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Antidiabetic and Hypolipemic Effects of Total Aqueous Extract of *Pseudarthria hookeri Wight & Arn.* (Fabaceae) on Hemoglobin Glycation in Alloxan induced Diabetic Rats

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ABSTRACT

Pseudarthria hookeri (Fabaceae) is a plant used in traditional medicine in Ivory Coast to treat diabetes. This study aims to evaluate antidiabetic and hypolipemic effects of total aqueous extract of *Pseudarthria hookeri* (EAPh) on hemoglobin glycation in diabetic rats. Diabetes mellitus was induced by a single intraperitoneal injection of alloxan (200 mg/kg body weight) to Wistar rats. The diabetic rats were treated orally with daily doses of extracts (800, 1000 and 1200 mg/kg bw) and glibenclamide (10 mg/kg bw) for 28 days, respectively. Blood samples from control and test group animals were used for dosing biochemical parameters. The results showed that EAPh administered orally at doses of 1000 and 1200 mg/kg bw for 28 days, leads to a significant decrease ($P < 0.01$) in blood glucose levels of diabetic rats. In addition, a significant decrease in serum total cholesterol and triglycerides levels was observed in diabetic rats treated with EAPh (1200 mg/kg bw) associated with an increase ($P<0.01$) in serum HDL cholesterol levels after 28 days of treatment. Moreover, after 90 days of treatment, EAPh (1200 mg/kg bw) induced a significant decrease (Hba1c < 7 %) in glycated hemoglobin percentage of diabetic rats. Our present study clearly revealed that EAPh possesses potent antihyperglycemic and hypolipemic effects comparable to those of glibenclamide in diabetic rats. These results justify the use of *Pseudarthria hookeri* (Fabaceae) in traditional medicine to effectively treat diabetes and lipid disorders associated with this pathology.

1. INTRODUCTION

Diabetes mellitus (DM) is mainly characterized by chronic hyperglycemia resulting from defects in insulin secretion, action or both[12]. Persistently high glucose levels lead to irreversible glycation and advanced glycation end products formation (AGEs). The extent of glycated hemoglobin is proportional to glucose levels and is associated with oxidative stress induced by AGEs interaction with receptors on tissues[25]. Indeed, the nonenzymatic glycation of hemoglobin increases in response to a chronic or prolonged exposure to glucose[20,18,27]. Moreover, diabetes is also associated with dyslipidemia characterized by both hypercholesterolemia and hypertriglyceridemia, two risk factors for cardiovascular disease. Currently, there are over 366 million diabetics worldwide 3.2 million deaths per year[31]. On account of the high incidence of vascular complications associated with diabetes mellitus, various studies are being conducted to find out remedies against it. Thus, an ethnobotanical survey carried out in Ivory Coast, identified *Pseudarthria hookeri* (Fabaceae) as a plant used by traditional healers to treat diabetes[5]. The aim of this study is to evaluate the antidiabetic and hypolipemic effects of total aqueous extract of *Pseudarthria hookeri* (EAPh) on hemoglobin glycation in alloxan induced diabetic rats.

2. MATERIALS AND METHODS

2.1 Plant Materials

Fresh leaves of *Pseudarthria hookeri Wight & Arn.* were collected from Marahoue, in West-Central of Ivory Coast, during the month of June 2014. It was identified by comparison with specimen No. 2026 of 3 October 1953, deposited in the herbarium of the department, authenticated by Laurent Ake-Assi, Emeritus Professor of Botany at Felix Houphouet-Boigny University (Abidjan, Ivory Coast).

2.2 Animals

Healthy Wistar rats *(Rattus norvegicus),* weighting between 150 and 200 g were used for this present study. They were obtained from the Animal house at Felix Houphouet-Boigny University (Abidjan, Ivory Coast) and kept at constant temperature (24 ± 2°C) with 50-55 % of humidity and a .photoperiod of 12 hours of daylight and 12 hours of darkness. They were fed *ad libitum* with pellet (Ivograin, Abidjan, Ivory Coast) and allowed free access to clean

water. All the procedures were conducted in accordance with the guidelines for Care and Use of Laboratory Animals published by the National Institutes of Health.

2.3 Methods

2.3.1 Preparation of the total aqueous extract of *Pseudarthria hookeri* (EAPh)

250 g of fresh leaves of this plant were dropped in 1.5 liters of distilled water. The mixture is boiled for one hour (1 hr). The decoction obtained was filtered twice on cotton and whatman filter paper. The filtrate was oven dried at 50°C for 72 hours. After drying, the dark powder obtained is the total aqueous extract of *Pseudarthria hookeri* (EAPh).

