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EVALUATION OF THE IMPACT OF INSULIN, OCIMUM GRATISSIMUM AND VERONONIA AMYGDALINA ON HEMOGLOBIN CONCENTRATION IN TYPE 1 DIABETIC RATS: A COMPARATIVE STUDY

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ABSTRACT

Decreased hemoglobin (Hb) concentration has been implicated in patients with diabetic nephropathy, due to impaired ability of kidney to produce erythropoietin (EPO). Several in vivo studies have also reported varying results on the effect of herbal remedies like Ocimum gratissimum (OG) and Veronia amygdalina (VA) on hemoglobin concentration. So far, there is no sufficient evidence or data for an effective therapy for anemia in type 1-diabetes (T1D). To compare the impact of insulin, OG and VA on Hb concentration in type 1- diabetes, type I diabetes was induced using 65 mg/kg STZ. Once diabetes once established by testing with a glucometer, insulin, OG and VA were administered and compared with negative and positive controls. Hb concentrations for the various groups were measured using Ion-Exchange Resin Method and the results analysed using One Way ANOVA. Insulin caused significant (P 0.05) increase in Hb concentration when compared to controls, OG and VA treated groups. However, there was a significant (P 0.05) increase in Hb concentrations in non-diabetic rats treated with VA, when compared with negative control. It could be concluded that Insulin is effective in treating anemia in uncomplicated T1D while VA significantly increase Hb in healthy states and could be evaluated for the treatment of anemia in humans.

KEY WORDS : Insulin, Ocimum gratissimum, Veronia amygdalina, Hemoglobin and Type 1-diabetes.

Introduction

Recent studies have included anemia as a complication of diabetes arising from impaired renal ability to produce erythropoietin (EPO) in diabetic nephropathy (Christopher H. et al, 2010), chronic inflammatory activity, increased levels of Advanced Glycated End products (AGEs), EPO hypo-responsiveness, effects of oxidative stress and anti-diabetic medications, (Antwi-Bafour et al., 2016, Craig KJ et al., 2005, Montemarano N. et al., 2013, Forte V. et al., 2011, Baisakhiya S., Garg P., and Singh S., 2017, Singh DK, Winocour P., and Farrington K., 2009). The decline in hemoglobin levels was observed to be 2-3 times higher than the general population with anemia from other causes and this decline appeared to be age dependent occurring in the early to mid- 20s, putting these patients at greater risk of complication and contributing to patient's comorbidity with vascular disease and adverse outcome (Barbieri J. et al, 2015; Gupta A. et al, 2017; Makadiya R. et al, 2013; Bonakdaran S, Gharebaghi M and Vahedian M, 2011; Adejumo BI. et al, 2012; He BB. et al, 2015). While other studies have associated diabetes with high hemoglobin concentration (Conway B, Fried L and Orchard T, 2008; Baqiyyah N. Conway, Rachel G. Miller and Trevor J. Orchard, 2010) arising from hemoconcentration secondary to diabetes-induced increase in osmolality, generalized response to hypoxia secondary to vascular disease or a response to testosterone, which has been reported to be increased in type 1 diabetes (Meyer K. et al, 2000; Christensen L. et al, 1997). Additionally, insulin and insulin-like growth factor 1 and 2 have been shown to stimulate erythropoiesis in astrocytes (Masuda S, Chikuma M. and Sasaki R. et al, 1997), while elevated fetal Hb arising from diabetic ketosis-induced increase in β-hydroxy-butyrate has also been observed in children and adult with T1D (Peters A. et al 1998; Diem P. et al 1993).

The development of diabetes-induced anemia results in micro and macrovascular complications in different organ systems which may impair circulatory functions and worsen clinical outcomes in diabetic patients (Thomas MC. et al, 2006; Thomas MC, 2007; Samuel TR. et al, 2018; Salma M. and AlDallal NJ 2018; Periasamy S, Xavier AA, Gowtham R., 2016; Holland DC and Lam M., 2000). T1D is caused by impaired insulin secretion from the pancreas that limits cellular uptake of glucose by cells resulting in complications. Insulin administration improves glucose dys-regulation and stimulates erythropoiesis by bone marrow cells, increasing hemoglobin in the early stages of the disease. However, in the latter stages of the disease as evidenced by diabetic nephropathy, a decline in Hb concentration has been observed even with insulin administration (Christopher H et al, 2010). Despite these findings, a study by Christopher H., Felix K. and Justo LB., 2016 demonstrated an improvement in Hb concentrations in diabetic nephropathy with insulin analog (like Glargine and Lispro) treatment.

Some studies have reported that herbal therapies like OG reverses anemia secondary to alloxan-induced diabetes mellitus in rats probably due to its antioxidant activity, by increasing hemoglobin level (Shehu-Tijani TS et al. 2016). While some studies report an increase Hb concentration in OG treated non-diabetic rats, others report a reduction in Hb level (Jimoh OR, 2008; OE Ofem, EJ Ani, and AE Eno, 2012). Also, while some evidence exists on VA's ability to increase Hb concentration in non- diabetic state Ejike et al, (2021), there is little evidence of the effect of OG and VA on Hb concentration in diabetes. This in vivo study was designed to compare the Hb boosting effect of insulin, OG and VA in diabetic and non-diabetic states with a view to ascertaining more effective and inexpensive way of ameliorating anemia in diabetic or non-diabetic states.

