Nigeria and south east of Burkina Faso^{1,2}. The leaves are short, 1 – 2 times as long as broad, large, thick and coriaceous, round –cordate at base, acute or obtuse at base. They are usually broadly ovate or elliptic, being 4.5 - 17 cm long and 3 – 12.5 cm broad, with petioles 6 -18 mm long¹.

Globimetula cupulata is a medicinal plant used by some traditional herbalists in the management of high blood pressure and diabetes mellitus³. Little information exists in the literature regarding the effect of the plant on blood glucose levels. Reports are lacking on the effect of this plant on tissue histology. Thus the present study was undertaken to explore the effects of G. cupulata leaf extracts on blood glucose levels and pancreatic histology of experimental animals. The study therefore, is aimed at investigating the scientific basis to the claims on the usefulness of the leaves of G. cupulata to lower blood glucose concentrations.

MATERIALS AND METHODS

(a) Animals

Twenty four (24) male Wistar albino rats weighing 156.3 – 337.1g were

obtained from the Animal House of the College of Health Sciences, University of Uyo, Uyo, Nigeria. The rats were kept in clean and only dry plastic cages, with 12hrs light-dark cycle at 25 + 2 °C and 45-55% relative humidity. The animals were fed with pelletized commercial rat chow (Pfizer Livestock Co. Ltd, Aba, Nigeria), without any restriction to food and drinking water during the experimental period.

The rats were assigned into 4 groups of 6 rats each. Rats in group 1 were the control which did not receive any extract. Animals in the test groups (2, 3 and 4) were orally fed once daily with 300mg/kg, 600mg/kg and 900mg/kg body weight (wt) extracts respectively. Administration of the extracts was carried out for 14 days. All animals were treated in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals⁴.

Collection of Plant Materials

Samples of G. cupulata leaves were obtained from Itak Ikot Akap village in Ikono Local Government Area of Akwa Ibom State in Nigeria. The plant material was authenticated by a taxonomist Dr (Mrs.) M.E. Basse of the Department of Botany and Ecological Studies, University of Uyo, Uyo, Nigeria. A voucher specimen with number 'Agbai UUH 849

(Ikono)' was deposited in the herbarium of the University of Uyo. The samples were washed with clean tap water to remove dirt on the leaves. After the leaves were kept for 2 hrs for the water to dry off, a sharp stainless steel knife was used to cut them into small pieces. They were then dried to constant weight in an oven (Stuart Scientific, UK) at 55 C for 24 hours. After drying, the leaves were ground into fine powder (which passed through a 30 – mesh sieve) and stored in air containers at 4 C until when required.

(c) Preparation of Aqueous Extract of G. cupulata leaves

One hundred grams (100g) of the powdered material were brewed in

750ml of boiled tap water and allowed to stand for at least 30 minutes. The decoction was then filtered (through a plug of cotton wool) and stored in a clean bottle to be administered to rats. Twenty millilitre (20ml) aliquots of the decoction were dried at 50 C to constant weight using a rotary evaporator. The residue left was then used to determine (by gravimetric method) the concentrations of the mistletoe extract administered to different groups of the experimental animals. One milliliter (1 ml) of the extract produced a residue of 50.0mg. 1.10ml of the solution administered to 184.3g rat was equivalent to 300mg/kg body wt extract. Other doses per wt of rats were determined accordingly.

(d) Animal Treatments

The 4 group of rats were as follows: GLE -1, GLE -2, GLE -3 and NC. The groups GLE -1, GLE -2 and GLE -3 were administered with 300, 600 and 900mg/kg body wt of the extract, while the NC group which served as normal control was not given any extract. All treatments were given for 14 days, by oral gavage.

(e) Collection and Treatment of Blood Samples

After 14 days, the animals were anaesthetized under chloroform vapour. Blood samples were obtained by cardiac puncture. Aliquots of the blood were poured into screw-cap sample bottles containing fluoride /oxalate anticoagulant for blood glucose determination. All analyses were carried out within 24 hrs of blood collection.

(f) Blood Glucose Analysis

Blood glucose concentration was estimated by glucose oxidase method, using a reagent kit from Randox Laboratory Ltd, UK.

(g) Histopathological Study

On the last day of experiment, the tail parts of the pancreas were removed and kept in 10% formaldehyde. Tissue processing was carried out by autotechnicon and the prepared 5µ thick sections were mounted on slides and stained with hematoxylin and eosin (H & E). Stained sections were morphologically evaluated.

