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Antidiabetic potential of *Hibiscus sabdariffa* extract in alloxan-induced diabetic rats

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Abstract

Plants are known to possess relatively high efficacy in the treatment of several chronic diseases with fewer adverse effects. In the recent years, numerous medicinal plants have been reported to be effective in treating diabetes. Hence, the present study aims to evaluate the antidiabetic property of hydroalcoholic extract of *Hibiscus sabdariffa* (HS) calyces in diabetic rats.

Diabetes was induced by single intraperitoneal injection of alloxan (150 mg/kg, b.w), in male Wistar rats. Diabetic rats were administered daily oral doses of HS (100, 200 and 400 mg/kg body weight) and Glibenclamide (10 mg/kg) for 21 days. Then, blood glucose levels, oral glucose tolerance test and lipid profiles were determined. Treatment with HS resulted in a significant dose dependent reduction of blood glucose levels accompanied by a significant improvement in body weight. The extract also enhanced the glucose tolerance and significantly decreased cholesterol, triglycerides, and low-density lipoproteins levels while the high-density lipoproteins level significantly increased.

From the results obtained, it can therefore be concluded that *Hibiscus sabdariffa* has an antidiabetic effect in alloxan-induced diabetic rats.

Keywords: Diabetes mellitus; Alloxan; *Hibiscus sabdariffa* calix; Lipid profile; Blood glucose

1. Introduction

Diabetes mellitus (DM) is a metabolic disease characterized by hyperglycemia, dyslipidemia, and impaired glucose tolerance, which can lead to a variety of chronic complications [1, 2]. DM results from either deficiency of insulin secretion secondary to pancreatic β-cells destruction or the development of insulin resistance or from both [3-5].

About 537 million adults (20-79 years) are living with diabetes (1 in 10) and this number is predicted to rise to 643 million by 2030 and 783 million by 2045 [6]. DM is responsible for 6.7 million deaths in 2021, over 3 in 4 adults with

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diabetes live in low-and middle-income countries. In Africa, 1.24 million adults are living with diabetes and the total number of people with diabetes is predicted to increase by 29% to 55 million by 2045 [6].

Currently available treatment options for diabetes mellitus are achieved through oral hypoglycemic agents (metformin, sulfonylureas, and thiazolidinediones), insulin therapy, dietary guidance, and regular physical activity [7, 8]. Unfortunately, their use is limited by adverse effects including gastrointestinal disorders, hypoglycemia, pancreatic degeneration, liver damage in the body [9], failure to achieve glycemic control, a different drug response among individuals [8, 10] and cardiovascular disorders [9]. Moreover, antidiabetic drugs have become very expensive due to the high cost of treatment and these constraints lead people in developing countries to turn to traditional medicine for their primary health care keeping. Thus, the development of novel antidiabetic drug products from natural sources with fewer side effects, better efficacy and an affordable price becomes an important issue in the treatment of DM. Numerous studies have revealed the existence in plants of several classes of bioactive compounds responsible for the prevention and treatment of chronic health pathologies [11, 12].

Hibiscus sabdariffa L. belongs to the Malvaceae family, is used worldwide as a food and local medicine for a variety of illnesses including hypertension, diabetes, urinary problems and for cardiovascular complications [13, 14]. Phytochemical studies on the calyces have shown that they are a major source of flavonoids (anthocyanins, anthocyanidins, quercetin), phenolic compounds (protocatechuic acid, eugenol), polysaccharides, organic acids (maleic, citric, oxalic, tartaric, ascorbic, hibiscus acid), vitamins (ascorbic acid, thiamine, riboflavin), and β-carotene [13, 15, 16]. Previous scientific reports have proven the use in traditional medicines of *H. sabdariffa* extract in the treatment of diabetes in animal models [17, 18]. Methanolic extract of *H. sabdariffa* calyces showed positive effect in the regeneration of pancreatic beta-cells in streptozotocin-induced diabetes type-1 in rats [17]. In addition, hypoglycemic and hypolipidemic properties were found in alloxan-induced diabetic rats after oral administration of leaves [18] or seed [19] methanolic extract. However, efficacy studies of *H. sabdariffa* calyces extract against diabetes are relatively few even as calyces of *H. sabdariffa* have been shown to better promote the antioxidant capacity compared to the leaves [20]. Therefore, the present study aimed to evaluate the antidiabetic potential of hydroalcoholic extract of *H. sabdariffa* calyces in alloxan-induced diabetic rats.

