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Original Article

Glucose lowering effect of leaf extracts of Viscum Album in normal and diabetic rats

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Abstract

BACKGROUND: The use of plants in the treatment of diabetes mellitus is a well-established practice in traditional medicine. *Viscum album* has been recommended for the treatment of several diseases. This study evaluated the glucose lowering effect of leaf extracts of this plant in normal and streptozotocin induced diabetic rats.

METHODS: Leaf extracts of *Viscum album* were prepared with 80 % ethanol and administered to normal and diabetic New Zealand white rats. The LD_{50} was determined by the Karbar method. The glucose lowering effect was assessed in these animals in comparison to normal saline and glibenclamide. Blood glucose was estimated with the aid of a glucose sensor.

RESULTS: The LD_{50} was 1520 mg/kg. A dose dependent lowering effect of the fasting blood glucose was observed in both the normal and diabetic rats, with maximum lowering occurring 6 hours after extract administration. The patterns of effect were similar to that produced by glibenclamide.

CONCLUSIONS: Leaf extracts of *Viscum album* showed a significant glucose lowering effect in normal and diabetic rats. Extracts from this plant could therefore, be useful in controlling blood glucose level.

KEY WORDS: Animal models, diabetes mellitus, *Viscum album*, hypoglycemic effect, streptozotocin.

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In Africa, as in many parts of the world, application of traditional medical practices to the treatment of diabetes is quite popular [1]. Several plant species have been used for this purpose including Vernonia amygdalina, Momordica charantia, Trigonella foenum graecum, Panax-ginseng and Dioscorea dumetorum. Pharmacological studies on many of these plants have shown that they possess glucose-lowering effects [2,3]. Mistletoe (*Viscum album*) grows as a partial parasite on a variety of trees such as pine, apple, plum and spruce in both tropical and temperate countries. This plant is widely distributed in Africa, Asia, Europe and Australia. Previous studies have demonstrated that extracts from this plant possess pharmacological properties among which are immunomodulatory, anti-inflammatory, cardiovascular and antimicrobial effects [4,5]. For many years, it has been used in the treatment of many medical conditions and as a homeopathic remedy. It has been suggested as remedy for cancer, AIDS and cardiovascular disorders [6-8], though its efficacy in these conditions has not been proved. Although the glucose lowering effect of *Viscum album* has been

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reported [9], there could be quantitative and/or qualitative differences in the pharmacological activities of extracts from this plant grown in different geographical regions with different environmental conditions. In this study therefore, we investigated the glucose lowering potential of ethanolic leaf extracts of subspecies of this plant, growing as parasites on trees in Eastern Nigeria, in normal and diabetic rats.

Methods

Plant Collection and Preparation: Fresh leaves of mistletoe were collected from the botanical garden of a traditional herbalist residing in Aba, Nigeria. The plant was shade-dried for 2 days. Mr. Ibe from the Department of Forestry, Michael Okpara University of Agriculture, Umudike, Nigeria, identified the plant. Fresh leaves from the plant were washed, air dried in the laboratory at room temperature (29-30^{o}C) and milled to a coarse powder using a grinder. 200 g of the powder were extracted by maceration with 600 ml of 80 % ethanol at room temperature in a rubber-corked bottle for 4 days. This was then, filtered with a clean muslin cloth. The filtrate was evaporated to dryness in a rotary evaporator at 40-50^{o}C giving a yield of approximately 10%.

Animals: 92 New Zealand white albino rats weighing 120 – 125 g were used for this study. They were purchased from the Michael Okpara University of Agriculture Umudike and habituated in the animal house of the Department of Pharmacology and Therapeutics, Abia State University Teaching Hospital, Aba, Nigeria for three days with 12 hours light-darkness cycles. They were fed with standard animal feed and water ad libitum.

Experimental Protocol: (i) *LD_{50} Determination*: Twelve of the animals were used for LD_{50} determination using the method of Karber [10].