(h) Statistical Analysis

All data were expressed as means ± SD. Student's t – test was used to compare the mean values of test groups and control. Differences in mean values were considered significant at p < 0.05.

RESULTS

The blood glucose concentrations in the experimental animals were 4.25 – 4.75 mmol/L before the start of the study. In comparison with the NC group, which had 4.15 mmol/L glucose, the other experimental groups had significantly lower final blood glucose concentrations of 2.60 – 3.05 mmol/L. (p < 0.05). No significant differences in blood glucose concentrations were observed between groups which received the plant extracts (p < 0.05). However, there were dose-dependent decreases in blood glucose concentrations with increasing levels of extracts.

Figure 1

TABLE 1

Plasma Glucose Concentrations	Experimental Group			
	NC 0 mg/kg body wt	GLE-1 300 mg/kg body wt	GLE-2 600 mg/kg body wt	GLE-3 900mg/kg body wt
Initial (mmol/L)	4.25 ± 0.45	4.75 ± 0.26	4.48 ± 0.50	4.60 ± 0.91
Final (mmol/L)	4.15 ± 0.26 ^a	3.05 ± 0.57 ^b	2.63 ± 0.15 ^b	2.60 ± 0.14 ^b
% reduction	2.35	35.79	41.18	43.48

Hypoglycemic Effects of Aqueous Extract of Globimetula cupulata leaf *

Legend: NC = Normal Control; GLE-1 = Globimetula leaf extract Group 1

GLE-2 = Globimetula leaf extract Group 2; GLE-3 = Globimetula leaf extract Group 3

*Values are means \pm standard deviation (n = 5). Values in same row with different superscripts in a horizontal row represent means that are significantly different (p < 0.05).

Treatment of the GLE groups of rats with 300, 600 and 900mg/kg body wt of *G. cupulata* leaf extracts resulted in significant reductions (p < 0.05) of blood glucose (by 35.79, 41.18 and 43.48 % respectively), when compared with the NC (a group without extract treatment), which had 2.35% reduction.

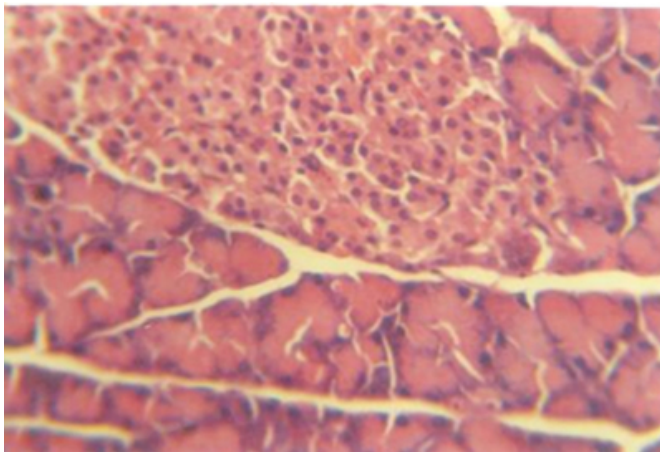
Effects of Consumed Extracts on Histopathology of Pancreas: Histomorphologic Changes of Pancreas.

The cellular integrity and architecture were intact in the NC group (Figure 1). Pancreas of the GLE-1 group which consumed 300mg/kg body wt extract (Fig. 2), showed some similarity to group NC which did not consume any extract.

FIGURE: Histological Sections of Pancreas

Figure 2

1 NC (Normal control, 0mg/kg body wt extract)



Pancreas of Normal Health Rat, H & E Staining (x 40)

Figure 3

2 GLE- 1(Globimetula leaf extract Group 1)



Pancreas of Rat Treated with 300mg/kg body wt extract, H & E Staining (x 40)

There was a relative increase of granulated and normal beta cells in the GLE-2 group which consumed 600mg/kg body wt extract (Fig 3), when compared with the GLE-1 group. However, the increases in size and number of islets especially around the central vessel were higher in the GLE-3 group (which consumed 900mg/kg body wt extract) than in the other experimental groups.

