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Evaluation of the Anti-diabetic Property of *Morinda lucida* Leaves in Streptozotocin-diabetic Rats

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Abstract

The hypoglycaemic and anti-hyperglycaemic activities of a methanol extract of *Morinda lucida* Benth. (Rubiaceae) leaves were studied in normal and streptozotocin-diabetic rats.

In normal rats, the extract demonstrated a significant ($P < 0{\cdot}05$) and dose-dependent hypoglycaemic activity within 4 h after oral administration. The plasma glucose level of 400 mg kg^{-1} of the extract at 4 h was $42{\cdot}5 \pm 0{\cdot}4$ mg/100 mL (control $67{\cdot}4 \pm 1{\cdot}2$ mg/100 mL). After 12 h, the plasma glucose level of rats administered 50, 100, 200 or 400 mg kg^{-1} extract fell to $51{\cdot}9 \pm 1{\cdot}2$, $47{\cdot}3 \pm 0{\cdot}8$, $43{\cdot}1 \pm 0{\cdot}4$ and $40{\cdot}0 \pm 0{\cdot}5$ mg/100 mL, respectively. In hyperglycaemic rats, the extract produced a significant ($P < 0{\cdot}05$) anti-diabetic effect from day 3 after oral administration, with 400 mg kg^{-1} extract-treated animals having a plasma glucose level of $248{\cdot}7 \pm 5{\cdot}3$ mg/100 mL compared with glibenclamide (10 mg kg^{-1})-treated animals with a plasma glucose level of $251{\cdot}5 \pm 5{\cdot}8$ mg/100 mL.

These results suggest that the leaves of *Morinda lucida* have a strong glucose lowering property when administered to streptozotocin-treated rats.

The pathogenesis of diabetes mellitus and the possibility of its management by the oral administration of hypoglycaemic agents have stimulated great interest in recent years. Only two groups of oral hypoglycaemic agents are available for clinical use, sulphonylureas and biguanides (Al-Awaidi et al 1985; Mariam et al 1996). For a long time diabetics have been treated orally with several medicinal plants or their extracts based on folk medicine (Akhtar & Ali 1984). Based on the WHO recommendations on diabetes mellitus (WHO 1980), investigations of hypoglycaemic agents of plant origin used in traditional medicine are important.

Morinda lucida Benth. (Rubiacea) is used for a variety of ailments, including fevers, malaria, inflammation and pain. The plant is used in the treatment of hypertension, and as a diuretic and a laxative. Treatment with the plant was also reported to induce vomiting and diarrhoea (Watt & Breyer-Brandwijic 1962; Oliver-Bever 1986; Iwu 1993).

Various types of anthraquinones and anthraquinols have been shown to be present in *M. lucida* (Adesogan 1973; Rath et al 1995). *M. lucida* has been reported to possess antimalarial activity in-vivo against *Plasmodium berghei berghei* in mice (Obih et al 1985; Makinde et al 1994) and *Plasmodium falciparum* in-vitro (Koumaglo et al 1992; Awe & Makinde 1998). Asuzu & Chineme (1990) demonstrated trypanocidal activity of *M. lucida*-leaf extract in mice.

An anti-hepatotoxic effect of the leaf extract of *M. lucida* was reported against paracetamol-induced liver intoxication in mice (Udem et al 1997). Previously, we reported the anti-inflammatory, analgesic, antipyretic and CNS-depressant effects of *M. lucida* methanolic leaf extract (Awe et al 1998). We have reported also on the promotion of gastric emptying and intestinal transit of a charcoal meal by the plant extract (Olajide et al 1998) and its smooth muscle contractile and purgative effects (Olajide et al 1999).

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It has been observed that a concoction containing the plant had the ability to lower the blood sugar in a diabetic patient. Kamanyi et al (1994) studied the hypoglycaemic activity of root extract of *M. lucida*. In this study, we have evaluated the methanolic leaf extract of *M. lucida* for hypoglycaemic and anti-hyperglycaemic effects.

Materials and Methods

Preparation of plant material

Morinda lucida leaves were collected from a tree growing behind the Department of Pharmacology and Therapeutics, University of Ibadan, Ibadan, Nigeria. Collection was made in March, and samples were authenticated by a botanist at the Forest Research Institute of Nigeria (FRIN) where voucher specimens were deposited. Fresh leaves of *M. lucida* were air-dried and reduced to coarse powder. The powdered plant material, 320 g, was extracted with methanol in a soxhlet apparatus. The extract was evaporated under reduced pressure at 40°C until all the solvent had been removed, to give an extract sample with a yield of 18·2%. The extract was stored in a refrigerator, and was prepared in 0·9% normal saline for pharmacological studies.

