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Research Article

Hypoglycemic Effect of Asheitu Adams Bitter in Diabetic Experimental Animals

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Abstract

Asheitu Adams bitter (AAB) is a common polyherb in Nigeria used for treating several diseases/ailments, including diabetes. It is also believed to be useful food/dietary supplement for health. But, there is only little or no scientific data to validate its anti-diabetic potential. Thus, this study was conducted to evaluate the hypoglycemic effect of AAB in diabetic experimental rats. Successive triple intraperitoneal (i.p.) injection of streptozotocin at 3 days interval (i.e. 40 mg/kg, 45 mg/kg and 35 mg/kg b. wt.) was administered to the rats after overnight fasting to induce type 1 diabetes in the animals. Diabetic rats were then orally administered AAB (15 or 30 mg/kg b.wt.). Findings showed that treatment with 30 mg/kg of AAB reduced significantly blood glucose concentration at ($p < 0.05$) in diabetic animals. Moreover, AAB down-regulated expression of insulin and pancreatic duodenal homeobox-1 genes in glucose concentration-dependent manner but, up-regulated expression of glucose-6-phosphate dehydrogenase. In a nutshell, AAB possessed the ability to regulate expression of some glucose-controlling genes in a glucose concentration-dependent manner for it to elicit its hypoglycemic effect in diabetic rats, and could thus be a potential therapy/food supplement for diabetes.

Keywords: Diabetes; Dietary Supplement; Glucose-controlling Genes; Hypoglycemic, Polyherb

Introduction

Diabetes is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both [1,2]. It is still identified as one of the main threats to human health in the 21st century [3-5] and according to Siddiqui., *et al*. [3], it is still one of the major diseases challenging the health of man worldwide. In fact, it is the most common endocrine disorder in industrialized countries [2]. Diabetes is an emerging epidemic of this century that is threatening to the existence of life both in the developed and developing countries. According to Ezuruike and Prieto [5], its prevalence is on a steady increase worldwide, and globally, it is one of the six major causes of death with various systemic complications.

The treatment of diabetes has been of great challenge to the world for a while now. It is usually treated by insulin hormone therapy or by administering glucose-lowering agents such as alpha-glucosidase inhibitors, sulfonylureas, biguanides, and thiazolidinediones, but the development of adverse effects/events i.e. undesirable side effects such as weight gain, edema, gastrointestinal toxicities and hypoglycemia has always been some of the limitations of these hypoglycemic agents or anti-diabetic agents [6]. According to Rotenstein., *et al.* [7], all existing therapies have limited efficacy, limited tolerability and/or significant mechanism-based side effects hence; many research institutes and pharmaceutical companies are involved in drug development to find molecules with good therapeutic potentials and less adverse effects/events [8]. Consequently, today many Nigerians use different polyherbal medicines commonly called herbal medicines as alternative forms of therapy to synthetic drugs [5] alone or alongside hypoglycemic synthetic drugs for the management of diabetes without any experimental/empirical data to validate the usage of the polyherbs. According to their producers, most of them are used as food or dietary supplements to improve health and wellness. Therefore, this present study was conducted to investigate the hypoglycemic effect of Asheitu Adams bitter (AAB) in diabetic experimental rats.

Materials and Method

Drugs and chemicals

Streptozotocin (STZ) was obtained from Sigma Aldrich, and metformin from Teva Pharmaceutical, Wales. All other chemicals used for the study were of analytical grade. All diagnostic kits were procured from Lab-care diagnostics Ltd., India.

Experimental animals

Male wistar rats, average weight 96.37 ± 11.42g were used for this study. The rats were got from Animal Facilities, University of Ibadan, Oyo State, and were acclimatized for two weeks before the commencement of the experiment. They were maintained in line with the regulations guiding the use of animals for experimental research stated by Animal Welfare Act (Laboratory Animal Welfare Act) as amended in 2013, which is also in line with National Institutes of Health guide for the care and use of Laboratory animals. They were fed standard pelleted laboratory animal feed and water *ad libitum* at 22 ± 2°C and 55 ± 5% relative humidity in a light controlled (12h light/12h dark) room.

