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Evaluating the Impact of *Ocimumgratissimum* and *Verononiaamygdalina* on Glycated Hemoglobin in Niacin - Induced Type 2- Diabetes

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Abstract: ***The cost of conventional anti-diabetic drugs, their accessibility coupled with increased non- compliance among patients necessitates the need for a more acceptable, accessible and cheap alternative to lowering Glycated Hemoglobin (GHb) in type 2- diabetes. To evaluate the impact of Ocimumgratissimum (OG) and Verononiaamygdalina (VA) on GHb in type 2- diabetes, type II diabetes was induced using 100 mg/kg niacin, l hour before the administration of 65 mg/kg STZ. Once diabetes once established by testing with a glucometer, OG and VA were administered and compared with negative and positive controls. GHb concentrations for the various groups were measured using Ion-Exchange Resin Method and the results analysed using One Way ANOVA.VA showed a significant (p<0.001) reduction in glycated hemoglobin as well as a significant (p<0.05) reduction in GHb was observed in the group treated with OG+ VA. Thus, daily treatment with OG and VA drastically reduces glycated hemoglobin levels in type 2 diabetes under niacin coverage; improving clinical outcomes of type 2 diabetic rats.***

Keywords: *Ocimumgratissimum* (OG), *Verononiaamygdalina* (VA), Type 2 diabetes, Niacin, Glycated hemoglobin

1. Introduction

The growing prevalence of Type 2 diabetes (T2D) or Non-Insulin Dependent Diabetes Mellitus (NIDDM) in Sub-Sahara Africa from <1% in 1960 to 6.4-16% in 2014 with a predicted increase in the prevalence of T2D has necessitated the need for the use not only non- conventional therapies but preventive measures of abating the disease (Goedecke JH and Ojuka EO). T2D cause disease complications in various systems such as cardiovascular (Saydah SH. et al, 2002), renal (Brown WV., 2008), retinal and neural tissues, thus increasing the burden of managing the disease with increased morbidity and mortality. The reason for this growing prevalence has been attributed to increase in restaurant food (Supriya K., Patricia FC and Julie RP, 2010) and sweetened beverage consumption among other factors like genetics.

Whatever the cause, the marker for a more reliable hyperglycemic measurement is glycated Hb. Glycated hemoglobin is the non-enzymatic condensation of glucose with hemoglobin at the α and β amino groups of β-chains to form amino-1-deoxyfructose. The rate of glycosylation depends on blood glucose concentration, which is increased during the incidence of Diabetes Mellitus (DM) (Chandellia and Krishnaswamy, 2002). The development or formation of advanced glycated end product (AGEs) in DM has been implicated in the pathophysiology of glycated hemoglobin species. Free radicals like H_2O_2 enhances the release of free Iron (Fe^{3+}) from HbA1 than the non- glycosylated forms (HbO). The free iron then acts as a fenton reagent that produces more free radicals to degrade cell constituents especially DNA. Formation of carbonyl content as an index of oxidative stress is higher in HbA1 than HbO and causes adverse structural modifications of hemoglobin. This is demonstrated by reduced o-helix content, more surface accessible hydrophobic tryptophan residues, increased thermo ability and weaker heme-globin linkage in HbA1 than in its non glycated analog. The glycation-induced structural modification of hemoglobin may be associated with its functional modification leading to oxidative stress in diabetic patients (Khalid R, 2007).

Glycated hemoglobin is used as a biological marker to measure the average glucose concentration over a period of 2-3 months in DM. The higher the plasma glucose concentration the greater the fraction of hemoglobin that is glycated. In diabetes mellitus, higher amounts of glycated hemoglobin, indicating poorer control of blood glucose levels which have been associated with cardiovascular disease, nephropathy, and retinopathy. Monitoring HbA1 in type 2 diabetic patients may improve outcomes. (Larson, 1990)

Herbal extracts, like *Verononiaamygdalina* and *Ocimumgratissimum* are considered less toxic when compared to their synthetic counterparts and it is used in about 80% of the world's population, particularly in developing countries because of better cultural acceptability, safety, efficacy, affordability and tolerability (Pan and Umamaheswari, 2000). *V. amygdalina* has a high reputation for use in the traditional management of diabetes mellitus. Scientific studies have also reported/confirmed its antihyperglycemic (Akah P. Njoku O. Nwanguma A. and Akunyili D, 2004) and hypoglycemic (Gyang, S. S. Nyam E. N. and Sokomba, 2004) action in diabetic and non-diabetic rats respectively. The aqueous leaf extract, also has protective effect on the livers (Atangwho, I. J. Ebong P. E. Egbung G.E. Eteng M.U. and Eyong E.U, 2007) of alloxan diabetic rats, which may impact on the glycogenesis role of the liver. The aqueous extract of OG, shows anti-diabetic properties in streptozocin-induced diabetic rats (Mohammed A. Tanko Y. Okasha M.A. Magaji R. A. and Yaro A.H, 2007).