2. Material and methods

2.1. Chemicals and reagents

Alloxan monohydrate and Glibenclamide were purchased from MilliporeSigma (Oakville, ON, Canada). Alloxan was used to induce diabetes in rats and Glibenclamide was used as a standard hypoglycemic drug. All chemicals used were of analytical grade.

2.2. Plant material and extraction

Red calyces of *H. sabdariffa* were purchased from a local market in Bertoua, East-Cameroon, and authenticated by botanist experts. The plant was cleaned, air-dried for 1 week at room temperature and mechanically ground into fine powder. Then, 1000 g of a powdered calyces were soaked in 1 L of ethanol and distilled water (70:30) solution for 24 days with occasional shaking and stirring. The mixture was then filtered with Whatmann no. 1 filter paper and the filtrate was concentrated using a rotatory evaporator at 40°C. The remained aqueous residue was then lyophilized into powder of *H. sabdariffa* hydroalcoholic extract (HS), weighed, labelled, and stored at room temperature in a sealed container for future use.

2.3. Phytochemical Screening

H. sabdariffa calyces' extracts was analyzed qualitatively for the presence of secondary metabolites like terpenoids, flavonoids, phenols, tannins and saponins [21, 22]. Based on the intensity of coloration or the precipitate formed during the test, secondary metabolites proportion was characterized as strongly present (+++), present (++), weakly present (+), and absent (−) when the test result was negative.

2.4. Animals

Healthy adult male Wistar rats (120-160 g) were used in the present experiment. Animals were housed in animal facility of the Pharmacological Research Laboratory of Medicinal Plants, Department of life Sciences, Higher Teacher Training College, University of Bertoua (UBe). They were kept in polycarbonate cages under standard conditions of temperature (25 ± 2 °C), humidity (55-65%) and light (12-h light/dark cycle), with *ad libitum* access to food and water. All rats were acclimatized to experimental conditions for 1 week before start of the study. Animal care and experimental procedures

described throughout this study were carried in accordance with the guidelines of the Cameroon National Ethical Committee (Ref No. FW-IRB00001954).

2.5. Diabetes induction

Diabetes was induced by a single intraperitoneal injection of freshly prepared alloxan (150 mg/kg) dissolved in 0.9% saline after overnight fasting for 12 h except the control group. Then, rats were allowed to free access of 5% glucose solution for 24 hours to prevent hypoglycemia [23]. After 3 days, the fasting blood glucose levels in the rats were assessed to validate the successful induction of diabetes mellitus. The blood glucose values were measured in mg/dl and only rats with fasting blood glucose levels of 250 mg/dl or higher were considered as diabetic-induced rats and included in this study [24].

2.6. Experimental design

Thirty six rats were randomly divided into six groups of six animals each described as follows: 1-Normal rats (Nor) received distilled water, 2-Alloxan-induced diabetic rats (Dia) received distilled water, 3-Alloxan-induced diabetic rats treated with 10 mg/kg of Glibenclamide (Gli), 4-Alloxan-induced diabetic rats treated with 100 mg/kg of *H. sabdariffa* (HS100), 5-Alloxan-induced diabetic rats treated with 200 mg/kg of *H. sabdariffa* (HS200), 6-Alloxan-induced diabetic rats treated with 400 mg/kg of *H. sabdariffa* (HS400). All treatments were started on the same day (day 1) of diabetic confirmation (3 days after alloxan administration) by daily oral administration for 21 consecutive days.