Induction of Diabetes

The method of Sharma*., et al.* [9] with few modifications was used to induce type 1 diabetes in the rats. Successive triple intraperitoneal (i.p.) injection of streptozotocin at 3 days interval i.e. 40 mg/kg, 45 mg/kg and 35 mg/kg of body weight dissolved in 0.1M citrated buffer (pH 4.5) was administered to the rats after overnight fasting. On the 9th day of subjection of animals to streptozotocin challenge, glucometer was used to estimate glucose level in animals' whole blood cell, and those found to have fasting blood glucose level of 200 mg/dl and above with polydipsia (increased thirst) and polyuria (increased urination) signs were categorized as diabetics and subsequently classified into the various diabetic groups for treatments.

Experimental design

Experimental rats were divided into five groups, with five animals in each group.

- Group 1 - Control (Non-diabetic)
- Group 2 - Diabetic control
- Group 3 - 15 mg/kg Metformin,
- Group 4 - 15 mg/kg Asheitu Adams bitter
- Group 5 - 30 mg/kg Asheitu Adams bitter.

Fasting blood glucose and weight of animals were monitored on weekly basis as treatments progress. At the end of the four weeks of the study, the animals were fasted overnight and anaesthetized using chloroform, and fasting blood sugar was determined, while key organs (pancreas and liver) were collected for insulin, pancreatic duodenal homeobox-1 (PDX-1) and glucose-6-phosphate dehydrogenase (G6PD) gene expressions.

Blood glucose determination

Blood glucose levels of the experimental animals were monitored on weekly basis till the last day of the experiment by a drop of blood collected from tail vein of the animals using Accu Check Glucometer, Germany.

Gene expression

Burgmann., *et al*. [10] protocol with slight modifications was used for gene expression analysis. Pancreas was used for insulin gene expression, and intestinal crypt for glucagon-like peptide 1 (GLP 1) and glucose transporter 2 (GLUT 2) gene expressions. RNA was isolated from the tissues with TRIzol reagent (Invitrogen) and was reverse transcribed into cDNA with the Prime Script RT reagent kit (TaKaRa). Real-time RT-PCR was performed with SYBR Green (TaKaRa). Primer pair sequences are as follows:

Target Gene	Forward 5'-3'	Reverse 5'-3'
B-actin	ACACTTTCTACAATGAGCTGCG	ACCAGAGGCATACAGGACAAC
Insulin	GAGGCTCTGTACCGTGGTGTG	ACCTCCAGTGCCAAGGTTT
GLP-1	ACCGTTTACATCGTGGCTGG	CCCTGTGAATGGCGTTCTC
GLUT-2	CCTGGCGTCTTCAGAGAGTG	ACCGAGGAAGGAATCGGTTT

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics, Version 21 Software. Data were statistically analyzed using one-way analysis of variance (ANOVA) and p value of ≤ 0.05 was considered statistically significant. Results are presented in histogram and bar chart.

Results

Figure 1 results show that initial blood glucose concentration of control group is significantly different from the initial blood glucose concentrations of other experimental groups.