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Several studies have showed that VA and OG have hyperglycemic lowering effect and anti-diabetic properties but the impact of OG and VA on GHb species (HbA1C) especially in T2D has yet to be investigated, as well as the accompanying mechanisms by which these herbs exert their GHb lowering effect. This experiment was designed to investigate GHb lowering effect of VA and OG in T2D under niacin coverage.

2. Materials and Methods

Fresh leaves of OG and VA were rinsed with water to remove sand and debris. The leaves were cut into small pieces and allowed to dry in an ambient temperature for two days. The dried leaves were pulverized into fine powder. About 425 g each of the powdered plant materials were macerated in 3000 m1 of distilled water for about l2 hours and stirred at regular intervals. The mixture of each extract was filtered and their filtrates were concentrated to dryness in a water bath at 45°C. The extracts were weighed and refrigerated at 4°C until required for use.

The lethal dose (LD50) of the plant extracts were determined by method of Lorke (1983) using 30 mice of both sexes weighing between 20 g to 25 g. The animals were weighed and grouped into 10 groups of 3 mice per group. The dosage of administration ranged between 500 mg/kg to 5000 mg/kg body weight. The route of administration was intra-peritoneal. After a single administration the animals were observed within 24 hours for physical signs of toxicity, excitation, increased respiratory rate, writhing, convulsion and death.

Thirty (30) female albino wister rats were randomized into 6 groups of 5 animals per group and were induced with type II diabetes after fasting for 12 hours. Body weights and glucose levels were deduced prior to induction. Type II diabetes was induced using 100 mg/kg niacin, l hour before the administration of 65 mg/kg STZ dissolved in 0.l ml fresh cold citrate buffer at pH 4.5. The state of diabetes was observed after 48 hours for symptoms of polyuria, polyphagia and polydipsia and confirmation done after a week by testing blood glucose levels using a glucometer. The extracts (the doses for OG and VA were 208 mg/kg and 52 mg/kg bw respectively) and drug (5 mg/kg bw of glibenclamide) were administered one week after induction of diabetes. Body weights and blood glucose levels were measured to confirm the establishment of diabetes before administration. The drugs and extracts were administered daily for 28 days and facilitated by the use of a syringe and esophageal canula. Blood glucose content and body weight were monitored at weekly intervals throughout the 28 days.

Thirty (30) adult female Albino wistar rats weighing 110-200 g were randomly divided into 6 groups of 5 rats per group as shown below:
Group1: Negative control
Group2: Positive control
Group3: DM2 + Glabenclamide
Group4: DM2 + OG
Group5: DM2 + VA
Group6: DM2 + OG + VA

At the end of 28 days, blood samples were collected via cardiac puncture and a quantitative analysis was made using Ion Exchange Resin Method which is based on the property of non GHb binding to a weak cation exchange resin, leaving GHb free in the supernatant.

Principle: Whole blood was mixed with lysing reagent to prepare a hemolysate. This was then mixed with a weakly binding cation exchange resin. The non GHb binds to the resin leaving GHb free in the supernatant. The GHb percentage is determined by measuring the absorbance of the GHb fraction and the total Hb using the formular below:
Absorbance of GHb% = Absorbance of THb x 7.2 x temp. factor (TF)
For assay at 23^0C, Tf =1.0; at 30^0C, Tf = 0.9

Statistical analysis
Data collected during the study were expressed as mean ± Standard Error of the Mean SEM. The data were statistically analyzed using ANOVA with multiple comparisons versus control group.

3. Results

AIC concentrations in experimental type 2 diabetic rats under Niacin coverage treated with OG, VA and Glibenclamide is shown in the chart below:

