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HYPOGLYCEMIC AND HYPOLIPIDEMIC ACTIVITIES OF *HALLEA STIPULOSA* STEM BARK AQUEOUS EXTRACT ON DEXAMETHASONE INDUCED INSULIN RESISTANCE IN RATS.

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ABSTRACT

The aim of this study was to examine the hypoglycemic and hypolipidemic activities of aqueous extract of *Hallea stipulosa* on dexamethasone induced insulin resistance in rats, and to evaluate *in vitro* the inhibitory activity of this plant extract on α- glucosidase enzyme. Insulin resistance was induced by subcutaneous injection of dexamethasone (1 mg/kg) for 10 days, one hour before administration of different treatments. Body weight and blood glucose level were measured on day 1, 5 and 10. At the end of treatment, glucose tolerance test was performed to the rats, blood samples were collected for triglyceride, total cholesterol, LDL-c and HDL-c level determination. Organs (heart, liver pancreas and kidney) were also collected for relative organ weight determination. The results showed a significant increase ($p < 0.001$) in serum triglyceride, total cholesterol, LDL-c, blood glucose level, liver weight and a significant decrease ($p < 0.001$) in HDL-c and body weight observed in dexamethasone control group compared to normal control group. *Hallea stipulosa* at doses 25, 50 and 100 mg/kg significantly reversed the alteration induced by dexamethasone. *Hallea stipulosa* significantly reduced postprandial glucose and showed appreciable α-glucosidase inhibitory effect in a concentration-dependant manner (IC_{50}=13.14). These results suggest that *Hallea stipulosa* stem bark aqueous extract may be useful for the treatment of type 2 diabetes mellitus.

KEYWORDS: *Hallea stipulosa*, dexamethasone, α-glucosidase, rats.

INTRODUCTION

Diabetes mellitus (DM) is characterized by hyperglycemia and alterations of carbohydrate, protein, and lipid metabolism, caused by a defect in insulin production or it action.[1] It is a growing world –wide epidemic [2] with a prevalence of 6.4% (285 million adults) in 2010, and a prevalence projection of 7.78% (439 million adults) by 2030.[3]

Type 2 diabetes is characterized by insulin resistance in the major target tissues combined with insufficient insulin secretion, this leading to an impaired uptake and metabolism of glucose.[4] This is followed by abnormal lipid and protein metabolism that can lead to serious complications including nephropathy, retinopathy, neuropathy and coronary artery disease.[5,6,7] The aim of oral therapy in type 2 diabetes is to reach normoglycemia and prevent later complications.[8] One therapeutic approach involves controlling postprandial hyperglycemia by inhibiting the alpha-glucosidase enzyme in the digestive tract, delaying and prolonging the overall carbohydrate digestion time.[9] Slowing carbohydrate digestion reduces the rate of glucose absorption and consequently prevent spikes in the postprandial blood glucose and insulin levels.[10, 11]

Many progresses in the management of DM using synthetic drugs have been made, but these drugs are expensive and still have many adverse effects. Therefore, the search for improved and safe natural anti-diabetic agents with minimal side effects is ongoing.[12] Among these natural products we have plants. *Hallea stipulosa* is a tree widely distributed throughout Africa.[13,14] In Cameroon, the decoction of stem bark is used traditionally to treat diabetes, diarrhea, cancer and hypertension, and as diuretic for local populations. Various phytochemical studies have already done on the stem bark of this plant. Isorotundifoline [15], Mitraphylline [16], ursolic acid, quinovic acid [17, 18] and β-sitosterol [19] were isolated.

The aim of this study was to evaluate *in vitro* α-glucosidase inhibitory activity and hypolipidemic and hypoglycemic activities of aqueous extract of the stem barks of *H. stipulosa* on dexamethasone induced insulin resistance in rats.

MATERIALS AND METHODS

Chemicals

All the reagents were obtained commercially and were of analytical reagent grade. Dexamethasone sodium phosphate, acarbose, α- glucosidase and pnitrophenyl-D-glucoside (pNPG) were purchased from Sigma-Aldrich, St Louis, USA. D-glucose and sucrose were purchased from Edu-Lab Biology Kit, Bexwell, Norfolk PE38 9GA, UK. All the reagents were obtained commercially and were of analytical reagent grade.

Plant material

H. stipulosa stem barks were collected in Foumban (West Cameroon) in June 2003 and a voucher specimen was authenticated by comparison to specimen No. 21076/ SRF/ CAM of the Cameroon herbarium.

Preparation of the aqueous extract of stem barks of *Hallea stipulosa*

A decoction was prepared by boiling 100 g of a dried powder with 500 ml of distilled water for 20 min. The decoction once cooled at room temperature was filtered. Then the filtrate was concentrated by evaporating water at 40°C an oven for 48 h.

Experimental animals

Adult Wistar rats weighing between 200 and 250 g, raised in ambient environment, fed on standard laboratory diet, receiving water *ad libitum*, in the animal house of the Department of Animal Biology, Faculty of Science at the University of Dschang, Cameroon were used. All experiments were conducted in compliance with ethical guide for care and use of laboratory animals.