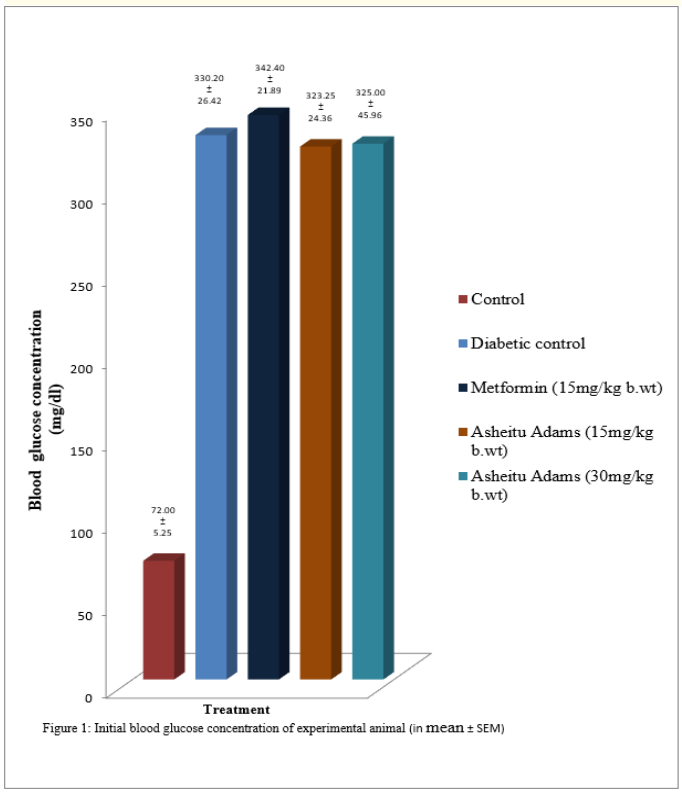

Figure 1: Initial blood glucose concentration of experimental animal (in mean ± SEM)

Figure 1: Initial blood glucose concentration of experimental animal (in mean ± SEM).
Key: Aisheitu: Asheitu Adams Bitter Polyherb; b.wt: Body Weight.

Conversely, figure 2 reveals that control group final blood glucose concentration is not statistically significant different from that of 30 mg/kg AAB group, but differs significantly from the final blood glucose concentrations of other groups.

Furthermore, figure 3 shows down-regulation of pancreatic insulin gene expression at AAB 15 mg/kg and 30 mg/kg of body weight in comparison with other experimental groups.

More also, figure 4 shows down-regulation in gene expression of PDX-1 in AAB (15 and 30 mg/kg) groups in comparison with other groups.

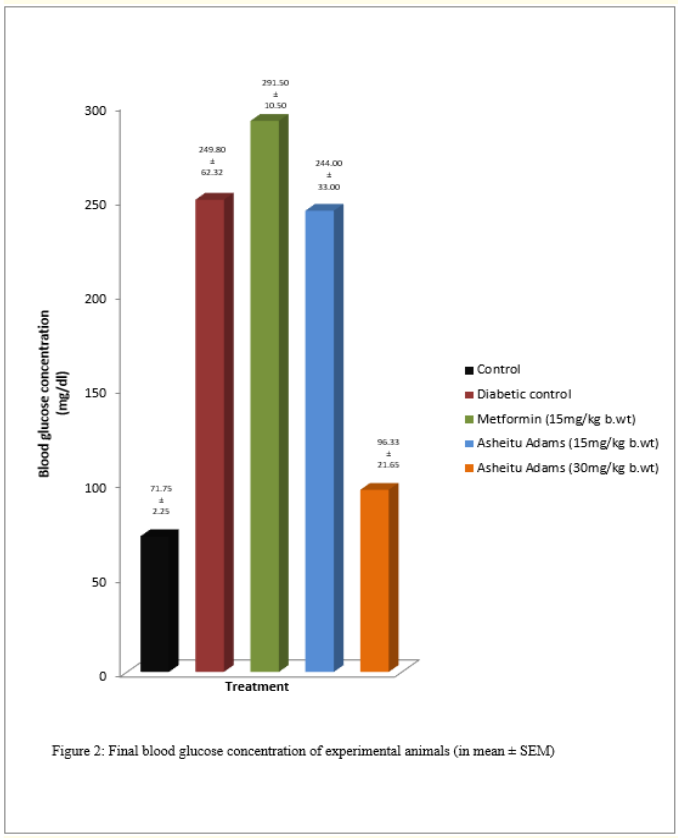

Figure 2: Final blood glucose concentration of experimental animals (in mean ± SEM)

Figure 2: final blood glucose concentration of experimental animal (in mean ± SEM).
Key: Aisheitu: Asheitu Adams Bitter Polyherb; b.wt: Body Weight.