2.7. Blood glucose level and lipid profile determination

A glucometer (Accu-Chek Aviva, Roche, Mannheim, Germany) and compatible blood glucose test strips were used to measure the blood glucose levels in experimental rats. After overnight fasting for 12 h, blood samples were collected from the tail vein and glucose levels were assessed fifth times: before alloxan induction (baseline) and then on days 1, 7, 14 and 21 after diabetes induction. At the end of the study period (day 21), oral glucose tolerance test (OGTT) was performed to determine pancreas capacity to produce insulin after treatment. Rats from all groups were administered a glucose solution (2 g/kg b.w) by oral gavage, then the blood glucose levels were measured at baseline (0), 30, 60, 90 and 120 min intervals. In addition, lipid profile of blood samples, including triglyceride (TG), total cholesterol (TC), low-density lipoprotein-cholesterol (LDL) and high-density lipoprotein-cholesterol (HDL) were measured using automatic biochemical analyzer (BC300, Medgroup).

2.8. Body weight and relative weight organs

The body weight of all experimental groups was measured fifth times: before alloxan induction (baseline) and then on days 1, 7, 14 and 21 after diabetes induction with an ordinal weighing scale. At the end of study, the animals were euthanized with ketamine/xylazine (80/10 mg/kg, i.p), and a mid-line incision was made through the anterior abdominal walls of the rats. Then, liver, heart, splenic, pancreas and kidney were removed, washed in saline, drained, and weighed using sensitive balance (Mettler Toledo, Germany). Then, these organs were stored in 10% formalin solution for histological procedure. The relative weight of each organ (%) was calculated on the basis of organ-to-body weight ratio.

2.9. Data analysis

Data were presented as mean ± standard error of the mean (SEM) and analyzed using GraphPad Prism 9.5.0 (GraphPad Software LLC, Boston, MA, USA). Differences between groups were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's test multiple comparisons. Area under the curve (AUC) was calculated using GraphPad Prism 9.5.0. Statistical significance was set to $P < 0.05$.

3. Results

3.1. Phytochemical Analysis

The result of the qualitative phytochemical screening of *H. sabdariffa* calyces extract is shown in Table 1. *H. sabdariffa* was found to be rich in tannins, flavonoids, phenols, and terpenoids. There is the absence of saponins.

Table 1 Phytochemical composition of *Hibiscus sabdariffa* calyces extract

Phytochemical compound	Result	Intensity
Terpenoids	Present	+
Flavonoids	Present	++
Phenols	Present	++
Tannins	Present	+++
Saponins	Absent	-

+++: strongly present; ++: present; +: weakly present; –: absent

3.2. Effects of *H. sabdariffa* extract on blood glucose levels

After injection of alloxan, all rats showed an increase of blood glucose levels compared to the normal group (Figure 1A). Subsequently, treatment with HS (100, 200 and 400 mg/kg) and Glibenclamide (10 mg/kg) showed a reduction of the blood glucose levels over time from day 7 to 21 compared to the continuous increase observed during the same period in the diabetic group. The blood glucose levels in the normal group showed a relatively stable level from the beginning to the end of the study period, while the diabetic group showed a steady increase in blood glucose levels.

The area under curve (AUC) corresponding to glucose levels during the three weeks of treatment is shown in Figure 1B. Diabetic groups showed a significant increase of blood glucose levels compared to the normal group ($p < 0.001$). In contrast, alloxan-induced diabetic rats treated with HS (100, 200 and 400 mg/kg) showed a significant and dose-dependent reduction of blood glucose levels as compared with diabetic groups ($p < 0.001$). Alloxan-induced diabetic rats treated with 10 mg/kg of Glibenclamide also showed a significant decrease of blood glucose levels as compared with diabetic groups ($p < 0.001$).

