GLUCOSE TOLERANCE TEST IN HYPERGLYCEMIC GUINEA PIGS TREATED WITH AQUEOUS *VERNONIA AMYGDALINA*

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SUMMARY: We have determined the comparative response in hyperglycemic guinea pigs to a glucose tolerance test (GTT) following treatment with either unboiled or boiled aqueous extract of Vernonia amygdalina (bitter leaf) or glucophage solution. The animals were divided into four groups, fasted overnight and each received glucose solution by intraperitoneal injection (ip.) following fasting to induce hyperglycemia. One group served as control, the second group received unboiled extract of bitter leaf, the third group received boiled extract of bitter leaf, while the fourth group received a glucophage solution all by i.p. Following treatment, blood samples were collected from each group at 30 min, 60min, and 180 min and glucose concentration was determined in each sample by the glucose oxidase method.

The results show that at 30 min, control blood glucose level spiked to 17.4 mmol/L from a Fasting Blood Glucose level of 5.6mmol/L; at 60 min, the level dropped to 8.1 mmol/L and then stabilized to Fasting (Baseline) level of 5.5 mmo/L at 180min. This curve (pattern) is consistent with classical GTT protocol. In the GTT pattern of animals treated with unboiled aqueous bitter leaf extract (b.l.e), blood glucose level rose to 9.8 mmol/L at 30min, nose dived to 5.7 mmol/L at 60min (a Baseline value), and stabilized at 5.6mmol/L after 180min. On the other hand, in the GTT pattern of the boiled aqueous b.l.e. and glucophage solution, excess blood glucose was not cleared to Baseline (control) level even after 180 min: for boiled b.l.e, blood glucose level rose to 12.9 mmol/L at 30 min, dropped to 6.7 mmol/L and stabilized at 6.2 mmol/L at 180 min, a level higher than baseline. Similarly, for glucophage, a spike of blood glucose level of 13.1 mmol/L occurred at 30 mins, (near identical with boiled b.l.e), dropped to 7.2 at 60 min (higher than unboiled b.l.e) and increased to 9.5 mmol/L after 180 min (similar pattern to boiled b.l.e).

These results clearly show that under these GTT conditions, the unboiled water b.l.e. cleared the excess blood glucose molecules more rapidly and more quantitatively than either the boiled water b.l.e. or glucophage, and therefore may be a more effective antihyperglycemic preparation.

Key Words: Vernonia amygdalina, Antihyperglycemia, Glucose Tolerance Test, Unboiled and Boiled Water extracts, Blood Glucose Level, Glucophage.

INTRODUCTION

Diabetes, especially the Type II insulin-independent, is now a world-wide health problem, and Nigeria is not an exception, where there is the preference for the traditional approach with the use of natural herbs, such as the aqueous extracts of Vernonia amygdalina ("bitter-leaf," personal experience). The World Health Organization (WHO) defines Type II diabetes, on the basis of labora-

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tory findings, as a Fasting Venous plasma glucose concentration that is greater than 7.8 mmol/L (140 mg/dl) or greater than 11.1 mmol/L (200mg/dl) two hours after a carbohydrate meal or after an oral ingestion of 75g of glucose even if the fasting concentration is normal (15). Glucose Tolerance Test (GTT), in medical practice is used in the diagnosis of diabetes to determine how quickly administered glucose is cleared from the blood; in diabetes-insulin resistance and sometimes also in reactive hypoglycemia (15). The glucose is most often given orally (OGTT), but also intravenously (IVGTT) for the investigation of early insulin secretion abnormalities (15). For simple diabetes screening, the most important sample is the 2 hour sample, but for research purposes, samples may be taken at many different time schedules (15). The anti-hyperglycemic effect of Vernonia amygdalina or 'bitter-leaf' has been reported by various authors, however, the common denominator in these animal studies was the use of alloxan or streptozotocin which destroy the β-cells of the pancreas where insulin is produced-(14). This would eliminate the secretion of insulin and thereby induce hyperglycemia (diabetes II) in the animals. Nwanjo (10) investigated the effect of aqueous extracts of bitter leaf in streptozotocin-induced hyperglycemic rats and reported a significant reduction in Fasting blood glucose levels in the treated diabetic rats. Similarly, Osinubi (13) reported that aqueous bitter leaf extract produced significant reductions in blood glucose concentrations of normal (normoglycemic) and diabetic hyperglycemic Sprague-Dawley rats one to two hours after acute treatments; and that its blood glucose lowering potential in both sets of rats compared favourably to that of a known hypoglycemic drug, chlorpropamide (13).