(ii) *Induction of Diabetes*: Thirty of the 80 remaining animals were made diabetic by intraperitoneal injection of streptozotocin (Aldrich Chemical Company Limited, USA) at a dose of 65 mg/kg body weight. The streptozotocin was diluted to 10% with normal saline [11]. These animals were then fed normally for five days. The diabetic state was confirmed by fasting blood glucose higher than or equal to 126 mg/dl [12]. The remaining rats served as the normoglycemic controls.

(iii) *Demonstration of the Glucose Lowering Effect of the Extract*: Fifteen diabetic rats and fifteen normoglycemic ones were separated into 6 groups of 5, each as follows:

Group I: 5 normal rats treated with 750 mg/kg extract.
Group II: 5 normal rats treated with 5 mg/kg glibenclamide.
Group III: 5 normal rats treated with 5 ml normal saline.
Group IV: 5 diabetic rats treated with 750 mg/kg extracts.
Group V: 5 diabetic rats treated with 5 mg/kg glibenclamide.
Group VI: 5 diabetic rats treated with 5 ml saline.

The animals were fasted for 12 hours during which they had free access to water. They were then treated as above. Blood samples were collected from each animal at 0, 2, 4, 6, 8 and 10 hours after administration of the drugs/extract.

(iv) *Study of the Dose Response Relationship*: The remaining 50 rats were also separated into 10 groups of 5 each. The animals were fasted as above and the groups were treated with plant extracts as follows:

Groups I: 5 normal rats treated with normal saline.
Group II: 5 normal rats treated with 250 mg/kg plant extract.
Group III: 5 normal rats treated with 500 mg/kg plant extract.
Group IV: 5 normal rats treated with 750 mg/kg plant extract.
Group V: 5 normal rats treated with 1000 mg/kg plant extract.
Group VI: 5 diabetic rats treated with normal saline.
Group VII: 5 diabetic rats treated with 250 mg/kg plant extract.
Group VIII: 5 diabetic rats treated with 500 mg/kg plant extract.

Group IX: 5 diabetic rats treated with 750 mg/kg plant extract.
Group X: 5 diabetic rats treated with 1000 mg/kg plant extract.

Blood samples were collected from each animal by cardiac puncture after 1 hr and blood glucose level was determined as described below.

***Measurement of Blood Glucose*:** Blood glucose levels were determined with the help of MediSense Precision QID blood glucose sensor (Abbott Laboratories MediSense Products, Maidenhead, UK). Results were presented as means ± SEM. Differences between means were computed by one-way ANOVA using the SPSS statistical soft ware. A P value less than 0.05 was considered significant.

Results

The LD_{50} of the ethanolic extract of *Viscum album* was 1520 ± 0.15 mg/kg. Doses of the extract between 250 and 1000 mg/kg significantly reduced the fasting plasma glucose in both normal and diabetic rats, with maximum reduction occurring at a dose of 750 mg/kg as shown in figures 1 and 2 (P<0.05). Compared with saline, the extract and glibenclamide significantly lowered the fasting plasma glucose over time (P<0.05). Maximum reduction occurred in 6 hours in both normal and diabetic rats (figures 3 and 4), and the patterns of glucose lowering were similar to that produced by 5 mg/kg glibenclamide, which however produced significantly higher reductions in plasma glucose than the leaf extract between 2 and 10 hours of administration (P<0.05, figures 3 and 4).