2.3.2 Experimental diabetes induced in rats

The rats were fasted overnight (12 hours) and fasting glucose levels were measured by a glucometer Accu - Chek Active (Roche diagnostics, Germany). Blood samples were collected from tail vein by the technical described by Kraus[16]. After overnight fasting (12 hrs), the normal rats having glucose levels less than 1.2 g/L (≤ 1.2 g/L) were used. Diabetes was induced in rats by a single intraperitoneal injection of alloxan (Alfa Aesar, Germany) dissolved in 9‰ sodium chloride at a dose of 200 mg/kg body weight. Two weeks after alloxan injection, rats with fasting blood glucose levels ≥ 2 g/L were considered diabetic[17]and selected for the study.

2.3.3 Assessment of fasting blood glucose levels in diabetic rats

Thirty (30) rats were randomly divided into 6 groups of 5 rats (five rats per group).
Group I: Normal control rats received distilled water. Group II: Diabetic control rats were given distilled water. Group III, IV and V were treated orally with daily doses of 800, 1000 and 1200 mg/kg body weight of EAPh respectively, for 28 days. Group VI: Glibenclamide at a dose of 10 mg/kg body weight was used as a standard drug every day, for 28 days. Fasting blood glucose was withdrawn from tail vein[16]and blood glucose levels from control and test group animals were measured by a glucometer Accu-Chek on day 0 (before treatment), 7th, 14th, 21st and 28th day of post treatment.

2.3.4 Estimation of serum lipid profile and glycated hemoglobin in diabetic rats

2.3.4.1 Dosing of lipid profile

Blood samples from the experimental rats were collected from tail vein[16]. The collected blood samples were centrifuged at a speed of 4500 rpm for 10 min to get serum which stored at -20°C for analysis of lipid parameters using a spectrophotometer (Biolabo, France). The serum samples were analyzed for lipid profile markers including Total cholesterol, HDL-cholesterol and triglycerides. So Total cholesterol was estimated by CHOD/POD method[3], HDL cholesterol[19] and triglycerides were determined by using GPO/POD method[8, 11].

2.3.4.2 Assay of glycated hemoglobin (HbA1c)

For our study, blood samples were collected in tubes containing anticoagulant (EDTA) and determined by the immunoturbidimetric assay using a Cobas Hitachi C311 (Roche, Germany). From hemolyzed blood, total hemoglobin concentration was determined by measuring photometrically hemoglobin released from erythrocytes at 525 nm. The concentration of glycated hemoglobin (HbA1c) was determined by measuring the absorbance at 625 nm of the complex formed from poly-hapten and antibody antiHbA1c. The ratio of the two absorbances gives the percentage of HbA1c.

2.3.4.3 Experimental design

Twenty (20) Wistar rats weighing 150 - 200 g were divided into four (4) groups.

Group I (normal control) and group II (Diabetic control), received distilled water.

Group III and group IV were treated orally with glibenclamide (10 mg/kg body weight) and EAPh at oral dose of 1200 mg/kg body weight, respectively. The drugs were given every day for 28 days and 90 days. Serum levels of Total cholesterol, HDL cholesterol and triglycerides were measured on day 0 (before treatment), 7^{th}, 14^{th}, 21^{st} and 28^{th} day of post treatment. After 90 days of treatment, the blood from control and test group animals was collected in EDTA tubes and the percentages of glycated hemoglobin were determined.

2.3.5 Statistical analysis of results

The computer program GraphPad InStat (San Diego CA, USA) was used for statistical analysis of results. Each value was given as mean followed by the standard error of the mean (M ± SEM). The difference between two values was determined by the Student-Newman-Keuls. It is significant when $P < 0.05$. The computer program GraphPad Prism (San Diego CA, USA) was used to draw the graphs.

3. RESULTS

3.1 Effects of EAPh on fasting blood glucose levels in diabetic rats

The values of fasting blood glucose level before (day 0) and after treatment for four weeks (28 days) in normal rats, control diabetic rats, treated diabetic rats are presented in figure 1.