Moreover, figure 5 shows up-regulation and down-regulation in G6PD gene expression for 30 mg/kg AAB group and diabetic control group respectively in comparison with other experimental groups.

Discussion and Conclusion

Persistently high blood glucose level is termed hyperglycemia and is an indication of diabetes [2]. Therefore, the significantly high blood glucose concentration of experimental animals in all groups except the control group as shown in figure 1 implies that; all animals used in this study were diabetic except the control group hence, the reason for the significantly low initial blood glucose concentration of the control animals. However, AAB at 30 mg/kg had through some mechanisms caused reduction in blood glucose concentration of diabetic animals thus, bringing their blood glucose

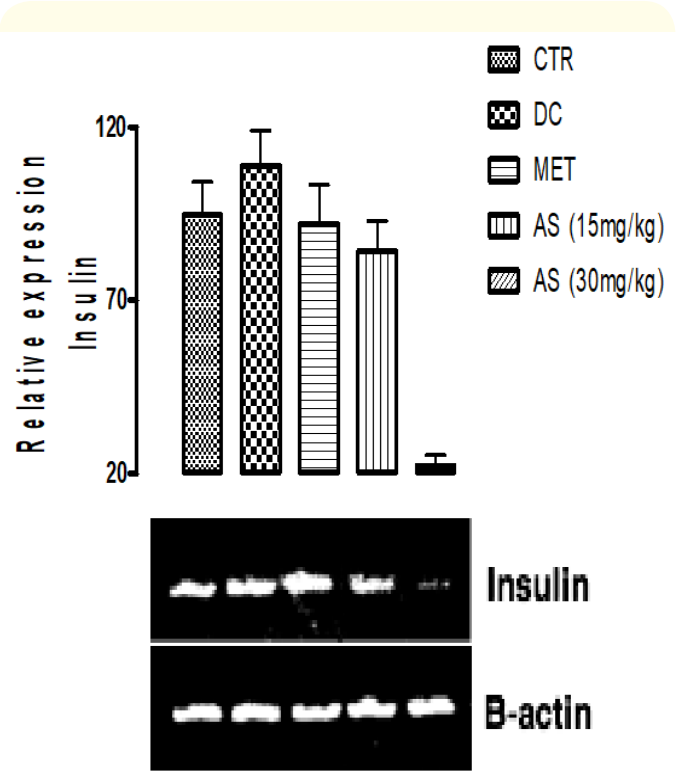

Figure 3: Pancreatic insulin gene expression of experimental animals after treatments.
Key: CTR: Control Group; DC: Diabetic Control Group; MET: Standard Control (Metformin) Group; AS (15 mg/kg): Asheitu Adam Bitter (15 mg/kg Body Weight) Group; AS (30 mg/kg): Asheitu Adam Bitter (30 mg/kg Body Weight) Group; Insulin: Insulin Gene Gel Picture.