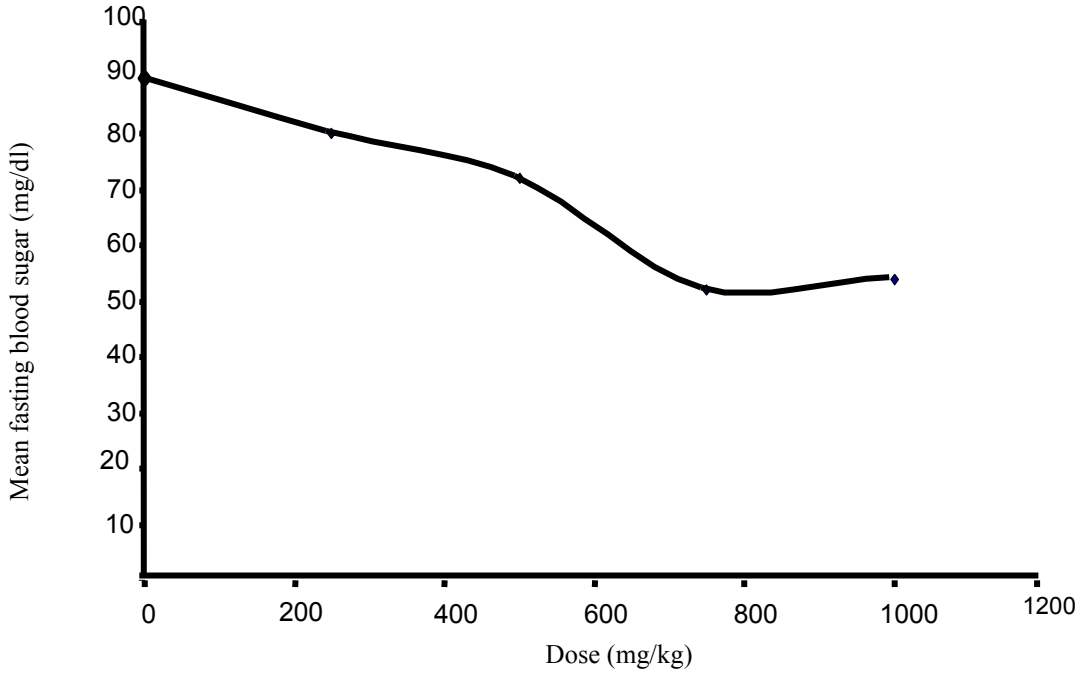

Figure 1. Dose-response relationship of Viscum album on fasting blood sugar level of normoglycemic rats. Each point is the mean ± SEM.

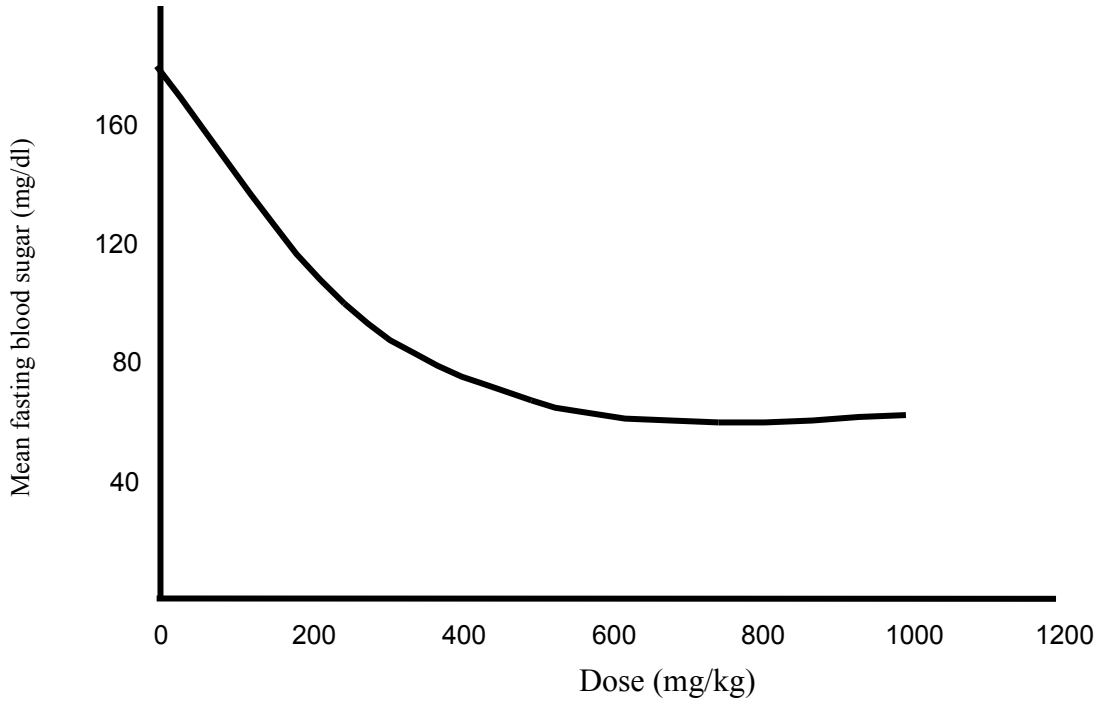

Figure 2. Dose-response relationship of Viscum album on fasting blood sugar level of streptozotocin-treated rats. Each point is the mean ± SEM.