Although the active anti-hyperglycemic components in bitter leaf have not been identified, they are thought to work by bringing about cell regeneration whilst enhancing insulin sensitization as trials involving hyperglycemic mammals resulted in the restoration of complete insulin activity within six months (9). However, aqueous extracts of bitter leaf have been reported to contain saponins, alkaloids (9) and the anti-oxidant, flavonoids (5). On the other hand, though the potential toxicity of bitter leaf has not been adequately studied, some potentially carcinogenic and mutagenic compounds, such as marmesin, imperatonin, dictamine and heraclenin (furocoumarins) have also been isolated and characterized from aqueous extracts of Vernonia anygdalina and Garcina cola (11). In that study, we showed that aqueous extracts of both bitter leaf and bitter cola altered glucose-6-phosphatase activity and consequently perhaps gluconceogenesis or glycogen metabolism, and regenerative, and total DNA concentrations in regenerating and normal rat liver in a structure-activity-relationship of these furocoumarins in bitter leaf and bitter cola to aflatoxin B1, a known and potent human hepato-carcinogen. This, we concluded, could also impair DNA function (11).

After an extensive internet search, there was no reference to any study that examined the comparative anti-hyperglycemic effect of unboiled versus boiled water extracts of Vernonia amygdalina, on the one hand, and the glucose tolerance response of hyperglycemic laboratory animals when treated with aqueous extract or the unboiled form. More so as GTT measures the rate of clearance of excess glucose molecules from the blood without harming the pancreas (14).

As mentioned earlier, there is a widespread preference in Nigeria for the traditional approach to the management of hyperglycemia through the oral ingestion of either the unboiled or boiled aqueous extracts of bitter leaf; however, there is uncertainty as to which is more effective as most prefer the boiled form as in a soup.

In this study, it was therefore of interest for us to determine the efficacy of the unboiled-versus the boiled-aqueous extract of bitter leaf under the novel GTT condition, based strictly on the differential rate of reduction of high blood glucose concentration in hyperglycemic guinea pigs.

MATERIALS AND METHODS

All the experiments described in this article were carried out in compliance with the regulations of the Federal Republic of Nigeria guiding Animal Care and Use.

Preparation of bitter leaf aqueous (unboiled and boiled) extracts

Unboiled Aqueous Extract

Fresh mature bitter leaves were weighed, washed, and sheared by hand into a 1000ml beaker. Appropriate volume of distilled and deionized water was added to a final concentration of 35% and the leaves were hand-squeezed in manual extraction for 10 minutes. This was filtered through two layers of wire-gauze and the dark-greenish filtrate was collected in a separate

Table 1: Blood Glucose Concentrations (mmol/L) of Guinea-pigs at 30 mins, 60 mins and 180 mins following treatment with glucose solution only or glucose solution and unboiled bitter leaf extract (b.l.e.) under the Glucose Tolerance Test (GTT) condition.

Blood Glucose Concentration (mmol/L)				
Time after Treatment (mins)	Fasting	Glucose solution only (Control)	Glu.soln.+ unboiled b.l.e	% change over control
0	5.6 ± 0.60	-	-	-
30	-	17.4 ± 1.51	9.8 ± 1.22	- 43.78 (decrease)
60	-	8.1 ± 1.0	5.7 ± 0.65	-26.6 (decrease)
180	-	5.5 ± 0.52	5.6 ± 0.71	+ 1.8 (increase)

beaker and stored at room temperature for 20 minutes before use.

Boiled Aqueous Extract

The same procedure was followed as in the preparation of the unboiled extract except that the leaves were sheared into appropriate volume to a final concentration of 35%, and boiled, for 15 minutes, allowed to cool, and then filtered before use.

Treatment of Animals

Twenty mature male guinea-pigs weighing between 200gm-250gm were divided into four groups (A,B,C and D) of five animals per group. All animals were fasted overnight (about 10 hours) but given drinking water ad libitum, prior to treatment. Following fasting, all the animals received 0.5ml of a 50% glucose solution each by intra peritoneal injection (i.p.); Group A served as the control (Baseline) and each animal in this group received the glucose solution only. Group B animals each received same dose of glucose solution and 0.5ml of unboiled bitter leaf extract immediately after, also by i.p; Group C animals each received same glucose solution and 0.5ml of boiled bitter leaf extract immediately after, also by i.p; Group D animals each received same glucose solution and 0.5ml of a 35% glucophage solution immediately after, also by i.p.