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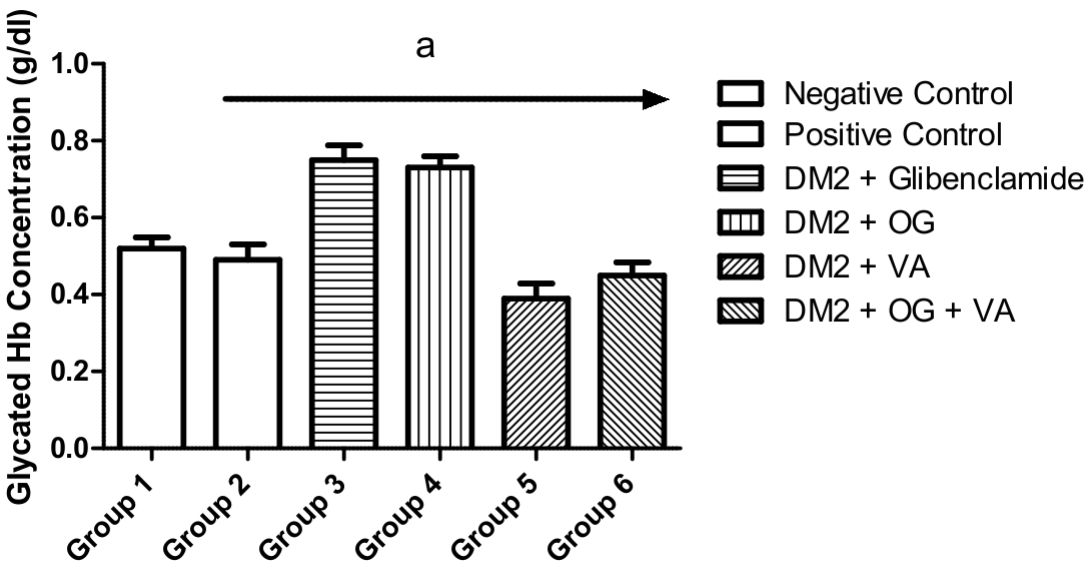

VA significantly ($p<0.001$) lowered GHb when compared with negative and positive controls as well as when compare with glibenclamide groups. Also the combined use of OG and VA significantly ($p<0.05$) decreased GHb concentrations when compared with negative and positive controls but higher than VA treated groug.

4. Discussion

Niacin is reputed to increase insulin resistance and obesity in the long-term by continuously stimulating pancreatic beta cells and their eventual burning out; thus very effective in the induction of type 2 diabetes when administered concomitantly with STZ, (Gary and Grundy,1990). In this study, T2D rats treated with OG showed a significant ($p<0.05$) reduction in GHb/Alc concentration when compared with positive control group. This reduction ($p<0.001$) was even more when treated with VA. The underlying mechanism for this may reside in the ability of VA to reduce ROS/oxidative stress via its antioxidant property with niacin, (Micheal et 'al, 2010) to bring about a reduction in Hb glycation. Since insulin in T1D, causes an in increase in both THb and GHb, it could be inferred that the nicotinamide-induced increase in insulin release in T2D (induced by nicotinamide), brought about by improvement in beta cell integrity and its regeneration by nicotinamide, could be responsible for interfering with the GHb-lowering ability of VA in T2D (induced by nicotinamide). However, niacin effects (insulin resistance and burning out of beta cells) are associated with a decrease in insulin concentration, which allows/permits VA to lower GHb in T2D (induced by niacin).

Thus, the discovery that GHb is lowered by VA only in the absence/reduced levels of insulin, is most remarkable. Insulin can therefore be said to exert a non-permissive effect on the GHb lowering action of VA.

T2D, when treated with a combination therapy of OG + VA, also showed a significant ($p<0.01$) reduction in GHb when compared to positive control, OG and glibenclamide treated groups. VA seems to potentiate the GHb lowering effect of OG.

Phytochemical compounds in OG and VA like terpenes, saponins tannins, flavonoids, phenols, renins, balsan, steroids and cardiac glycosides, (Ezekwe CI and Obidao O, 2001), may in part be responsible for the observed significant activity of these extract either singly or in synergy with one another to lower total cholesterol and triglycerides level (Stanley et al, 2017) thereby correcting insulin resistance.

Glibenclamide, like other sulphonylureas, is effective in mild diabetic states and ineffective in severe diabetic animals where pancreatic beta cells are completely destroyed (Qamar F., Afroz S., Feroz Z., Siddiqui S. and Ara A., 2011). Glibenclamide has been reported to produces hypoglycemic effect by stimulating insulin secretion from beta cells of pancreatic islets (Tavafiet'al, 2011; Gosh and Suryawanshi., 2001) and since T2D induced by niacin is characterized by hypoinsulinemia due to beta cell destruction by niacin, glibenbenclamide showed no effect on GHb when compared to controls and herbal remedies.

5. Conclusion

T2D is characterized by normal insulin levels, insulin resistance and treatable by sulphoylureas like glibenclamide which act to increase beta cell secretion of insulin. Glibenclamide action serves little purpose in increasing insulin sensitivity as evidenced by the persistent increase in GHb concentration- a marker of hyperglycemia, hence requiring a more realistic and purposeful non-conventional use of herbal remedies. VA and OG, from the fore-going experimental result, can conveniently serve as an alternative treatment for T2D especially in pancreatic beta cell destruction, where they effectively decrease insulin resistance by correcting total cholesterol and triglycerides levels.

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