The fasting blood glucose levels (1.02 ± 0.05) did not show significant difference in the normal control rats for 28 days of experimentation. In alloxan induced diabetic rats, fasting blood glucose levels showed a significant increase of 97.06 % (from 1.02 ± 0.05 to 2.01 ± 0.06 g/L on day 0) as compared to normal control rats and it attained 2.10 ± 0.09 g/L on day 28. The administration of EAPh at a dose of 800 mg/kg body weight has no significant effect on blood glucose levels in diabetic rats. However, when diabetic rats were treated with 1000 mg/kg bw of EAPh, blood glucose levels significantly decreased (from 2.01 ± 0.06 to 1.62 ± 0.06 g/L) and with 1200 mg/kg bw of EAPh (from 2.01 ± 0.06 to 1.4 ± 0.08 g/L) from 14^{th} to 28^{th} day of treatment. The effect of the extract is dose dependent respectively corresponding to 39.39 % and 59.81 % ($P < 0.01$) inhibition of hyperglycemia induced by alloxan. With glibenclamide at a dose of 10 mg/kg bw, blood glucose levels significantly lowered (from 2.01 ± 0.06 on day 0 to 1.2 ± 0.05 on day 28) in diabetic rats representing 79.41 % ($P < 0.001$) inhibition of hyperglycemia induced by alloxan in rats. It appears that oral administration of EAPh and glibenclamide to diabetic rats reversed the changes in the levels of blood glucose to near normal.

3.2 Effects of EAPh on serum lipid profile in diabetic rats

3.2.1 Effects of EAPh on serum Total cholesterol in diabetic rats

Total cholesterol levels (0.86 ± 0.18 g/L) were not changed in the normal control rats for 28 days of experimentation. In diabetic rats, Total cholesterol levels showed a significant increase of 62.79 % ($P < 0.01$) when compared with normal control rats, from 0.86 ± 0.18 to 1.40 ± 0.24 g/L after four weeks. However, with 1200 mg/kg bw of EAPh, Total cholesterol levels in diabetic rats were significantly decreased ($P < 0.01$), from 14^{th} to 28^{th} day of treatment. A significant reduction in serum Total cholesterol level was observed in diabetic rats treated with 1200 mg/kg bw of EAPh (from 1.40 ± 0.24 to 0.96 ± 0.2 g/L) and glibenclamide (from 1.4 ± 0.24g/L before treatment to 0.88 ± 0.22 g/L on day 28) respectively corresponding to 81.48 % and 96.30 % ($P < 0.001$) inhibition of

hypercholesterolemia induced by administration of alloxan in rats. The results are shown in figure 2.

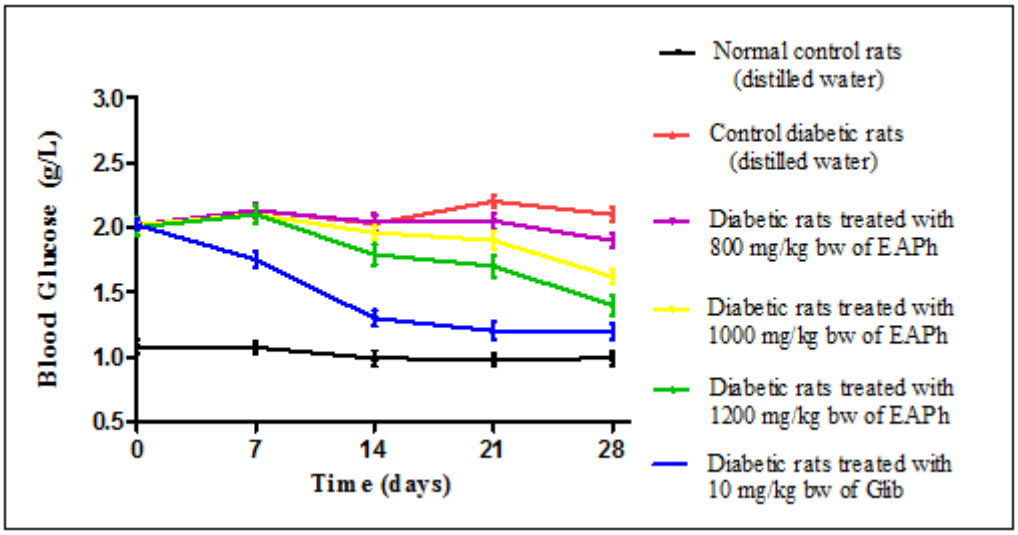

Figure 1: Dose-response effects of aqueous extract of *Pseudarthria hookeri* (EAPh) and glibenclamide (Glib) on fasting blood glucose levels in diabetic rats for 28 days of treatment