According to the GTT protocol (WHO, 2007), blood glucose concentrations are measured in blood samples at zero hour (Fasting), 30 minutes (1/2 hr), 60 minutes (1hr),120 min (2hrs), and 180 min (3hrs) and although the 2hr sample is considered a target -sample in clinical human tests, varying the time schedules can be adopted, especially for research purposes (15). We therefore skipped the 2hr-sampling, and sampled at 1/2hr, 1hr and 3hr after treatment for measurement of glucose concentration in each of the animals. Zero-hr (Fasting) blood glucose concentration was determined earlier in each of the four groups.

Blood Glucose Quantification

Blood glucose levels were measured in all the collected samples by the glucose oxidase method that is widely used in clinical laboratory procedures (12). To ensure reproducibility, each assay was carried-out in triplicates, and the Arithmetic Means with their corresponding Standard Deviations were calculated. The results are therefore expressed as Mean ± SD. Changes over Control values were calculated as Percentage Increase or Decrease.

RESULTS

Table 1 shows time-related decreases in blood glucose concentrations in animals treated with glucose solution only (as control) or glucose solution and unboiled aqueous bitterleaf extract (b.l.e.), As is shown, the Fasting blood glucose concentration obtained at zero (0hr) was 5.6mmol/L. In guinea-pigs treated with glucose solution only, after overnight fasting, a sharp increase in the blood glucose concentration was obtained at 30 mins of 17.4mmol/L, but at 60 mins and 180 min the levels decreased dramatically to 8.1mmol/L and 5.5 mmol/L respectively. This pattern of response is consistent with the Glucose Tolerance Test (GTT) protocol (15), which suggests a normal β-cell pancreatic function as insulin seems to have been secreted adequately in response to

Table 2 : Blood Glucose Concentrations (mmol/L) of Guinea-pigs treated with glucose solution only or glucose solution and boiled bitter leaf extract (b.l.e.) at 30 mins, 60 mins and 180 mins following treatment under the Glucose Tolerance Test (GTT) condition.

Time after Treatment (mins)	Glucose solution only (Control)	Glu.soln.+ unboiled b.l.e	% change over control
30	17.4 ± 1.51	12.9 ± 1.73	- 25 (decrease)
60	8.1 ± 1.0	6.7 ± 0.72	- 17 (decrease)
180	5.5 ± 0.52	6.2 ± 0.65	+ 12.7 (increase)

glucose solution-induced high blood glucose.

Comparatively, blood glucose concentrations in the animals treated with unboiled b.l.e., in addition to following the same GTT response as above, were also quantitatively lower than the control values at 30min and 60 mins, 9.8 mmol/L against 17.4mmol/L, and 5.7mmol/L against 8.1mmol/L, respectively. This represents 43.7% and 26.6% decreases over the control, respectively. Furthermore, at 180mins, glucose level in these animals stabilized at baseline level of 5.6mmol/L. However, interestingly, in this group of animals that were treated with unboiled b.l.e., the blood glucose level returned to control (Baseline) level at 60 min (1hr), which clearly suggests an accelerated clearance of excess glucose molecules from the blood of glucose solution - treated guinea-pigs by components of the unboiled b.l.e. in 60mins. The relevance of this result would be discussed shortly.

In Table 2 the effect of boiled b.l.e. on glucose clearance is presented: blood glucose concentrations at 30 min, 60 min and 180 mins following treatment of guinea pigs with boiled b.l.e. are shown against the control values. There is a similar spike of glucose concentration at 30min of 12.9mmol/L from the zero (0hr) level of 5.6mmol/L. However, when compared to the control, the percent decrease of 25% is almost half that induced by unboiled b.l.e. which caused a 43.7% decrease over the control. At 60min, blood glucose dropped to 6.7mmol/L and surprisingly, did not change much at 180min at a value of 6.2mmol/L. This is noteworthy because contrary to the value of unboiled b.l.e.-treated guinea pigs, boiled b.l.e. did not attain baseline (control) level, even at 180mins, whereas blood glucose level dropped to base line (control) level in the unboiled b.l.e.-treated guinea pigs at 60min, (5.7mmol/L) and remained at baseline level after 180min (5.6mmol/L). In addition, the changes over control in blood glucose in unboiled - against boiled b.l.e.-treated guinea pigs showed larger percent decreases at two of the three treatment times, 30mins and 60mins. The GTT response for boiled b.l.e. therefore is not consistent with WHO protocol.