Animals

Male Wistar rats (170–220 g) were used for the study. They were bred and housed in the Pre-Clinical Animal House, College of Medicine, University of Ibadan, which was well ventilated with a 12-h light/dark cycle. They were fed on a commercial diet (Ladokun Feeds Ltd, Ibadan) and water was freely available.

Blood collection

The blood was collected from the orbital plexus in heparinized tubes and plasma was separated within 30 min of collection for estimation of glucose levels.

Effect of extract on normoglycaemic rats

The procedure described by Sharma et al (1997) was used. Rats were fasted for 18 h and were divided into groups of six animals each. The extract (50, 100, 200 or 400 mg kg^{-1}, p.o.) was administered to animals in each group. The control group received 10 mL kg^{-1} saline. Plasma glucose was estimated using the glucose oxidase method with a commercial kit (Sigma, USA) at 0, 4, 8 and 12 h after administration of extract.

Effect of extract on streptozotocin-hyperglycaemic rats

Groups of rats fasted for 18 h were made hyperglycaemic by injecting streptozotocin (Sigma, USA) dissolved in citrate buffer (pH 4·3), at a dose of 50 mg kg^{-1}. After 10 days, their plasma glucose concentration was estimated and moderately streptozotocin-diabetic rats (Chattopadhyay 1993), having plasma glucose levels above 250 mg/100 mL, were divided into groups of six animals each. One group received glibenclamide (10 mg kg^{-1}, p.o.) and a control group received 10 mL kg^{-1} normal saline. Other groups received the extract at oral doses of 50, 100, 200 or 400 mg kg^{-1}. Treatment was continued for 10 consecutive days. Before the treatment (0 day), and after the treatment (3, 5, 7 and 10 days) plasma levels were estimated using the glucose oxidase method (Sharma et al 1997).

Statistical analysis

The results are presented as means ± s.e.m., and statistical significance between treated and control groups was analysed by means of Student's *t*-test. $P < 0{\cdot}05$ was considered significant.

Results

Activity in normoglycaemic rats

The hypoglycaemic effect of the extract was observed within 4 h after oral administration at all the dose levels (Table 1). Twelve hours after the administration of the extracts (50, 100, 200 and 400 mg kg^{-1}, p.o.) a dose-dependent activity of the extract was observed, with plasma glucose levels of 51·9 ± 1·2, 47·3 ± 0·8, 43·1 ± 0·4 and 40·0 ± 0·5 mg/100 mL, respectively.

Activity in streptozotocin-hyperglycaemic rats

In hyperglycaemic rats, the extract exhibited significant ($P < 0{\cdot}05$) anti-hyperglycaemic effect from 3 days after its continuous oral administration. In untreated streptozotocin-diabetic rats, an increase in plasma glucose level was observed from day 3 onwards and over the experimental period was significantly higher ($P < 0{\cdot}05$) compared with the extract- or glibenclamide-treated groups. The extract of *M. lucida* at 400 mg kg^{-1} exhibited a slightly stronger anti-diabetic activity than the standard drug, glibenclamide (10 mg kg^{-1}) (Table 2).

Table 1. Effect of *Morinda lucida* leaf methanol extract on plasma glucose levels of normoglycaemic rats.

Group	Dose (mg kg^{-1})	Time after treatment			
		0 h	4 h	8 h	12 h
Control	–	68·2 ± 0·7	67·4 ± 1·2	68·3 ± 1·3	66·4 ± 1·0
M. lucida	50	67·6 ± 0·6	56·5 ± 1·3*	53·9 ± 1·2*	51·9 ± 1·2*
M. lucida	100	66·6 ± 1·3	51·6 ± 1·0*	48·3 ± 0·98	47·3 ± 0·8*
M. lucida	200	67·8 ± 0·8	46·3 ± 0·5*	44·6 ± 0·5*	43·1 ± 0·4*
M. lucida	400	67·2 ± 0·7	42·5 ± 0·4*	41·4 ± 0·5*	40·0 ± 0·5*

Each value represents the plasma glucose level (mg/100 mL) (n = 6), mean ± s.e.m. *$P < 0·05$ compared with untreated control.