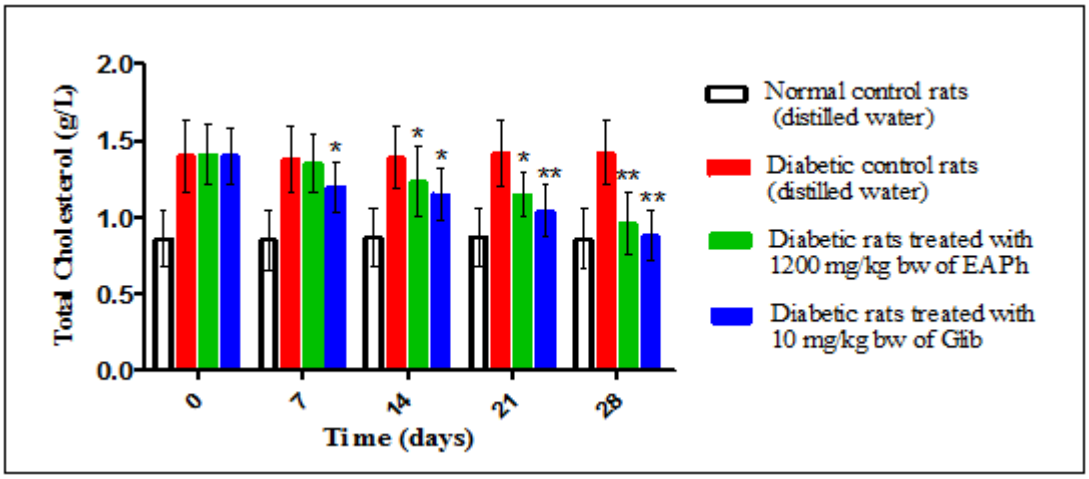

Figure 2: Effects of aqueous extract of *Pseudarthria hookeri* (EAPh) and glibenclamide (Glib) on serum total cholesterol in diabetic rats for 28 days of treatment

*Each value is mean± SEM for five rats in each group (N = 5); * P <0.05; ** P <0.01 compared to diabetic control rats.*

3.2.2 Effects of EAPh on serum HDL cholesterol in diabetic rats

HDL cholesterol levels (0.42 ± 0.02 g/L) did not show significant difference in the normal control rats for 28 days of experimentation. In alloxan induced diabetic rats, HDL cholesterol

levels showed a significant decrease of 28.57 % ($P < 0.05$) as compared to normal control rats and attained 0.30 ± 0.04 g/L for four weeks. However, oral administration of EAPh, at a dose of 1200 mg/kg bw, leads to a significant increase ($P < 0.01$) of HDL cholesterol levels in diabetic rats from 14th to 28th day of treatment. A HDL cholesterol level improvement was observed in diabetic rats treated with the extract (from 0.30 ± 0.04 to 0.39 ± 0.03 g/L) and glibenclamide (from 0.30 ± 0.04 to 0.41 ± 0.03 g/L) respectively representing 75 % and 91.67 % ($P < 0.001$) inhibition of hypocholesterolemia induced by administration of alloxan to rats. The results are shown in figure 3.

