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Hypoglycemic efficacy of *Ocimum gratissimum* and *Vernonia amygdalina* compared with insulin and glibenclamide in type I and type II diabetic rat models

Uduak Akpan Okon*, Titilope Helen Olatunbosun

Department of Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, University of Uyo, P.M.B. 1017 Uyo, Akwa Ibom State, Nigeria

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ABSTRACT

Objective: To compare the efficacy of *Ocimum gratissimum* (*O. gratissimum*) and *Vernonia amygdalina* (*V. amygdalina*) with those of insulin and glibenclamide.

Methods: Type I and II diabetes mellitus (DM) were induced by a single intraperitoneal injection of 65 mg/kg of streptozotocin and intraperitoneal administration of nicotinamide (100 mg/kg) along with streptozotocin, respectively. The state of diabetes was confirmed weekly by testing blood glucose level using a glucometer.

Results: The weekly blood glucose levels were higher in type I DM than in type II DM. Type I DM plus *O. gratissimum* showed a weekly progressive significant reduction in blood glucose compared to type I DM control. Type I DM control showed a duration dependent significant higher blood glucose concentration compared to normal control. Type I DM plus *V. amygdalina* also showed a time dependent significant lower glucose level compared to normal control and type I DM control. Combination treatment of type I DM (*O. gratissimum* plus *V. amygdalina*) showed a significantly elevated glucose concentration compared to normal control which was similar to type I DM control. Insulin treatment in type I DM showed a weekly progressive significant reduction of glucose concentration compared to normal control and type I DM control. Type II DM control showed a fairly constant blood glucose concentration throughout the duration of treatment that was significantly higher than that of the normal control. Type II DM plus *O. gratissimum* showed a fairly steady significant reduction of glucose concentration compared to type II DM control and normal control. Type II DM plus *V. amygdalina* also showed a fairly constant significant reduction of glucose concentration compared to type II DM control and normal control. Type II DM (*O. gratissimum* plus *V. amygdalina*) showed a slightly progressive significant reduction of glucose concentration compared to normal control and type II DM control. Type II DM with glibenclamide showed almost steady significant reduction in glucose concentration compared to normal control and type II DM control.

Conclusions: From the result, it is evident that *O. gratissimum* and *V. amygdalina* administration produces more potent hypoglycemic activity than insulin and glibenclamide in type I and II DM models, respectively.

1. Introduction

Hyperglycemia caused by insufficiency of secretion or action of endogenous insulin is the hallmark of diabetes mellitus (DM). The etiology of this disease is not clearly understood. However, viral infection, autoimmune disease and environmental factors have been implicated. Two types of DM have been described, based

formerly on age of onset, more recently on clinical presentation and necessary treatment regimen. Type I DM is associated with absolute insufficiency of insulin and is treated with insulin replacement. Type II DM is due to the relative lack of insulin or tissue resistance to insulin and is mainly treated with diet modification and oral sulphonylureas. The experimental model obtained by the combined administration of nicotinamide and streptozotocin (STZ) is considered as type II DM, while the model of treatment with STZ only is considered as type I DM. Some cases of type II DM may eventually require insulin if other medications fail to control blood glucose adequately.

Insulin causes cells in the liver, skeletal muscles and fat tissues to absorb glucose from the blood. In the liver and skeletal muscles,

*Corresponding author: Okon Uduak Akpan, Department of Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, University of Uyo, P.M.B. 1017 Uyo, Akwa Ibom State, Nigeria.

Tels: +23 48023147921; +23 7034741970

E-mail: chairmo2013@gmail.com

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glucose is stored as glycogen, and in fat cells, it is stored as triglycerides. Insulin retards the use of fat as an energy source by inhibiting the release of glucagon. Insulin is reported to have anabolic action on protein metabolism by stimulating protein synthesis and retarding protein degradation[1].

Glibenclamide is an antidiabetic drug in the class of the sulfonylurea, closely related to sulfa drugs. It is used in the treatment of type II DM. As of 2010, glibenclamide and metformin were the only two oral antidiabetics in the World Health Organization model list of essential medicines[2]. Recent research showed that glibenclamide improves outcome in animal stroke models by preventing brain swelling[3] and neuroprotection[4].