FIGURE: Histological Sections of Pancreas

Figure 4

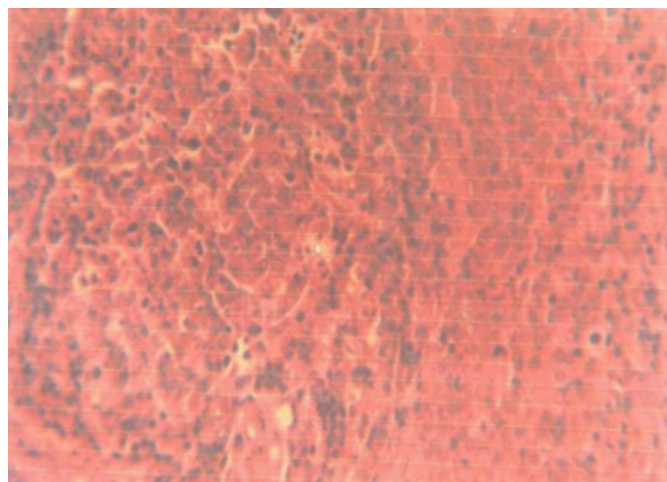
3 GLE -2 (Globimetula leaf extract Group 2)



Pancreas of Rat Treated with 600mg/kg body wt extract, H & E Staining (x 40)

Figure 5

4 GLE – 3 (*Globimetula* leaf extract Group 3)



Pancreas of Rat Treated with 900mg/kg body wt extract, H & E Staining (x 40)

DISCUSSION

The initial blood glucose concentrations of the experimental animals (4.25 – 4.75 mmol/L) is considered normoglycemic for the study^{5,6}. Furthermore, the percentage reduction in glucose levels was high for all groups treated with extract, when compared with that of the control. The results suggested hypoglycemic effects of the extracts. The findings may indicate the presence of some hypoglycemic agents in the leaves of *G. cupulata*, which have been concentrated in the extracts. The hypoglycemic effects of plants may be due to the presence of insulin-like substance in plants^(7,8), stimulation of β cells to produce more insulin⁽⁹⁾, inhibition of endogenous glucose production⁽¹⁰⁾, increasing glucose metabolism or regenerative effect of plants on pancreatic tissue¹¹. The maintenance of blood glucose levels within normal (narrow) limits (normoglycemia) is important, because it is essential to have a continuous supply of glucose to the brain, red blood cells and renal medulla, which have obligatory requirements for glucose in meeting their fuel needs¹².

The pancreas is the primary organ involved in sensing the organism's dietary and energetic states via glucose concentration in the blood. In response to elevated blood glucose, insulin is secreted. Insulin deficiency (or diabetes mellitus) causes excessive elevation of blood glucose and underutilization leading to hyperglycemia¹³. Hyperglycemia is the primary clinical manifestation of diabetes mellitus, a major degenerative disease affecting 3 – 5 % of the world's population¹⁴. It is thought to contribute to diabetic

complications by altering vascular cellular metabolism, vascular matrix molecules (especially glucose and lipids) and circulating lipoproteins¹⁵. The histopathological study of the pancreas indicated increased volume density of islets and increased percentage of beta cells, in the rats that received the extracts, which may be a sign of regeneration potential. Signs of regeneration of β cells, potentiation of insulin secretion from surviving β cells of the islets of Langerhans and decrease of blood glucose have been reported following consumption of some plant extracts^(11, 16, 17, 18).

G. cupulata leaves may have some chemical components that exert size and number-increasing effects on β cells, stimulate these cells to produce more insulin (pancreatotropic action) or may have some insulin-like substances. Beta cells secrete plenty of insulin to maintain glucose homeostasis and thus normally compensate for insulin resistance¹⁹. A higher dose of the extract has a greater potential restorative effect on the islet cells of rats than a lower dose of extract.

CONCLUSION

The findings of this study indicated that consumption of the *G. cupulata* leaf aqueous extract exerts significant hypoglycemic effect in rats. Histopathological studies of the pancreas of rat showed evidence of signs of β cell number and size increase in groups receiving extracts. These findings may support the traditional use of *G. cupulata* for controlling hyperglycemia in diabetics, in view of the restorative potential of the extract on pancreatic islet cells. Further investigation with longer period of higher dose may show clearer features of these findings.