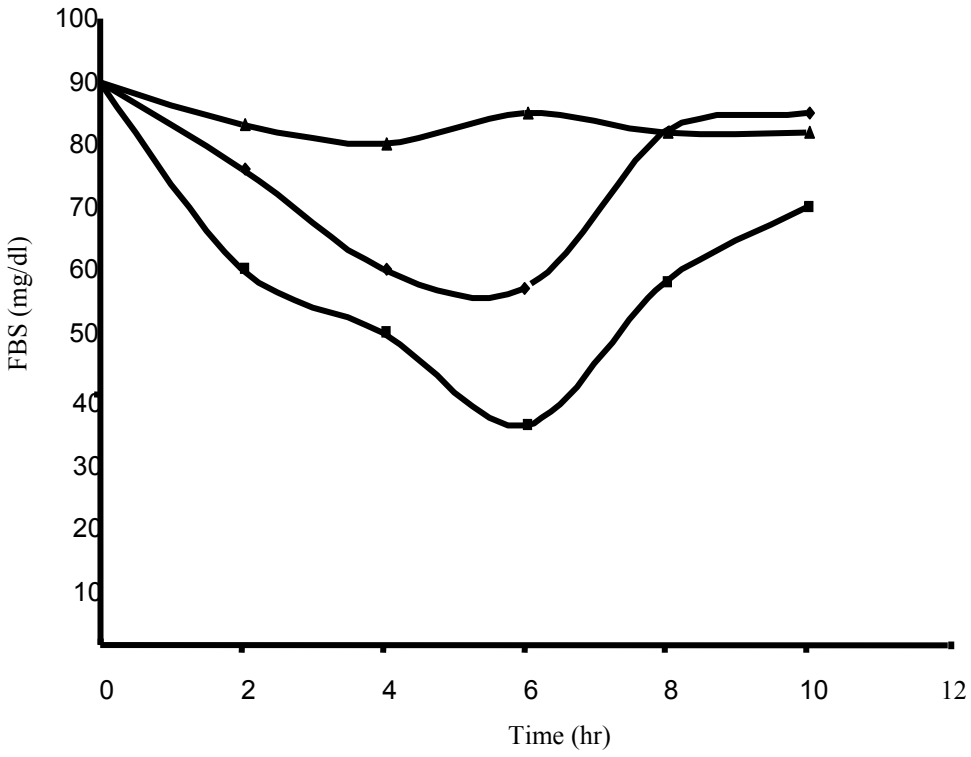

Figure 3. Effects of alcoholic extract of Viscum album (750 mg/kg), compared with glibenclamide (5 mg/kg) and normal saline (5 ml/kg) on fasting blood sugar level of normoglycemic rats. Each point is the mean ± SEM.