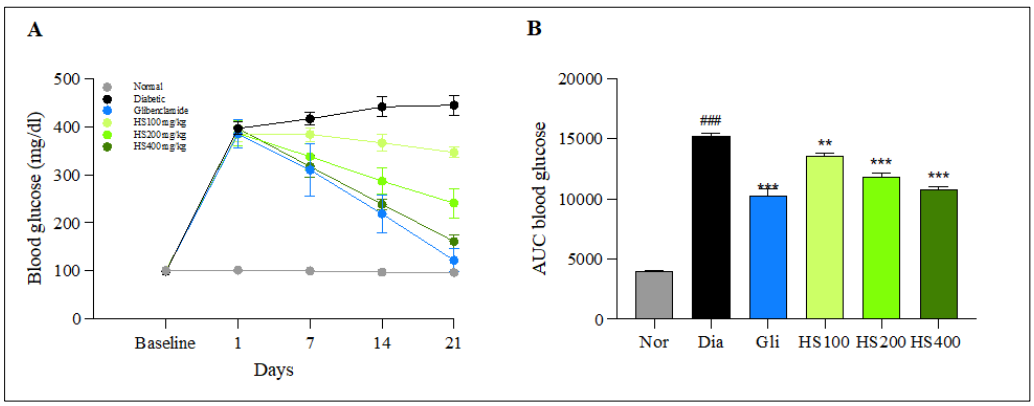

Figure 1 Effects of HS extract on blood glucose levels in alloxan-induced diabetic rats. Effect of the oral administration of HS and Glibenclamide on blood glucose level over time through 3 weeks of treatment (A) and its corresponding AUC (B). Nor: Normal control; Dia: Diabetic untreated; Gli: diabetic treated with 10 mg/kg glibenclamide; 100: diabetic treated with 100 mg/kg HS; 200: diabetic treated with 200 mg/kg HS; 400: diabetic treated with 400 mg/kg HS. Data are expressed as mean ± S.E.M (n = 6). ###: $P < 0.001$ vs normal, **: $P < 0.01$ vs diabetic,***: $P < 0.001$ vs diabetic

3.3. Effects of *H. sabdariffa* extract on oral glucose tolerance

Thirty minutes after oral administration of glucose, all groups of rats showed an increase of the blood glucose levels (Figure 2A). The normal group showed the early recovery after oral administration of glucose at 120 min interval while in diabetic group, blood glucose levels remain high even after 120 min interval. Treatments with HS (100, 200 and 400 mg/kg) and Glibenclamide showed a decrease of the blood glucose levels over 120 min when compared to diabetic group. The AUC of glucose showed a similar behavior of a glucose tolerance curve (Figure 2B). Diabetic group showed a significant increase ($P < 0.001$) of the glucose levels over 120min compared to normal group. Treatment with HS (100, 200 and 400 mg/kg) and Glibenclamide showed a significant decrease ($P < 0.001$) of the glucose levels over 120 min compared to normal group. Thus, HS improve glucose tolerance.