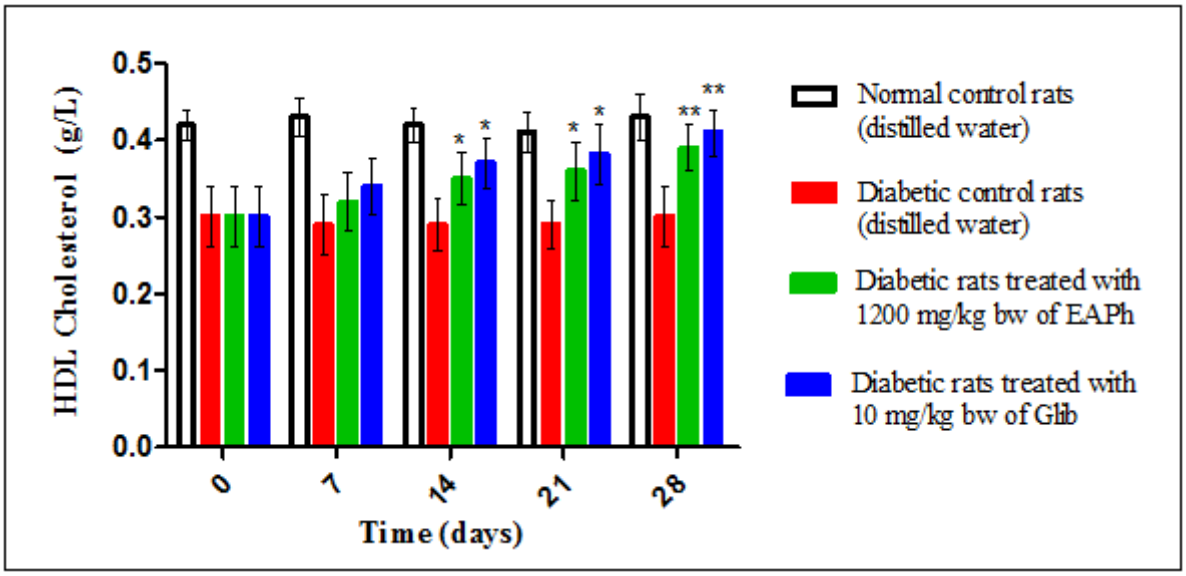

Figure 3: Effects of aqueous extract of *Pseudarthria hookeri* (EAPh) and glibenclamide (Glib) on serum HDL cholesterol in diabetic rats for 28 days of treatment

*Each value is mean± SEM for five rats in each group (N = 5); * P <0.05; ** P <0.01 compared to diabetic control rats.*

3.2.3 Effects of EAPh on serum triglycerides in diabetic rats

Triglycerides levels (0.99 ± 0.19 g/L) were not significantly changed in the normal control rats for 28 days of experimentation. When diabetes was induced in rats, triglycerides levels showed a significant increase of 56.57 % ($P < 0.01$) as compared to normal control rats and attained 1.55 ± 0.2 g/L for four weeks. However, with 1200 mg/kg bw of EAPh, triglycerides levels in diabetic rats were significantly decreased ($P <0.01$) from 7th to 28th day of treatment. Triglycerides levels significantly lowered in diabetic rats treated with 1200 mg/kg bw of EAPh (from 1.55 ± 0.2 to 1.10 ± 0.18 g/L) and glibenclamide (from 1.55 ± 0.2 to 1 ± 0.15

g/L) respectively representing 80.36 % and 98.21 % (P < 0,001) inhibition of hypertriglyceridemia induced by administration of alloxan in rats. The results are presented in figure 4.

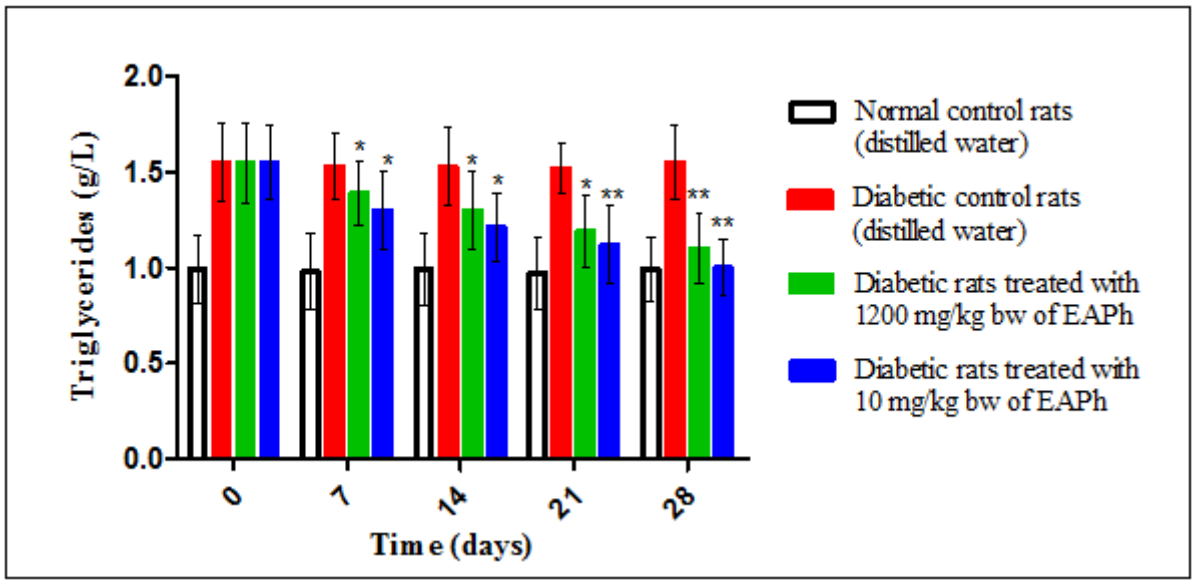

Figure 4: Effects of aqueous extract of *Pseudarthria hookeri* (EAPh) and glibenclamide (Glib) on serum triglycerides in diabetic rats for 28 days of treatment