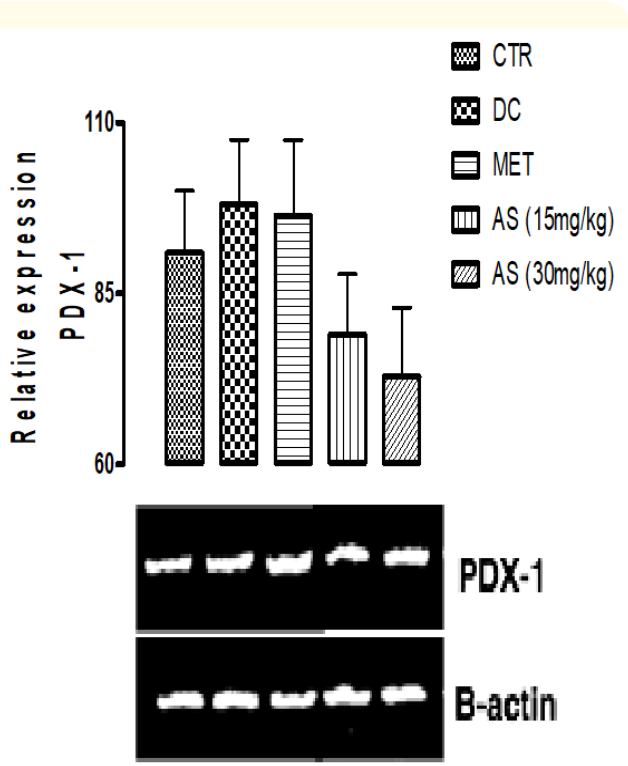

Figure 4: Pancreatic duodenal homeobox-1 gene expression of experimental animals after treatments.
Key: CTR: Control Group; DC: Diabetic Control Group; MET: Standard Control (Metformin) Group; AS (15 mg/kg): Asheitu Adam Bitter (15 mg/kg Body Weight) Group; AS (30 mg/kg): Asheitu Adam Bitter (30 mg/kg Body Weight) Group; PDX-1: Pancreatic Duodenal Homeobox-1 Gene Gel Picture.

concentration to almost the same level with the control group. This account for the observed statistically non-significant difference between the control group and the test group of 30 mg/kg AAB as revealed in figure 2. These results clearly demonstrate the ability of AAB to ameliorate hyperglycemic condition at this therapeutic dosage (30 mg/kg) and as well suggest its hypoglycemic effect and/or antidiabetic potential.

The possible and likely mechanisms for this observed hypoglycemic effect in diabetic rats could be; ability of AAB's constituents to regenerate β-cells of the pancreas, stimulation of the β-cells to produce insulin in glucose concentration-dependent manner, and binding of the insulin to its cognate receptor to regulate blood glucose level of the animals. These proposed mechanisms are likely and reasonable on the basis of the established finding of Akbarzadeh., *et al*. [11] and review of Ghasemi., *et al.* [12] that stated that "STZ administration at ≥ 55 mg/kg body weight selectively damages insulin-secreting β-cells of the pancreas". And when β-cells are damaged, there will be significant increase in blood glucose levels (hyperglycemia) as seen in figure 1 cum decreased insulin levels i.e. hypoinsulinaemia [3,13] as a result of the destruction of islet of Langerhans of β-cells of the pancreas.

Therefore, the observed near control group blood glucose concentration of animals administered 30 mg/kg of AAB in figure 2, which was not observed in the diabetic control group seems to attest to the β-cells regenerating ability of AAB in the animals after STZ administration that eventually led to its hypoglycemic effect in the diabetic animals, because the same effect was not seen in diabetic control group that was not treated with AAB.