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. Four hundred and Twenty-five grams each of the powdered plant materials were macerated in 3000 m1 of distilled water for l2 hours and stirred every 6 hours. 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 (LD-50) of the plant extracts were determined by method of Lorke (1983) using 30 mice of both sexes that weighed 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

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of 5 animals per group and were induced with type I diabetes after fasting for 12 hours. Body weights and glucose levels were deduced prior to induction. Type I diabetes was induced using 65 mg/kg streptozotocin (STZ) dissolved in 0.l ml freshly prepared 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 body weight respectively) and 0.16 IU of Insulin 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.

Forty (40) adult female Albino wistar rats weighing 110- 200 g were randomly divided into 8 groups of 5 rats per group as shown below:

Group 1: Negative control
Group 2: OG
Group 3: VA
Group 4: Diabetic control
Group 5: DM1 + OG
Group 6: DM1 + VA
Group 7: DM1 + OG + VA
Group 8: DM1 + Insulin

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 Hb binding to a weak cation exchange resin, leaving Glycated Hemoglobin (GHb) free in the supernatant as invented by James L. Sanders: Whole blood was mixed with lysing reagent – Potassium cyanide to prepare a hemolysate. This was then mixed with carboxymethyl dextran – a weakly binding cation exchange resin. The Hb binds to the resin leaving GHb free in the supernatant. The Total Hemoglobin (THb) concentration was determined by measuring the percentage absorbance of the GHb fraction divided by temperature factor and 7.2 (a constant) in accordance to the formula below:

Absorbance of THb = Absorbance of % GHb /7.2 x temp. factor (TF) (James L. Sanders, 1983)
For assay at 230C, Tf = 1.0; at 300C, Tf = 0.9
Where, THb = Total Hemoglobin and GHb = Glycated Hemoglobin

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.

Results

Hb concentrations in experimental Type 1 diabetic rats treated with OG, VA and Insulin is shown in the Table below:

Comparison of Total Hb, in experimental type 1 diabetic rats treated with OG, VA and Insulin.

	Control	OG	VA	Diabetic control	DM1 + OG	DM1 +VA	DM1 + OG +VA	DM1 + Insulin
Hb	0.44	0.48 ±0.020	0.51 ± 0.017	0.49 ± 0.024	0.45 ±0.039	0.43 ± 0.030	0.40 ± 0.033	0.58 ± 0.036

Total Hb: the mean ± SEM Total Hb values were 0.44 ± 0.001, 0.48 ±0.020, 0.51 ± 0.017, 0.49 ± 0.024, 0.45 ±0.039, 0.43 ± 0.030, 0.40 ± 0.033, 0.58 ± 0.036 g/dl for control, non- diabetic rats treated with OG, non- diabetic rats treated with VA, DM1, control, DM1 treated with OG, DM1 treated with VA, DM1 treated with both OG and VA and DM1 treated with insulin respectively. From the results obtained, DM1 control showed a significant increase in total hemoglobin concentration compared to control ($p<0.05$). Non diabetic rats treated with OG and VA showed no significant difference in normal hemoglobin concentration when compared with control. Total Hb concentration in DM1 treated with OG was significantly lower than DM1 ($p<0.05$), but treatment with VA showed a significant reduction compared to control ($p<0.001$). DM1 treatment with both OG, VA and insulin showed no significant difference. Insulin caused significant increase in Hb concentration when compared to controls (P 0.05), OG and VA treated groups. Also, there was a significant increase in Hb concentrations in non-diabetic rats treated with OG (P 0.05) and this increase was more in VA treated group, when compared with negative control.

Discussion

Insulin has been an age long first line treatment of especially T1D as it is reputed to facilitate cellular uptake of glucose by cells, decrease blood glucose concentrations and increase clinical outcome in patients. In recent times, it has been observed that diabetic patients develop anemia, especially during the later stages of diabetes, and are characterized by diabetic nephropathy in which the renal interstitial cells are unable to produce erythropoietin for Red Blood Cell (RBC) production, which adds to degrading action of reactive oxygen species (ROS) (generated from diabetic states) on cell constituents; in this case, RBC causing anemia. Recent findings report that anemia in latter stages of diabetes is not reversed even with insulin treatment. But our experiments give evidence of an insulin-induced increase in Hb concentration in T1D. These findings are consistent with those by Akoi et al, (1994) which suggested a direct action of insulin on erythroid progenators, indicating that insulin stimulates the formation of colony- forming unit (CFU)-erythroid and burst forming unit erythroid, and directly stimulates the proliferation of late stage of primitive erythroid progenitor cells and mature erythroid progenitor cells through the sharing of receptors, (Miyagawa et al, 2000).

OG and VA had no significant effect on Hb concentration in diabetic treated groups, as also reported by P.B. Enyievi, (2020) and Du-Bois Asante et al, (2016) which we suggest is due to the non-permissive effect of insulin on VA's action on Hb. However, VA increases Hb concentrations in non-diabetic rats as reported also by Ejike et al, (2021) and Nubila T. et al, (2013).

Conclusion

Treatment of Type1 Diabetes-induced anemia with insulin in rats, is most effective during the early stages of diabetes, when renal interstitial cell integrity can guarantee sufficient production of EPO for RBC formation. VA increased the production of Hb in the normal rats and could be further evaluated for the treatment of anemia in humans.

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