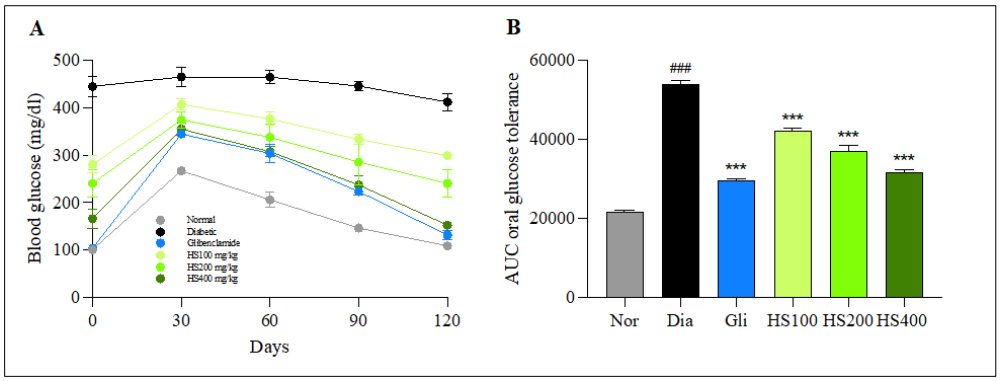

Figure 2 Effects of HS extract on oral glucose tolerance in alloxan-induced diabetic rats. Effect of the oral administration of HS and Glibenclamide on oral glucose tolerance over time through 3 weeks of treatment (A) and its corresponding AUC (B). Nor: Normal control; Dia: Diabetic untreated; Gli: diabetic treated with 10 mg/kg glibenclamide; 100: diabetic treated with 100 mg/kg HS; 200: diabetic treated with 200 mg/kg HS; 400: diabetic treated with 400 mg/kg HS. Data are expressed as mean ± S.E.M (n = 6). ###: $P < 0.001$ vs normal, ***: $P < 0.001$ vs diabetic

3.4. Effects of *H. sabdariffa* extract on lipid profile

Lipid profile was determined at the end of the experiment in days 21 as shown in Figure 3. The triglycerides (TG), total cholesterol (TC) and low-density lipoproteins (LDL) levels increased (Figure 3A, B and C) while the level of high-density lipoproteins (HDL) rather decreased (Figure 3D) in the diabetic group compared to the normal group. However, treatment with HS (100, 200 and 400 mg/kg) significantly ($P < 0.001$) decreased dose dependent the TG, TC and LDL levels respectively. Only HS at 200 and 400 mg/kg significantly increased ($P < 0.05$ and $P < 0.001$, respectively) the HDL level, respectively compared to the diabetic group. Treatment with Glibenclamide resulted in a significant decrease ($P < 0.001$) in TG, TC and LDL levels with a corresponding increase ($P < 0.001$) in HDL when compared to the diabetic group.

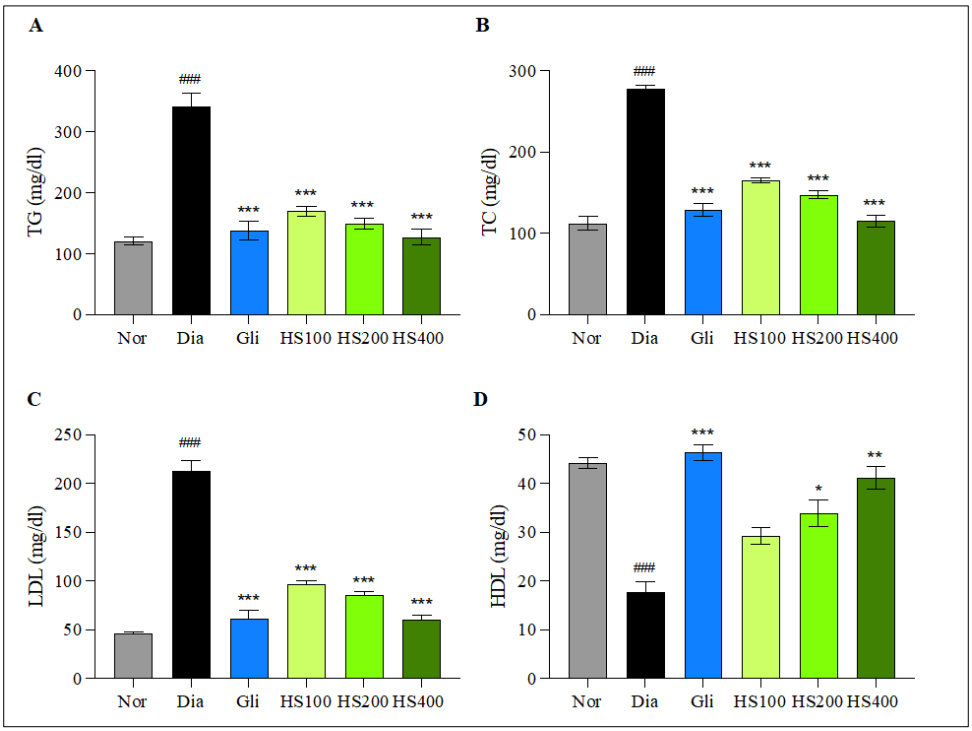

Figure 3 Effects of HS extract on lipid level of TG (A), TC (B), LDL (C) and HDL (D) in alloxan-induced diabetic rats. Nor: Normal control; Dia: Diabetic untreated; Gli: diabetic treated with 10 mg/kg glibenclamide; 100: diabetic treated with 100 mg/kg HS; 200: diabetic treated with 200 mg/kg HS; 400: diabetic treated with 400 mg/kg HS. Data are