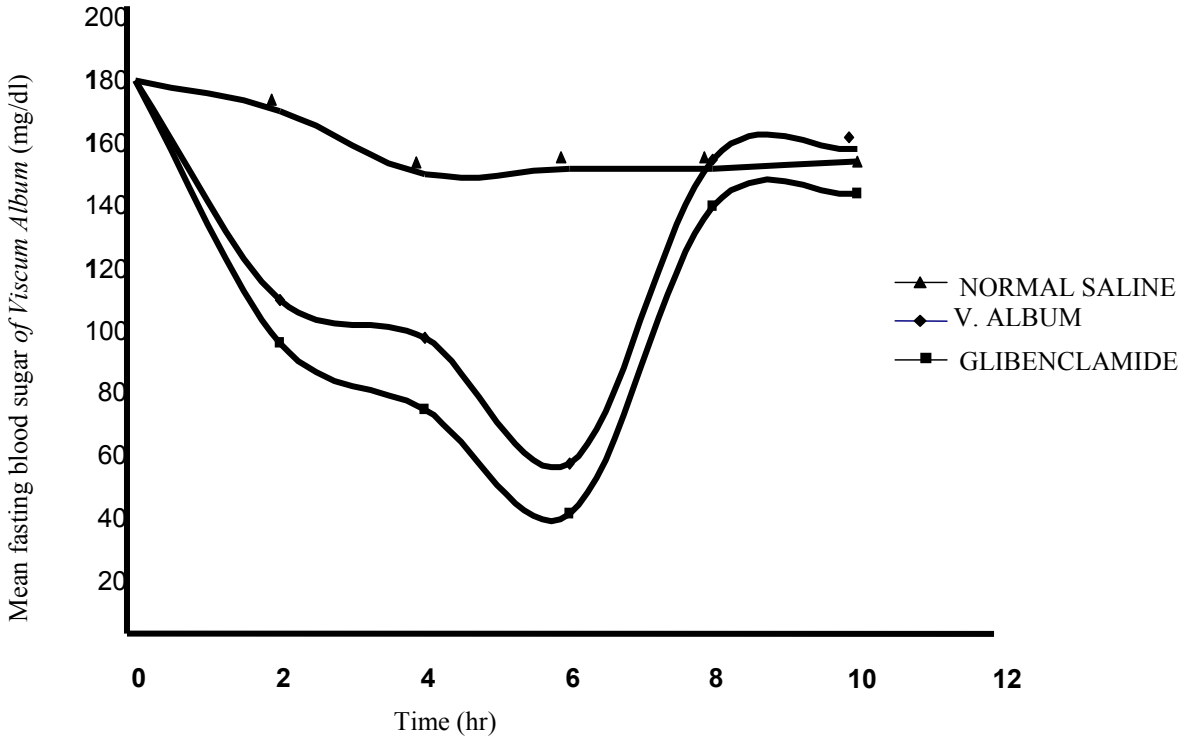

Figure 4. Effects of alcoholic extract of Viscum album (750 mg/kg), glibenclamide (5 mg/kg) and normal saline (5 ml/kg) on fasting blood sugar of streptozotocin-treated rats. Each point is the mean ± SEM.

Discussion

The alcoholic leaf extract of the plant, *Viscum album*, produced marked decrease in fasting blood glucose level in normal as well as in streptozotocin-induced diabetic rats, and therefore could be useful in the management of diabetes mellitus. This result compares favorably with findings by Ohiri and colleagues [13]. Hypoglycemic effect of the extract became evident in 2 hours and implies that this extract could be useful in the control of postprandial glucose excursions that occur in diabetes. Different studies have shown that most plant extracts with hypoglycemic properties exert their effects via various mechanisms. For instance, green tea contains catechin-related compounds (flavonoids), which have been shown to exert some protective effect on the pancreatic beta cells [11-14]. *Momordica charantia, Allium sativum, Allium cepa* and *fenugreek* are reported to act by inducing the secretion of insulin by the pancreatic beta cells, by enhancing the regeneration of the beta cells, or by reducing the resistance of the target cells to effects of insulin[15-18]. Other reported mechanisms include inhibiting metabolic process that increases hepatic glucose output such as gluconeogenesis or glycogenolysis, while at the same time promoting oxidation of glucose through glycolysis[19]. A study has reported the ability of *Viscum album* extract to stimulate insulin secretion from the islets cells[20]. Whatever the mechanisms involved, this study has demonstrated that extracts from mistletoe growing on plants in Nigeria possess significant antidiabetic properties, and could be useful in the control of blood glucose level in diabetic patients. Biochemical processes that play a role in diabetes are diverse and involve not only insulin but also other hormones such as glucagon, somastostatin, gastrointestinal-hormones and corticosteroids. Elucidation of the precise mechanism by which constituents of mistletoe extract interact with these processes to lower blood

glucose could be an interesting topic for further studies.

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