Table 3 shows blood glucose levels in guinea pigs treated with glucophage solution (an over-the-counter orthodox anti-hyperglycemic drug) after 30min, 60min and 180min against control values. As is shown, the GTT response is similar in character to that of the boiled b.l.e: at 30min, there was a spike, 13.1mmol/L from zero (0hr) value of 5.6mmol/L; at 60mins, it dropped to 7.2 mmol/L, but increased at 180min to 9.5mmol/L - a similar GTT profile of the boiled b.l.e. Percent changes over control are also similar: at 30min, 24.7%:25% decreases, glucophage and boiled b.l.e., respectively; at 60mins, 11.1%:17% decreases, respectively; then at 180min, 45.2%:12.7% increases, respectively. Therefore, as with the boiled b.l.e., the GTT response of glucophage under our conditions is not consistent with WHO protocol (15).

DISCUSSION

In most developing countries, such as Nigeria, there is a prevalence of traditional approaches to various health conditions (personal experience), and diabetes is not an exception. Indeed, Vernonia amygdalina, commonly known as "bitter leaf" in most African countries (in Nigeria), is widely used for the control and management of hyperglycemia and other

Table 3: Blood Glucose Concentrations (mmol/L) of Guinea-pigs treated with glucose solution only or glucose solution and boiled bitter leaf extract (b.l.e.) at 30mins, 60mins and 180mins following treatment under the Glucose Tolerance Test (GTT) condition or glucose solution and glucophage solution at 30 mins, 60 mins and 180 mins. e.t.c.

Blood Glucose Concentration (mmol/L)			
Time after Treatment (mins)	Glucose solution only (Control)	Glucose solution and Glucophage	% change over control
30	17.4 ± 1.51	13.1 ± 0.95	- 24.7 (decrease)
60	8.1 ± 1.0	7.2 ± 0.76	- 11.1 (decrease)
180	5.5 ± 0.52	9.5 ± 1.0	+ 45.2 (increase)

ailments (10). The International Diabetes Federation (6) has encouraged the use of such natural product as bitter leaf for medicinal purposes. Several studies have reported the anti-hyperglycemic effect of Vernonia amygdalina (1,2,13), however all showed induced damage to the pancreas when applied with either alloxan or streptozotocin (2,14). The relative efficiency of either unboiled - or boiled-water extract of bitter leaf in lowering high blood sugar has not been reported, till now; and although unboiled bitter leaf water extract cleared excess blood glucose molecules within 60min, the probable mode of action of the constituents is not clear, more so as some structurally different components have been reported, such as alkaloids, saponins and flavonoids (5, 8). The design of this study followed a unique Glucose Tolerance Test (GTT) protocol which had not been done before, as a way to hopefully shed light on the mechanism of action of bitter leaf components based on the responses of hyperglycemic guinea pigs to the test following treatment. Thus our results present some interesting information. First, Table 1 shows that the GTT pattern was achieved by the intraperitoneal injection of hyperglycemic guinea pigs with glucose solution following fasting, as blood glucose level increased, then reduced to baseline level at 30min, 60min and 180min respectively which is consistent with WHO protocol (15). Furthermore in Table 1, unboiled b.l e. is shown to respond rapidly to the test as it reduced the high blood glucose level to baseline at 60min. However in Tables 2 and 3 clear differences between the GTT responses of boiled b.l.e. and glucophage, respectively are shown to be slower and indeed poorer than that of the unboiled b.1.e. Boiling, it seems, may have altered the structure(s) of the active component(s) enough to impair its blood glucose clearance capacity. Therefore, the active principle(s) in bitter leaf may be heat labile, which further suggests that the component(s) may work in synergy in "helping" a "weakened" pancreas to work again, such as in β-cell regeneration thereby perhaps enhancing insulin sensitization.

Glucophage, on the other hand, is said to work through its metformin principle by suppressing hepatic glucose production, that is, hepatic gluconeogenesis (3, 7). Therefore its GTT response in Table 3 may not be entirely comparable to those of unboiled and boiled b.1.e. in Tables 1 and 2. These results suggest strongly that boiling or the application of heat may change the structures and thus alter the biological activities of the active components in bitter leaf in much the same way as the biological activity of protein is altered by denaturation as a result of heating. Perhaps the active principle is "protein-like" may be "insulin-like", consequently, unboiled b.1.e. seems to posses a more effective anti-hyperglycemic property than the boiled form. This may provide the scientific basis for choice of bitter leaf extract for use, although our explanation may not be the only plausible one. Nonetheless, we believe that the results may shed more light on the probable mode of anti-hyperglycemic action of Vernonia amygdalina (bitter leaf).

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