expressed as mean ± S.E.M (n = 6). ###: $P < 0.001$ vs normal, *: $P < 0.05$ vs diabetic, **: $P < 0.01$ vs diabetic,***: $P < 0.001$ vs diabetic

3.5. Effects of *H. sabdariffa* extract on body weight

The changes in the body weight are presented in Figure 4. As shown in Figure 4A, alloxan-induced diabetic rats showed a decrease of body weight three days after alloxan injection. Throughout the study period, diabetic group exhibited a progressive decrease of body weight while normal group showed a progressive increase of body weight. Rats treated with HS100 and HS200 continued showing a decrease body weight on day 7 before increasing on day 14 and 21. However, treatment with HS400 as well as with Glibenclamide increased body weight from day 7 to 21. The corresponding AUC of body weight during the three weeks of treatment is shown in Figure 4B. Diabetic group showed a significant decrease of body weight compared to the normal group ($p < 0.001$). In contrast, oral administration of HS (100, 200 and 400 mg/kg) extract and Glibenclamide in alloxan-induced diabetic rats significantly increased the body weight when compared with the diabetic group ($P<0.001$).

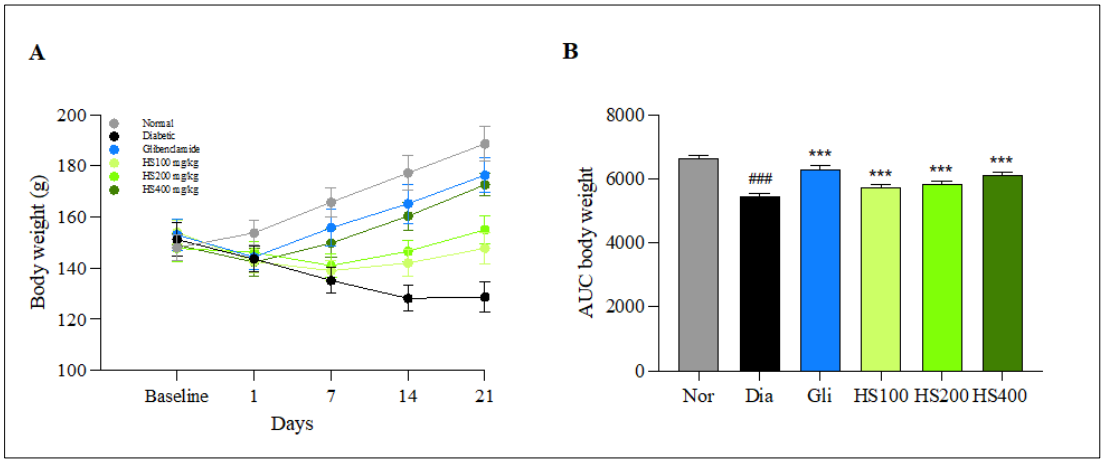

Figure 4 Effect of HS on body weight in alloxan induced diabetic rats. Effect of HS on the variation in the body weight (A) and it's corresponding AUC (B). Nor: Normal control; Dia: Diabetic untreated; Gli: diabetic treated with 10 mg/kg glibenclamide; 100: diabetic treated with 100 mg/kg HS; 200: diabetic treated with 200 mg/kg HS; 400: diabetic treated with 400 mg/kg HS. Data are expressed as mean ± S.E.M (n = 6). ###: P < 0.001 vs normal, ***: P < 0.001

3.6. Effects of *H. sabdariffa* extract on relative weight organs

The effects of *H. sabdariffa* on relative weight organs in all groups of rats are shown in Table 2. The relative weight of heart and spleen from all experimental groups did not show a significant difference. The relative weight of kidneys and livers did not show significant difference although the relative weight from diabetic group was found to be higher than those of the treated groups. However, they did not display significant different among them. The relative weight of the pancreas from normal, Glibenclamide and *H. sabdariffa* groups was higher than the relative weight of pancreas from diabetic group. However, there was no significant difference between the relative weight of the pancreas among these groups.