*Each value is mean± SEM for five rats in each group (N = 5); * P <0.05; ** P <0.01 compared to diabetic control rats.*

3.3 Effects of EAPh on glycated hemoglobin (HbA1c) in diabetic rats

The values of glycated hemoglobin (HbA1c) were 4.5 ± 0.7 % in the normal control rats during the 90 days of experimentation. In alloxan induced diabetic rats, percentages of glycated hemoglobin showed a significant increase of 117.78 % (P < 0.001) as compared to normal control rats, attained 9.8 ± 1.08 % after 90 days of treatment. However, the percentages of HbA1c in diabetic rats decreased after oral administration of EAPh (from 9.8 ± 1.08 % to 6.60 ± 0.18 %) and glibenclamide (from 9.8 ± 1.08 % to 6.2 ± 0.74 %) respectively corresponding to 60.38 % and 67.92 % (P < 0.01) reduction of HbA1c increased. It appears that oral administration of EAPh and glibenclamide to diabetic rats reversed the change of glycated hemoglobin percentage to near normal. The results are shown in figure 5.

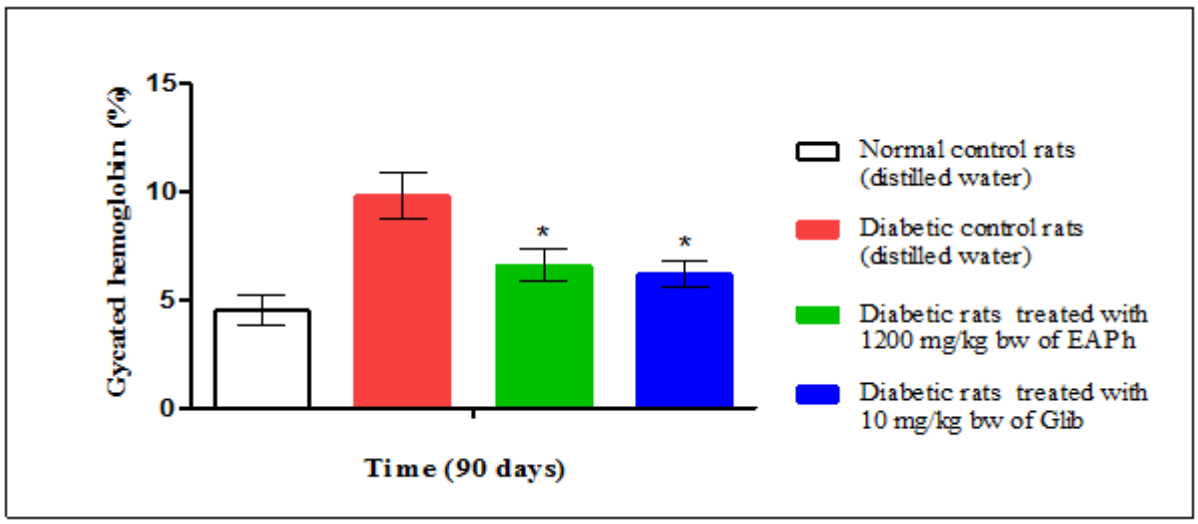

Figure 5: Effects of aqueous extract of *Pseudarthria hookeri* (EAPh) and glibenclamide on glycated hemoglobin (HbA1c) in diabetic rats for 90 days of treatment

*Each value is mean± SEM for five rats in each group (N = 5); * P <0.05; compared to diabetic control rats.*