The utilization of herbal extract from plants to treat various types of illnesses and diseases have increased over the years[5]. *Vernonia amygdalina* (*V. amygdalina*) and *Ocimum gratissimum* (*O. gratissimum*) are among the plants whose extract are utilized[6]. Different mechanisms of actions of antidiabetic plants have been proposed such as potentiation of insulin effect either by increasing the pancreatic secretion of insulin, inhibition of hepatic glucose production, inhibition of intestinal glucose absorption or correction of insulin resistance[7,8]. A combination of these extracts may result in significant antidiabetic activity, which is likely to be better than that of a single extract.

Most findings showed that sulfonylureas, including glibenclamide, produce hypoglycemia in normal as well as diabetic animals by stimulating the pancreatic beta cell to release more insulin[9]. It has been reported that a combination of these extracts worked through a similar mechanism like glibenclamide as the results obtained from the combinations were comparable[10]. Plants extract also exert hypoglycemic effect on DM by promoting regeneration of beta cells or by protecting these cells from destruction and activating insulin receptors to absorb the blood glucose and stimulate glucose consumption[11]. It has also been reported that these plants have various phytochemical constituents in common such as alkaloids, steroids, glycosides, saponins, tannins, terpenes and flavonoids[12]. In addition to antidiabetic properties of these plants, each of the plants has been reported of different activities geared towards alleviation of complications usually associated with diabetes. For example, *V. amygdalina* has been reported to be hepatoprotective and antioxidant[13] and *O. gratissimum* as antioxidant[14] and others, all of which appears to complement the antidiabetic activities of these plants.

In view of the widely reported hypoglycemic effect of *V. amygdalina* and *O. gratissimum* and the known standard treatment of insulin and glibenclamide for DM, the present study therefore focuses to compare the efficacies of this ethno-medicinal extract with the standard drugs. This we hope will further strengthen the interest in the future clinico-pharmaceutical development of these extract.

2. Materials and methods

2.1. Drugs

STZ, nicotinamide, glibenclamide (Doanil, Swiss Pharmaceutical Co., Ltd., 5 mg) and insulin (Actrapid, Novo Nordisk A/S, Bagsværd, Denmark, 100 IU/mL) were purchased from a reputable pharmaceutical company in Uyo, Akwa Ibom State, Nigeria.

2.1.1. Drug preparation

Two tablets of glibenclamide (5 mg each) were grounded into fine powder. The stock solution was prepared by dissolving 10 mg of glibenclamide in 20 mL of distilled water to give a concentration of 15 mg/mL. It was given at 100 mg/kg body weight.

2.2. Plant materials

2.2.1. Collection and identification

The fresh leaves of *O. gratissimum* and *V. amygdalina* were collected from the medicinal farm of the Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo, Akwa Ibom State, Nigeria. The plants were identified by the chief herbarium officer in the Department of Botany and Ecological Studies, University of Uyo, Akwa Ibom State, Nigeria.

2.2.2. Extracts preparation

The fresh leaves of *O. gratissimum* and *V. amygdalina* collected were rinsed with water and air dried. The leaves were cut into small pieces and sundried for two days and then transferred into Astell Hearson oven at a temperature range of 40–45 °C. The dried leaves were pulverized into fine powder to give a gram weight of 425 g each. The 425 g of each extract was macerated in 3000 mL of distilled water for about 12 h and stirred at regular intervals. The mixture of each extract was filtered and their filtrates were concentrated for dryness in a water bath at 45 °C to obtain a brown gummy paste. The weight of the dried extracts was 57 g each, and the stock solution of the extracts was prepared by dissolving 15 g of each extract in 10 mL of water to give a concentration of 1500 mg/mL, which were labeled appropriately and were refrigerated at 40 °C until they were required for use. The LD₅₀ was determined by method of Lorke[15].

2.2.3. Determination of phytoconstituents

The phytoconstituents of the extracts were determined and screened to reveal the presence of carbohydrates, tannins, alkaloids, saponins, phenolics, anthraquinones, cardiac glycosides

and others as described by Trease and Evans[16] and Sofowora[6].

2.4. Animal preparation, experimental groupings and treatment

A total of 72 female albino wistar rats weighing 110–200 g were used for the study. The animals were randomly divided into two type I DM and type II DM. Each of the types was subdivided into 6 groups of 6 rats each. Administration was done daily and was facilitated by the use of syringe and orogastric tube.