Table 2 Effect of *Hibiscus sabdariffa* calyces extract on relative weights of organs in alloxan induced diabetic rats

Treatment	Heart	Liver	Spleen	Pancreas	Kidney
Normal	0.43 ± 0.04	4.55 ± 0.31	0.29 ± 0.03	1.39 ± 0.05	0.83 ± 0.02
Diabetic	0.51 ± 0.04	5.23 ± 0.44	0.35 ± 0.04	1.09 ± 0.07	1.18 ± 0.08
Glibenclamide	0.40 ± 0.01	4.84 ± 0.42	0.33 ± 0.11	1.27 ± 0.05	0.84 ± 0.08
HS100	0.41 ± 0.06	4.65 ± 0.62	0.32 ± 0.02	1.49 ± 0.08	0.91 ± 0.10
HS200	0.39 ± 0.02	4.42 ± 0.34	0.29 ± 0.04	1.40 ± 0.11	0.88 ± 0.08
HS400	0.39 ± 0.02	4.38 ± 0.05	0.24 ± 0.01	1.16 ± 0.13	0.66 ± 0.07

Data are mean ± standard error of the mean; n = 6; HS100, HS200, HS400: Hibiscus sabdariffa extract doses at 100, 200 and 400 mg/kg.

4. Discussion

Several studies have demonstrated the beneficial effects of medicinal plants in reducing blood glucose level using different models of diabetes induction in animals [2, 23, 25]. In the present study, we chose alloxan monohydrate to induce diabetes as it has been widely used in the recent years to develop stable hyperglycemia in experimental animal model of diabetes [18, 19]. Alloxan monohydrate destroys and decreases the pancreatic β-cell population in the islets of Langerhans [26]. Following intraperitoneal administration of alloxan monohydrate (150 mg/kg b.w), all animals showed a significant increase of blood glucose levels and weight loss. These features indicated a successful induction of diabetes in experimental rats [27]. The significant increase of blood glucose levels after injection of alloxan probably result to the destruction of insulin or the destruction of the insulin producing beta cells (β-cells) of the pancreatic islets [26, 28]. Oral administration of *H. sabdariffa* extract, as well as Glibenclamide significantly decreased blood glucose levels in a dose dependent manner. Our results were in line with several studies using *H. sabdariffa* extract to manage blood glucose level in rats [17, 29, 30]. To assess pancreas capacity to produce insulin after treatment with *H. sabdariffa* extract in alloxan-induced diabetic rats, OGTT was determined at the end of study. Thus, alloxan-induced diabetic group showed an impairment of OGTT, which may be due the incapacity of pancreas to produce insulin to stabilize the blood glucose to its normal level. Treatment with *H. sabdariffa* extract and Glibenclamide enhanced the glucose tolerance in alloxan-induced diabetic rats.

The antidiabetic properties observed here, i.e. the decrease of blood glucose levels and the enhancement of glucose tolerance could be due to the presence of bioactive components revealed from the screening of *H. sabdariffa* extract and capable to reduce blood glucose level with a mechanism of action that may be similar to that of glibenclamide. In the present study, phytochemical screening showed the presence of triterpenes, flavonoids, phenols, and tannins compounds within the extract of *H. sabdariffa.* Several studies have demonstrated the involvement of phytochemical compounds such as triterpenes [31, 32], flavonoids [33-35], and tannins [36] for the management of diabetes. These compounds might act by several mechanisms such as enhancing insulin secretion [37] or regenerating insulin-producing pancreatic β-cell population in the islets of Langerhans [38-40]. Thus, *H. sabdariffa* extract may enhanced insulin secretion by stimulating the β-cell of the Langerhans islets of the pancreas as its also consists of flavonoids, phenols, and tannins. Therefore, our results strongly support the antidiabetic property of *H. sabdariffa* extract in alloxan-induced diabetic rats.