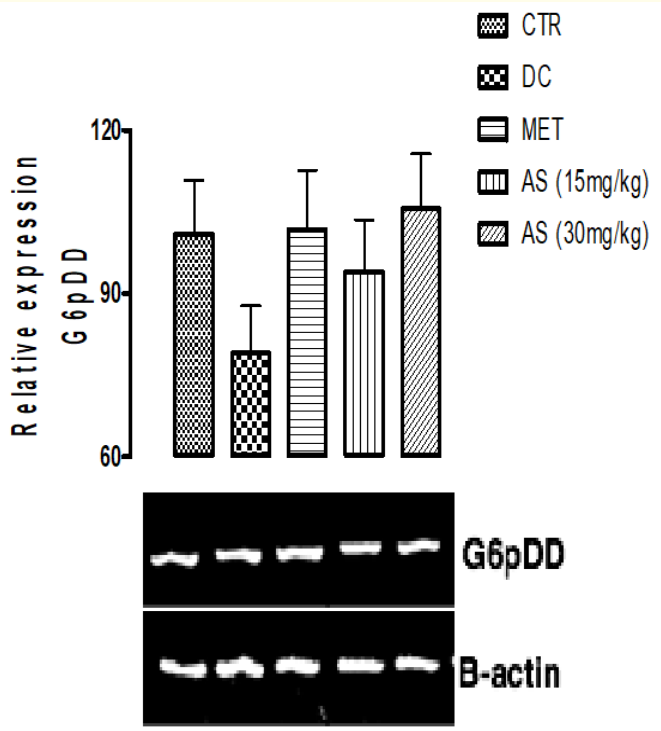

Figure 5: Liver glucose-6-phosphate dehydrogenase gene expression of experimental animals after treatments.
Key: CTR: Control Group; DC: Diabetic Control Group; MET: Standard Control (Metformin) Group; AS (15 mg/kg): Asheitu Adam Bitter (15 mg/kg Body Weight) Group; AS (30 mg/kg): Asheitu Adam Bitter (30 mg/kg Body Weight) Group; G6PDD: Glucose-6-phosphate Dehydrogenase Gene Gel Picture.

It is however unfortunate that metformin, a conventional antidiabetic drug did not ameliorate hyperglycemia in this research as the blood glucose level still remains high at the end of the study although, it lowered blood glucose level from 342.40 ± 21.89 mg/dl to 291.50 ± 10.50 mg/dl. This may be a pointer to the fact that metformin can only be used to treat type 2 diabetes [12] and not type 1 diabetes where destruction of beta cells is a major issue. It also tends to justify the statement of He and Wondisford [14] that metformin's acts remains only partially understood and controversial.

Furthermore, the down-regulation of pancreatic insulin gene expression in a glucose concentration-dependent manner in experimental animals treated with AAB (15 mg/kg and 30 mg/kg body weight) as shown figure 3 is also commendable. It is already a known fact that insulin is often secreted or produced and expressed in line with the concentration of available glucose in the blood. In other words, its production and expression are glucose concentration-dependent. Therefore, the ability of the pancreas of animals administered AAB to up- and down-regulate insulin gene expression when blood glucose concentrations were high and low respectively, unlike the unresponsive/insensitive highly expressed insulin gene of the diabetic control group, further suggests the re-instatement of β-cells of the pancreas to their normal functional and regulatory roles of producing a responsive or sensitive insulin that was expressed in accordance with available concentration of glucose in the blood. According to Thorens and Mueckler [15], when degenerated pancreatic β-cells are reinstated like this, rise in blood glucose concentration will trigger insulin secretion/expression and conversely, reduction in blood glucose concentration will inhibit insulin secretion/expression as seen with AAB treatments of figure 3. However, it is important to note that; the rise in blood glucose level of diabetic control animals and the up-regulation of the insulin gene did not bring about the resultant decrease in their blood glucose, indicating the unresponsiveness/insensitivity or defectiveness of this highly expressed insulin gene in the animals. Thus, insulin gene expression result of this study further give credence to the possible mechanism of action of AAB to exert the observed hypoglycemic effect on diabetic rats (as in figure 2). However, there is a feeling that AAB might cause dysregulation of insulin at high dosage because of the over down-regulation of insulin gene at 30 mg/kg shown in figure 3 although, this needs to be verified and confirm.