Table 2. Effect of *Morinda lucida* leaf methanol extract on plasma glucose levels in streptozotocin-diabetic rats.

Group	Dose (mg kg^{-1})	Plasma glucose levels (mg/100 mL)				
		Day 0	Day 3	Day 5	Day 7	Day 10
Control (untreated)	–	281·6 ± 5·3	290·1 ± 6·4	298·8 ± 6·1	303·0 ± 6·4	309·4 ± 5·8
M. lucida	50	292·2 ± 5·8	271·5 ± 4·4*	266·1 ± 12·8*	261·7 ± 2·6*	258·7 ± 2·0*
M. lucida	100	290·9 ± 4·2	260·0 ± 4·8*	260·0 ± 4·8*	250·4 ± 5·7*	247·0 ± 5·5*
M. lucida	200	288·4 ± 3·9	252·8 ± 6·4	252·5 ± 5·4*	247·8 ± 6·5*	244·3 ± 6·5*
M. lucida	400	292·2 ± 3·0	248·7 ± 5·3*	241·4 ± 5·4*	234·4 ± 4·8*	231·6 ± 5·2*
Glibenclamide	10	290·2 ± 5·5	251·5 ± 5·8*	243·2 ± 6·3	236·5 ± 5·9*	235·2 ± 5·6*

Each value represents mean ± s.e.m. (n = 6). *$P < 0·05$ compared with untreated control group.

Discussion

Although insulin has become one of the most important therapeutic agents known to medicine, efforts continue to find insulin substitutes from synthetic or plant sources for the treatment of diabetes (Choi et al 1991; Erenmemisoglu et al 1995). Over 150 plant extracts and some of their active principles including flavonoids are known to be used for the treatment of diabetes (Meiselman et al 1976; Choi et al 1991; Erenmemisoglu et al 1995).

The methanol extract of *M. lucida* leaves exhibited significant hypoglycaemic and anti-hyperglycaemic activities in normal and streptozotocin-hyperglycaemic rats, respectively. Kamanyi et al (1994) reported similar activities for the root extract of this plant. The mechanism of the blood sugar-lowering effect of *M. lucida* is yet to be established, but the results suggest that the plant methanol extract acts in a similar way as glibenclamide, the standard anti-diabetic drug, by stimulation of surviving β-cells to release more insulin. It has been demonstrated that glibenclamide and a natural hypoglycaemic product were effective in moderately streptozotocin-diabetic animals and ineffective in severe diabetic rats (Ivorra et al 1988; Sharma et al 1997) supporting this postulation.

The major constituents of *M. lucida* have been shown to contain anthraquinones (Adesogan 1973; Rath et al 1995) and tannins (Oliver-Bever 1986). Tannin-containing plants demonstrating anti-diabetic activity have included *Bridelia ferruginea* (Iwu 1980, 1983), *Rhizophora racemosa* (Jain & Sharma 1967) and *Sclerocarya birrea* (Oliver-Bever 1986). It is possible that the presence of tannins in *M. lucida* is responsible for the observed anti-diabetic effect of the plant extract. However, this is no evidence that tannins or any other components of *M. lucida* have shown this activity.

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References

Adesogan, E. K. (1973) Anthraquinones and anthraquinols from *Morinda lucida*. The biogenic significance of Oruwal and Oruwalol. Tetrahedron 29: 4099–4102

Akhtar, F. M., Ali, M. R. (1984) Study of the antidiabetic effect of a compound medicinal plant prescription in normal and diabetic rabbit. J. Pakistan Med. Assoc. 34: 239–244

Al-Awaidi, F. M,. Khattar, M. A., Gumaa, K. A. (1985) On the mechanism of the hypoglycaemic effect of a plant extract. Diabetologia 28: 432–434

Asuzu, I. U., Chineme, C. N. (1990) Effects of *Morinda lucida* leaf extract on *Trypanosome brucei brucei* infections in mice. J. Ethnopharmacol. 30: 307–313

Awe, S. O., Makinde, J. M. (1998) Evaluation of sensitivity of *Plasmodium falciparum* to *Morinda lucida* leaf extract sample using rabbit in vitro microtest technique. Indian J. Pharmacol. 30: 51–53

Awe, S. O., Olajide, O. A., Adeboye, J. O., Makinde, J. M. (1998) Some pharmacological studies on *Morinda lucida*. Indian J. Pharmacol. 30: 38–42