Lipid abnormalities are often the leading cause of cardiovascular disease in diabetic with poor control of blood glucose level [41]. In this study, diabetes induced by alloxan showed that the levels of triglycerides (TG), total cholesterol (TC), low-density lipoprotein (LDL) were increased, and high-density lipoprotein (HDL) level was decreased. This finding is in perfect agreement with the results obtained in case of lipid abnormality in diabetic patients [41] or animals model of diabetes [17, 18, 42-44]. Diabetic rats treated with *H. sabdariffa* extract and Glibenclamide for 21 days showed a significant decrease of TG, TC, LDL levels and a significant increase of HDL level when compared with diabetic group. The results indicates that *H. sabdariffa* effectively reverse lipid abnormalities in alloxan-induced diabetic rats and are in accordance with previous studies in animal models of diabetes [17, 18, 43, 44]. This hypolipidemic activity of *H. sabdariffa* extract may be mediated by their active phytochemical compounds (tannins, flavonoids, phenols, and triterpenes) capable to normalize cholesterol and acting by several mechanisms including stimulatory effect on insulin secretion from pancreatic β-cells, activation of lipoprotein lipase, insulin-mediated lipolytic activity or inhibition of lipogenic enzymes [45-47].

One of the main feature indicating a successful induction of diabetes in experimental rats is a weight loss [27]. Three days after alloxan injection, all rats showed a decrease in body weight. Diabetic group exhibited a continuous decrease in body weight over a period of 21 days. The decrease of body weight could be due to insulin deficiency or abnormalities in the catabolism of macronutrients such as fat and protein leading to extreme tissue protein loss and muscle wastage [23, 48]. The oral administration of *H. sabdariffa* extract and Glibenclamide in alloxan-induced diabetic rats for 3 weeks increased the body weight, which indicates a better control of high blood glucose in these rats. The increase in body weight may probably be due to the effect of the extract in controlling muscle wasting and tissue protein loss [48-50].

Finally, despite differences in the relative weight of organ found in each group, no significant differences were noted between experimental groups. However, the increase in the relative weight of liver from the diabetic group may well denote liver disarrangement in the form of fatty infiltration with attendant enlargement of the liver and tissue hyperplasia as result of absence of glycemic control [51, 52]. The reduction of the relative weight of the pancreas observed in diabetic group could be associated with the severity of the destruction of Langerhans islets by the alloxan [53]. Our result is in line with the decrease of the relative weight of pancreas seen in several studies as consequence of hyperglycemia [23, 54, 55].

5. Conclusion

Hydroethanolic extract of *H. sabdariffa* reduces in a dose-dependent manner the fasting blood glucose level, enhances the glucose tolerance, normalize lipid profiles improves body weight gain of diabetes rats with severe destroyed of Langerhans islets. Hydroethanolic extract of *H. sabdariffa* appeared then as an antidiabetic drug which may act through mechanisms that mimic Glibenclamide action. Although the study reveals the presence of polyphenolic compounds as possible active principles, the type of molecules and their possible mechanism of action still need to be investigated.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest.

Statement of ethical approval

The experimental protocols were approved by the University's Animal Care Ethics Committee

Author Contributions

- Research project: A. Conception, B. Organization, C. Execution.
- Manuscript: A. Writing of the first draft, B. Review and Criticism.

Hamadjida: 1A, 1B, 1C, 2A,2B; Metechie: 1C; Tchiengang: 1C; Ndji: 1B,1C,2B; Eteme: 1C; Njintang: 1C,2B; Mingoas: 2A,2B

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