2.4.1. Type I DM

Normal control rats which received distilled water only were Group I. STZ-induced diabetic rats left untreated were Group II. STZ-induced diabetic rats treated with 208 mg/kg body weight of *O. gratissimum* for 28 days were Group III. STZ-induced diabetic rats treated with 52 mg/kg body weight of *V. amygdalina* for 28 days were Group IV. STZ-induced diabetic rats treated with 208 mg/kg body weight of *O. gratissimum* and 52 mg/kg body weight of *V. amygdalina* for 28 days were Group V. STZ-induced diabetic rats treated with 0.16 IU of insulin for 28 days were Group VI.

2.4.2. Type II DM

Normal control rats which received distilled water only were Group I. STZ- and nicotinamide-induced diabetic rats left untreated were group II. STZ- and nicotinamide-induced diabetic rats treated with 208 mg/kg body weight of *O. gratissimum* were Group III. STZ- and nicotinamide-induced diabetic rats treated with 52mg/kg body weight of *V. amygdalina* were Group IV. STZ- and nicotinamide-induced diabetic rats treated with 208 mg/kg body weight of *O. gratissimum* and 52 mg/kg body weight of *V. amygdalina* were Group V. STZ- and nicotinamide-induced diabetic rats treated with 5 mg/kg body weight of glibenclamide were Group VI.

The experimental procedures involving the animals and their care were in line with the approved guidelines by the University of Uyo research and ethical committee established in accordance with the Helsinki declaration on animal research.

2.5. Induction of diabetes

Type I DM was induced in 30 female wistar rats by a single intraperitoneal injection of 65 mg/kg of STZ. The state of diabetes was observed by symptoms of polyurea, polyphagia, polydipsia, loss

of body weight, emaciation and wetting of their beddings and was confirmed one week after induction by testing blood glucose level using glucometer (ACCU-CHEK Advantage II, Roche Diagnostics GmbH, Germany and ACCU-CHEK Advantage II test strips).

2.6. Determination of blood glucose

Blood samples were collected by tail prick at weekly intervals. Glucometer with compatible test strips was used in the determination of blood glucose level.

2.7. Statistical analysis

Data collected during the study were expressed as mean \pm SEM. The data were statistically analysed using ANOVA with multiple comparisons versus control group. Computer software SPSS and Excel analyzer were used for the analysis.

3. Results

3.1. Type I DM

From the result obtained, the blood glucose level of Group II increased significantly ($P < 0.001$) compared to Group I from week 1 to 4. Group III and Group IV significantly decreased blood glucose level compared to Group II from week 1 to 4. Group VI significantly increased blood glucose level compared to Group I from week 1 to 4 but decreased blood glucose level significantly compared to Group II from week 1 to 4. Group V significantly ($P < 0.001$) increased blood glucose level compared to Group I from week 1 to 4 but decreased blood glucose level significantly compared to Group II from week 3 to 4 (Table 1).

3.2. Type II DM

From the results obtained, blood glucose level in Group II was significantly ($P < 0.001$) higher than Group I from week 1 to 4. Blood glucose level in Group II of type II DM was significantly ($P < 0.01$) lower than that of Group II of type I DM from week 1 to 4. Group III and Group IV significantly decreased blood glucose level from week 1 to 4 compared to Group II. Group V showed a significantly ($P < 0.001$) reduction in blood glucose level from week 1 to 4 when compared to

Table 1

Comparison of weekly blood glucose level in type I DM groups.

Groups	Before induction	After induction	Week 1	Week 2	Week 3	Week 4
Type I DM control	3.56 \pm 0.20**	26.48 \pm 1.67	24.86 \pm 1.82***	24.40 \pm 2.59***	31.40 \pm 1.11***	25.18 \pm 1.03***
Type I DM + <i>O. gratissimum</i>	3.40 \pm 0.32**	18.34 \pm 1.28 ^a	16.28 \pm 1.23*** ^a	14.26 \pm 1.33*** ^b	9.84 \pm 2.62 ^c	6.86 \pm 1.83 ^c
Type I DM + <i>V. amygdalina</i>	2.85 \pm 0.16*** ^a	23.38 \pm 1.00 ^a	14.98 \pm 1.84*** ^b	12.30 \pm 2.08*** ^a	9.58 \pm 2.96 ^c	5.18 \pm 1.01 ^c
Type I DM (<i>O. gratissimum</i> + <i>V. amygdalina</i>)	4.18 \pm 0.46	26.23 \pm 3.10 ^a	26.58 \pm 2.42***	25.70 \pm 1.07***	25.10 \pm 1.32*** ^b	23.63 \pm 3.99***
Type I DM + insulin	3.55 \pm 0.33**	27.78 \pm 2.81 ^b	21.17 \pm 2.97***	18.00 \pm 3.24**	14.55 \pm 2.80 ^{a, c}	12.00 \pm 2.69 ^{a, b}