4. DISCUSSION

A single intraperitoneal injection of alloxan in rats increases blood glucose which ranges from (1.02 ± 0.05 g/L to 2.01 ± 0.06 g/L) and persists during the 28 days of experimentation. Such animals are alloxan induced diabetic rats[4,30]. Indeed, alloxan monohydrate is a cytotoxin which causes, in a wide variety of animal species a massive destruction of pancreatic beta cells which are the leading provider of insulin secretion, inducing experimental diabetes[30,24,9]. Hyperglycemia persists for 28 days in diabetic control rats. However, diabetic rats treated with EAPh at doses ≥ 1000 mg/kg body weight, or with glibenclamide (10 mg/kg bw), showed on one hand a significant decrease of hyperglycemia and on the other hand glycemia tended to normalcy. These results showed antihyperglycemic and antidiabetic properties of *Pseudarthria hookeri.* Similar results were obtained by Mbagwu et *al.*[21], Agarwal *et al.*[1] and Guenzet et *al.*[13] respectively for *Phyllanthus amarus* (Phyllanthaceae), *Citrullus colocynthis* (Cucurbitaceae) and *Zygophyllum gaetulum* (Zygophyllaceae) which reduced hyperglycemia in diabetic rats. The effects of EAPh are similar to those of glibenclamide, a molecule from the family of sulfamides, recognized for it hypoglycemic and antihyperglycemic activities. In fact, sulfonylureas stimulate insulin secretion by pancreatic beta cells, entailing the storage of glycogen in the liver. Furthermore, they decrease glucagon secretion, by inhibiting the release of hepatic glucose and potentiate of insulin effects in liver[14, 22, 29, 32]. The decrease of

hyperglycemia in diabetic rats treated with EAPh could be explained by the stimulation of insulin secretion by the pancreas and/or probably due to an increased usage of peripheral glucose.

Total cholesterol and triglyceride levels increased in diabetic rats as compared to normal control rats. This result is in agreement with that of Betterridge et *al.*[7] who reported that insulin deficiency or insulin resistance might be responsible for hyperlipidemia because insulin has an inhibitory action on 3-hydroxy-3-methyl-glutaryl coenzyme A reductase (HMG-COA reductase), a key enzyme in cholesterol biosynthesis. Moreover, Khanna et *al.*[15], Ravi et *al.*[26]and Sharma et *al.*[28] suggested that abnormal high level of lipids in serum observed in diabetic people was due to an increase mobilization of fatty acids from adipose tissue. However, EAPh administered at a dose of (1200 mg/kg bw) significantly reduced hypercholesterolemia and hypertriglyceridemia observed in rats and after 28 days of treatment, Total cholesterol and triglycerides levels were tending to normalcy. Similar effect was reported by Eddouks *et al.*[10] showing a reduction of total cholesterol and triglycerides levels in diabetic rats treated with *Capparis spinosa* (Capparaceae). Therefore, EAPh has hypolipemic effects and could act by decreasing cholesterol biosynthesis and/or increase the catabolism of LDL cholesterol. In addition, HDL cholesterol significantly increased in diabetic rats treated with EAPh, so by increasing HDL cholesterol, EAPh could also prevent cardiovascular diseases.

Glycated hemoglobin (HbA1c) increased in diabetic rats (above 9%) as compared with normal control group (4.5 ± 0.7%). This indicates a poor glycemic balance for a chronic hyperglycemia in diabetic rats[23, 6]. The increased percentage of HbA1c in diabetic control rats could be due to the slow and irreversible binding of plasma glucose in excess on N-terminal ß chain hemoglobin A1 edge[20, 2]. The percentages of HbA1c decreased after the administration of EAPh and glibenclamide to diabetic rats. Similar results were reported by Guenzet *et al.*[13] which also demonstrated that *Zygophyllum gaetulum* (Zygophyllaceae) has antihyperglycemic effects and reduced HbA1c levels in diabetic rats.

5. CONCLUSION

This study showed that the aqueous extract of *Pseudarthria hookeri* (EAPh), administered at doses of 1000 and 1200 mg/kg bw reduced significantly hyperglycemia in diabetic rats. Therefore, EAPh possesses an antihyperglycemic and an antidiabetic property as

glibenclamide. Moreover, total cholesterol and triglycerides levels were significantly brought down in diabetic rats treated with EAPh (1200 mg/kg bw). EAPh is a hypolipemic substance which corrects lipid abnormalities associated with diabetes. In addition, EAPh normalizes HDL cholesterol in diabetic rats and lowered HbA1c levels (HbA1c < 7%) in diabetic rats and provides better glycemia control through its antihyperglycemic and hypolipemic properties. Thus, the aqueous extract of *Pseudarthria hookeri* (Fabaceae) proved to be an anti-diabetic substance that normalizes blood glucose balance and corrects lipid disorders in diabetes. This justifies the use of this plant by traditional healers to treat diabetes.

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