Chattopadhyay, R. R. (1993) Hypoglycaemic effect of *Ocimum sanctum* leaf extract in normal and streptozotocin-diabetic rats. Indian J. Exp. Biol. 31: 891–893

Choi, J. S., Yokozawa, T., Oura, H. (1991) Improvement of hyperglycaemia and methanolic extract of *Prunus davidana* stems and its main component, prunin. Planta Med. 57: 208–211

Erenmemisoglu, A., Kelestimur, F., Koker, A. H., Ustun, H., Tekol, Y., Ustdal, M. (1995) Hypoglycaemic effect of *Zizyphus jujuba* leaves. J. Pharm. Pharmacol. 47: 72–74

Ivorra, M. D., Paya, M., Villar, A. (1988) Hypoglycemic and insulin release effects of tormentic acid: a new hypoglycemic natural product. Planta Med. 54: 282–285

Iwu, M. M. (1980) Antidiabetic properties of *Bridelia ferruginea* leaves. Planta Med. 39: 247

Iwu, M. M. (1983) The hypoglycaemic properties of *Bridelia ferruginea*. Fitoterapia 54: 243–248

Iwu, M. M. (1993) Handbook of African Medicinal Plants. CRC Press Inc, Boca Raton, pp 209–210

Jain, S. R., Sharma, S. N. (1967) Hypoglycaemic drugs of Indian indigenous origin. Planta Med. 15: 439–442

Kamanyi, A., Njamen, D., Nkeh, B. (1994) Hypoglycaemic properties of the aqueous root extract of *Morinda lucida* (Benth) (Rubiaceae). Studies in the mouse. Phytother. Res. 8: 369–371

Koumaglo, K., Gbeassor, M., Nikabu, O., De Souza, C., Werner, W. (1992) Effects of three compounds extracted from *Morinda lucida* on *Plasmodium falciparum*. Planta Med. 58: 533–535

Makinde, J. M., Awe, S. O., Salako, L. A. (1994) Seasonal variation in the antimalarial activity of *Morinda lucida* on *Plasmodium berghei berghei* in mice. Fitoterapia 65: 124–130

Mariam, A., Asmawi, M. Z., Sadikun, A. (1996) Hypoglycaemic activity of the aqueous extract of *Orthosiphon stamineus*. Fitoterapia 67: 465–468

Meiselman, H. L., Halpern, B. P., Dateo, G. P. (1976) Reduction of sweetness judgements by extracts from the leaves of *Z. jujuba*. Physiol. Behav. 17: 313–317

Obih, P. O., Makinde, J. M., Laoye, J. O. (1985) Screening of various extracts of *Morinda lucida* for antimalarial action on *Plasmodium berghei berghei* in vivo. Afr. J. Med. Med. Sci. 14: 51–54

Olajide, A. O., Awe, S. O., Makinde, J. M. (1998) The effects of *Morinda lucida* Benth (Rubiaceae) extract on the gastrointestinal tract of rodents. Phytother. Res. 12: 439–441

Olajide, O. A., Awe, S. O., Makinde, J. M. (1999) Purgative effect of the methanol extract of *Morinda lucida*. Fitoterapia In press

Oliver-Bever, B. (1986) Medicinal Plants in Tropical West Africa. Cambridge University Press, London, p. 44

Rath, G., Ndonzao, M., Hostettmann, K. (1995) Antifungal anthraquinones from *Morinda lucida*. Int. J. Pharmacog. 33: 107–114

Sharma, S. R., Dwivedi, S. K., Swarup, D. (1997) Hypoglycaemic, antihyperglycaemic and hypolipidemic activities of *Caesalpinia bonducella* seeds in rats. J. Ethnopharmacol. 58: 39–44

Udem, S. C., Madubunyi, I. I., Okoye, J. O., Anika, S. M. (1997) Anti-hepatotoxic effects of the ethanolic extracts of *Combretum dolichopetalum* root bark and *Morinda lucida* leaf. Fitoterapia 68: 21–25

Watt, J. M., Breyer-Braudwijic, M. G. (1962) The Medicinal and Poisonous Plants of Southern and Eastern Africa. Livingstone, Edingburgh, p. 900

WHO (1980) The WHO Expert Committee on Diabetes Mellitus, Technical Report Series World Health Organization, Geneva