More also, the down-regulation of PDX-1 gene in glucose concentration-dependent manner by AAB (15 and 30 mg/kg) groups in comparison with diabetic control and standard control (metformin) groups, just like the control group as revealed by figure 4, is very similar to insulin gene expression result shown is figure 3. PDX-1 is a homeodomain-containing transcription factor essential for pancreatic development, beta-cell differentiation and maintenance of mature beta cell function [16,17] through regulating the expression of key endocrine beta-cell-specific genes such as insulin, glucokinase, islet amyloid polypeptide and the glucose transporter type 2 [18]. So, the down-regulation of PDX-1 gene for animals in AAB (15 and 30 mg/kg) groups, whose pancreas is just regenerating/recuperating from the devastating effects of STZ is understandable because, according to Zhou and Brunicardi [18], compartmentalization of PDX-1 is lost under pathological conditions and therefore, the oxidative stress condition created in these animals as a result of the administration of STZ should be possible reason for the down-regulation of the gene. Similar to what was observed in the diabetic control group of insulin gene expression result, the upregulation/high expression of PDX-1 gene in the diabe-

tic control animals did not bring about the expected hypoglycemic effect, which suggests that the gene might not be also responsive to glucose concentration or is defective. It as well bring to mind the finding that, an aberrant overexpression or up-regulation of PDX-1 gene in a number of studies resulted into human cancers such as pancreatic, gastric, colon, breast, prostate, colorectal, kidney cancer and paediatric solid pseudopapillary tumors etc [18].

Contrary to Weir*., et al*. [19] position, the observed down-regulation of PDX-1 gene in AAB treated animals did not result into β-cell failure and type 2 diabetes in this study since there is no hyperglycemia; a clinical condition linked with β-cell dysfunction and a major feature of diabetes, caused via reduced PDX-1 expression [17]. It is therefore suffice to say that; AAB made the PDX-1 gene of the animals to be responsive to glucose and also effective in order for AAB to carry out its hypoglycemic effect/anti-diabetic potential via the mechanism of a well-regulated PDX-1 gene expression in glucose concentration-dependent manner, which in turn resulted in well-regulated insulin production and gene expression, as well as effective insulin that resulted in the observed hypoglycemic effect of figure 2. This finding is in accordance with the submission of Kim and Hebrok [16] that, pancreatic β-cells of the islets are responsible for the transcription, synthesis, and release of insulin in response to ambient blood glucose level and that PDX-1 plays a central role in β-cell survival [16,20].

Moreover, G6PD gene up-regulation in 30 mg/kg AAB group, but down-regulation in diabetic control group when compared with other groups shown in figure 5 is for glucose concentration regulatory purpose. It implies that there were high G6PD activities in experimental animals at 30 mg/kg of AAB that expedite the reduction of blood glucose concentration in the animals to bring the blood glucose level close to that of the control group, while hyperglycemia was observed in diabetic control group (where G6PD was downregulated) as observed in figure 2. The seemingly possible reason for this observation is that G6PD as important enzyme involved in normal processing of carbohydrates would have enhanced pentose phosphate pathway to facilitate the conversion of gluose-6-phosphate (in the glycolytic pathway in response to insulin signal when blood glucose concentration is high) to 6-phosphoglucono-δ-lactone, while concomitantly converting NADP+ to NADPH [2]. Thus, glucose-6 phosphate is shunted into pentose phosphate pathway to fast track the removal of glucose from the blood in order to compliment the glycolytic pathway, because G6PD gene expression is glucose-6-phosphate concentration-dependent and glucose-6-phosphate concentration is in turn dependent on glucose concentration.

Although, the relationship between G6PD deficiency and diabetes is still a matter of debate, the experimental observations that hyperglycemia could lead to decrease in G6PD gene expression and activity [21] and also that G6PD deficiency could be a risk factor for the occurrence of diabetes [2] are in agreement with the finding of this study as seen in diabetic control group (see figures 2 and 5). Sequel to all these findings, that is, blood glucose concentration reducing ability of AAB and its capability to regulate the gene expression of insulin, PDX-1 and G6PD genes in glucose concentration-dependent manner, it is concluded that AAB has antidiabetic effects in experimental animals, and could be potential hypoglycemic agent for diabetes (hyperglycemic condition), in other word, an alternative therapy to synthetic hypoglycemic agents (conventional drugs) in the treatment of diabetes.

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Conflict of Interest

I declare there is no conflict of interest of any kind as regards this study.

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