Values were expressed as mean \pm SEM ($n = 5$). *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$ vs. normal control; ^a: $P < 0.05$; ^b: $P < 0.01$; ^c: $P < 0.001$ vs. type I DM control.

Table 2

Comparison of weekly blood glucose level in type II DM groups.

Groups	Before induction	After induction	Week 1	Week 2	Week 3	Week 4
Normal control	5.30 ± 0.30	-	4.58 ± 0.20	4.72 ± 0.26	4.68 ± 0.15	4.58 ± 0.20
Type II DM control	4.38 ± 0.29	15.72 ± 0.20 ^c	14.67 ± 0.26 ^{*c}	14.77 ± 0.24 ^{*c}	14.17 ± 0.09 ^{*c}	14.53 ± 0.20 ^{*c}
Type II DM + <i>O. gratissimum</i>	5.88 ± 0.43 ^b	5.54 ± 0.25 ^c	5.26 ± 0.65 ^c	5.34 ± 0.16 ^c	5.12 ± 0.43 ^c	4.94 ± 0.19 ^c
Type II DM + <i>V. amygdalina</i>	5.40 ± 0.36 ^b	6.40 ± 1.39 ^c	6.44 ± 2.17 ^{***}	6.70 ± 2.66 ^b	6.16 ± 0.71 ^c	5.89 ± 0.75 ^c
Type II DM (<i>O. gratissimum</i> + <i>V. amygdalina</i>)	4.79 ± 0.22 ^b	8.50 ± 1.39	8.30 ± 2.03 ^c	6.09 ± 0.80 ^c	6.06 ± 0.49 ^{*c}	5.67 ± 0.47 ^c
Type II DM + glibenclamide	4.43 ± 0.15 ^{*a}	8.38 ± 2.73 ^b	8.50 ± 3.26 ^{**}	8.68 ± 3.23 ^a	8.23 ± 3.05 ^c	7.23 ± 2.14 ^c

Values were expressed as mean ± SEM (n = 5). ^{*}: P < 0.05; ^{**}: P < 0.01; ^{***}: P < 0.001 vs. normal control; ^a: P < 0.05; ^b: P < 0.01; ^c: P < 0.001 vs. type II DM control.

Group II. Group VI showed a significant ($P < 0.01$) increase in blood glucose level from week 1 to 4 compared to Group I but significantly decreased ($P < 0.001$) when compared to Group II (Table 2).

4. Discussion

STZ is widely used as a strong inducer of diabetes in experimental animals. STZ selectively destroys the pancreatic cells that secrete insulin[17]. STZ enters the beta cells DNA and induces the activation of poly adenosine diphosphate-ribosylation. This leads to depletion of cellular nicotinamide adenine dinucleotide⁺ and adenosine triphosphate (ATP). Enhancing ATP dephosphorylation after STZ treatment supplies a substrate for xanthine oxidase, resulting in the formation of free radicals. Furthermore, STZ liberates toxic amount of oxide that inhibits the activity and precipitates DNA damage. As a result of the STZ action, beta cells undergo destruction by necrosis. STZ action on beta cells is ultimately accompanied by characteristic decrease in insulin level and increase blood glucose concentration leading to hyperglycemia[17].

When we consider hyperglycemia as the hallmark of DM, our emphasis must not be limited to the alteration or derangement of nutrient metabolism. Evidentially, hyperglycemia is implicated as the sole initiator of the cascade of pathogenic mechanisms that heralds the multisystemic diabetic complications. This explains why the base line of diabetic treatment revolves around establishment of euglycemia. The aim of this present study is therefore of great relevance in the quest to formulate a handy and potent alternative to the present standard but narrow regimen with its attendant limitations in the management of hyperglycemia in DM. This research work presented an exceptional result which should be of interest in future research work. The relative paradoxical elevation of the blood glucose level, by the combination treatment of *O. gratissimum* and *V. amygdalina* appears to be most curious finding from our result. Both *O. gratissimum* and *V. amygdalina* were on the other hand more potent than insulin and glibenclamide in the reduction of blood glucose level in type I and type II DM models.