References

1. Hutchinson, J. and Dalziel, J. M. (1958). Flora of West Tropical Africa, Volume 1 part 2, Crown Agents for Oversea Governments and Administrations, Millbank, London SW1, pp. 658 – 660. ISBN 0855 92027
2. Boussim, I.J., Guinko, C., Tuquet and Sallé, G. (2004). Mistletoes of the agroforestry parklands of Burkina Faso. *Agroforestry Systems* 60(1): 39 – 49.
3. Ojewole, J.A.O. and Adewole, S.O. (2007). Hypoglycemic and hypotensive effects of *Globimetula cupulata* (DC) van Tieghem (Loranthaceae) aqueous leaf extract in rats. *Cardiovascular Journal of South Africa* 18(1): 9 -15.
4. NRC (1985). National Research Council Guide for the care and use of laboratory animals. Publication no. 85 – 23 (Rev.). National Institutes of Health, Bethesda, MD.
5. Kaneko, J.J., Harvey, J.W. and Bruss, M.L. (1997). *Clinical Biochemistry of Domestic Animals*. 5th edn., New York, Academic Press, pp.895-899.
6. Jelodar, G., Mohsen M and Shahram S. (2007). Effect of

- walnut leaf, coriander and pomegranate on blood glucose and histopathology of pancreas of alloxan – induced diabetic rats. *African J. Traditional, Complementary and Alternative Medicines* (Afr. J. Trad. CAM) 4 (3): 299 – 305.
7. Collier, E., Watkinson, A., Cleland, C.F. and Roth, J. (1987). Partial purification and characterization of an insulin-like material from spinach and *lemna gibba* G3. *J. Biol. Chem.* 262: 6238 -6241.
8. Gray, A.M. and Flatt, P.R. (1999). Insulin – releasing and insulin – like activity of the traditional antidiabetic plant *Coriander sativum* (coriander). *Br. J. Nutr.* 81: 203-208.
9. Khan, A., Bryden, N.A., Polasky, M.N. and Anderson, R.A. (1990) Insulin- potentiating factor and chromium content of selected spices. *Biol. Trace Elem. Res.* 24:183-188.
10. Eddouks M, Jouad H, Maghrani M, Lemhadri A & Burcelin R (2003) Inhibition of endogenous glucose production accounts for hypoglycemic effect of *Spergularia purpurea* in streptozotocin mice. *Phytomedicine: International Journal of Phytotherapy & Phytopharmacology* 10 (6-7): 594-599.
11. Shanmugasundaram, E.R., Gopith, K.I., Radha, S.K. and Rajendram, V.M. (1990). Possible regeneration of the islets of Langerhans in streptozocin diabetic rats given *Gymnema sylvestre* leaf extract . *J. Ethnopharm* 30:265 -269.
12. Vasudevan, D.M. and S. Sreekumari, 2005. *Textbook of Biochemistry (For Medical Students)*. 4th Edn, Jaypee Brothers Medical Publishers (P) Ltd., New Delhi, India, pp. 101 – 114.
13. Standl, E., Ballestshoffer, B., Dahl, B. and Shagler, H. (2003). Predictors of 10 years macrovascular and overall mortality in patients with NIDDM: the Munich practitioner project. *Diabetologia* 39:540-545.
14. Schwartz, S. L. (2006). Diabetes and dyslipidemias. *Journal of Diabetes, Obesity and Metabolism* 8(4): 355 – 364.
15. Scoppola, A., Montecchi, F.R., Mezinger, G. and Lala, A. (2001), Urinary mevalonate excretion rate in type 2 diabetes: role of metabolic control. *Artherosclerosis* 156: 357-361.
16. Ayber, M.J., Riera, A.N., Grau, A. and Sanchez, S.S. (2001). Hypoglycemic effect of the water extract of *Smallanthus sonchifolius* (Yacon) leaves in normal and diabetic rats. *J. Ethnopharmacol.* 74: 125 -132.
17. Suba, V., Muragesan, T., Bhaskara, R.R., Ghosh, L., Pat, M., Mandal, S.C. and Saha, B.P. (2004). Antidiabetic potential of *Barleria lupulina* extract in rats. *Fitoterapia* 75:1-4.
18. Yadav, J.P., Saini, S., Kalia, A.N. and Dangi, A.S. (2008). Hypoglycemic activity of ethanolic extract of *Salvadora oleoides* in normal and alloxan – induced diabetes rats *Indian J. Pharmacol .* 40 (1): 23 – 27.
19. Farswan, M., Mazumder, P. M. and Percha, V. (2009). Protective effect of *Cassia glauca* Linn. on the serum glucose and hepatic enzymes level in streptozotocin induced NIDDM in rats. *Indian J. Pharmacology* 41(1): 19 -22.

Author Information

David Okon Edem, PhD

Department Of Biochemistry, University Of Uyo