Insulin acts by binding to the extracellular portion of the alphas subunits of the insulin receptor. This, in turn, causes receptor that activates the kinase domain residing on the intracellular portion of the beta subunits[18]. The activated kinase domain autophosphorylates tyrosine residue on the C-terminus[19]. Activation of insulin receptor leads to internal cellular mechanisms that directly affect glucose uptake

by regulating number and operation of enzymatic protein molecules in the cell[20]. Two types of tissues that are most strongly influenced by insulin as far as stimulation of glucose uptake is concerned, are muscle cells (myocytes) and fat cells (adipocytes)[21]. As expected, insulin treatment of type I DM model group was found to reduce the blood glucose levels. Remarkably, when compared to *O. gratissimum* and *V. amygdalina* treatment, the extracts were found to be more potent with respect to level of reduction and duration-dependent progress of the efficacies.

In this study, glibenclamide was used as the standard drug of treatment for type II DM model groups. Glibenclamide administration resulted in the reduction of the blood glucose levels. Again, when compared to the hypoglycemic activity of both *O. gratissimum* and *V. amygdalina* on the same group of experimental rats, these extracts were observed to show a more potent activity than glibenclamide with respect to level of reduction and duration-dependent progress of efficacy.

It has been reported that glibenclamide produces hypoglycemic effect by stimulating insulin secretion from beta cells of pancreatic islets[22,23]. Glibenclamide works by binding to and activating the sulfonylurea receptor 1, the regulatory subunit of the ATP-sensitive potassium channels (K_{ATP}) in pancreatic beta cells[4]. This inhibition causes depolarization of cell membrane, opening of voltage-dependent calcium channel. Ca^{2+} influx results in an increase in intracellular calcium in the beta cell and subsequent stimulation of insulin release.

Enhanced capacity for gut glucose uptake in diabetic animals, consistent with increase in brush border enzyme expression had been previously reported[24]. Akpan and Effiom had reported severe epithelial and mucosal gland erosion in *O. gratissimum*-treated type I diabetic rat model[8]. This was proposed to account for the apparent reduction in gut glucose absorption. Also in the same report, the possibility of some active phytochemical constituents of *O. gratissimum* affecting mitochondrial metabolism, leading to a decrease in ATP availability was noted, as glucose transport is an energy dependent process. The hypoglycemic action of *O. gratissimum* was said to be linked to the reduction in intestinal glucose absorption at the expense of the intestinal epithelial integrity[8]. We may therefore infer that in the combined treatment of *O. gratissimum* and *V. amygdalina*, the latter, *V. amygdalina* might have exerted a preservative effect on the intestinal epithelial membrane, and also counteract the effect of *O. gratissimum* on mitochondrial metabolism. Both of which will increase gut glucose absorption and raise blood glucose level

in the type I DM model. However, in type II DM, the predominant preservative action of nicotinamide on pancreatic beta cells might have accounted for the moderate hyperglycemia, thus confirming the proposition that pre-administration of nicotinamide- and STZ-induced diabetic rat models could simulate clinical type II DM. With this disposition already enhanced, following residual insulin secretion, any additional treatment is bound to produce improved glycemic control.

In clinical practice, insulin is predominantly used in the treatment of type I DM and in the treatment of type II DM when the oral hypoglycemic fails. However, insulin is basically administered as a first line drug to stabilize glycemic status in most acute presentation of DM, while glibenclamide is used as a maintenance treatment. A formulation involving nicotinamide, *O. gratissimum* and *V. amygdalina* may therefore offer a kind of go-between regimen in the management of DM irrespective of type.

O. gratissimum and *V. amygdalina* were more potent in type II DM than in type I DM, and effect of *O. gratissimum* was greater than that of *V. amygdalina*. The duration dependent efficacy of *O. gratissimum* and *V. amygdalina* was greater than that of insulin and glibenclamide in type I and type II DM, respectively. The combination treatment of *O. gratissimum* and *V. amygdalina* appears to be effective only in type II DM. Nicotinamide, *O. gratissimum* and *V. amygdalina* offer interesting prospect in the search for comprehensive treatment modality for DM.

Conflict of interest statement

We declare that we have no conflict of interest.

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