In vitro α- Glucosidase inhibitory activity

The α-glucosidase inhibitory activity was determined according to Dewi et al.[20] with slight modifications. One mg of α-glucosidase (from *Saccharomyces cerevisiae*) was dissolved in 100 ml of Tris-HCl buffer (pH 6.8) containing 200 mg of bovine serum albumin. In test tubes, 200 µl of a sample at different concentrations was pre-incubated with 100 µl of α-glucosidase for 10 min at 37 °C. The reaction was initiated by addition of 100 µl of *p*-nitrophenyl-*α*-D-glucopyranoside (5 mM). After 15 min of incubation at 37 °C, 800 µl of Na_2Co_3 (200 mM) was added to stop the reaction. Acarbose was used as a positive control and distilled water as control. α-Glucosidase activity was determined spectrophotometrically at 400 nm by measuring the quantity of p-nitrophenol release from pNPG. The α-glucosidase inhibitory activity was calculated from the difference in two absorbencies and expressed in percentage of inhibition (% inhibition) as follow

Inhibition (%) = 100 x (AC – AS) / AC

Where AC is the absorbance of control and AS is the absorbance of sample The concentration of the extract required to inhibit 50 % of α-glucosidase activity (IC_{50}) was calculated using the percentage scavenging activities at six different concentrations of the extract.

Oral glucose tolerance test

30 overnight fasted rats were divided into five groups of six rats each. Group 1 received distilled water; group 2 received Glibenclamide (5 mg/kg); groups 3, 4 and 5 received respectively 25, 50 and 100 mg/kg of aqueous extract of *Hallea stipulosa* (AEHS). 60 min after, all the rats received oral administration of D-glucose (3 g/kg body weight), then blood was collected at the level of the tail for blood estimation using ACCU-CHEK Active glucometer (Roche Diagnostics GmbH, Sandhofer Strass 116, 68305 Mannheim, Germany). Blood glucose was estimated just before the administration of substances (0 min) and at 30, 60, 90 and 120 min after D-glucose treatment.

Dexamethasone induce insulin resistance

36 overnight fasted rats were divided into six groups of six animals per group. Groups 1 (normal control group) and 2 (diabetic control group) received distilled water; group 3 received Metformin (40 mg/kg) and served as positive control; groups 4, 5 and 6 were administrated orally AEHS at the doses 25, 50 and 100 mg /kg respectively. One hour after drug treatment, the rats of groups 2 to 6 were subcutaneously administrated with dexamethasone sodium phosphate (1 mg/kg) each day all over the experiment (10 days).

Blood glucose level was estimated on the first day, just before all the treatments, the fifth and the tenth day. Oral glucose tolerance test was also performed in these rats on the 10^{th} day.

Thereafter, all the animals were anesthetized intraperitoneally with an administration of diazepam (10 mg/kg) and ketamin (50 mg/kg). Blood samples were collected by catheterization of abdominal artery and centrifuged at 3000 rpm for 15 min. Serum was separated and stored at – 20°C for biochemical analysis. Total protein was determined according to the methods described by Gornal et al. (1949); total cholesterol (TC), triglycerides (TG) and high density lipoprotein (HDL-c) concentrations were determined using commercial diagnostic Kit INMESCO, Germany. Low density lipoprotein cholesterol (LDL-c) concentration was calculated as follows: LDL-c (mg/dl) = TC/1.19 + TG/1.9 – HDL-c/1.1– 38 (Seyed-Ali *et al.*, 2008).

The TG/HDL-c ratio was also calculated.

Just after the blood collection, the heart, liver, pancreas, and the kidney were removed, cleaned with saline

solution (0.9 %) and weight for the calculation of relative organ weight (ROW).

$$ROW = 100 \times \frac{\text{Absolute organ weight (g)}}{\text{Body weight of rat on the day of sacrifice (g)}}$$

Statistical analysis

All the results were expressed as mean ± standard error mean (SEM). Data obtain were submitted to ANOVA, and means were separated by Turkey or Bonferroni tests at 95% confident limit, using Graph pad Prism Version 5.03.

RESULTS

α- glucosidase inhibitory activity of AEHS

AEHS inhibited α-glucosidase activity with the maximum inhibition of 27,713 ± 0.261 % at the concentration of 300 µg/ml, compared to 47.354 ± 2.077 % of acarbose at the same concentration. *H. stipulosa* stem bark's IC_{50} was 13.14 µg/ml whereas acarbose was 24.21 µg/ml (Table 1).

Table 1: Effect of *H. stipulosa* (HS) on percentage of α-glucosidase inhibition and the IC_{50} values

	Concentration (µg/ml)	Inhibition (%)	IC_{50} (µg/ml)
AEHS	1	19.910 ± 1.350	13.14
	3	21.973 ± 0.941	
	10	24.215 ± 0.993	
	30	24.574 ± 0.499	
	100	25.471 ± 0.435	
	300	27.713 ± 0.261	
Acarbose	1	19.641 ± 0.867	24.21
	3	21.704 ± 1.048	
	10	25.202 ± 1.027	
	30	36.054 ± 0.578	
	100	43.587 ±1.248	
	300	47.354 ± 2.077	

AEHS: Aqueous extract of HS. n = 5, data are presented as mean ± SEM

Effect of AEHS on glucose tolerance test in normal rats: The effect of extract on blood glucose level of healthy rats after administration of D-glucose are presented in Figure 1. Oral administration of *H. stipulosa* significantly decline ($p < 0.05$) the rise in blood glucose level after glucose administration from 90 to 120 min at dose 50 mg/kg and at 120 min for dose 100 mg/kg. Glibenclamide also significantly decline this rise in blood glucose level ($p < 0.05$ at 60 min and $p < 0.01$ from 90 to 120 min).

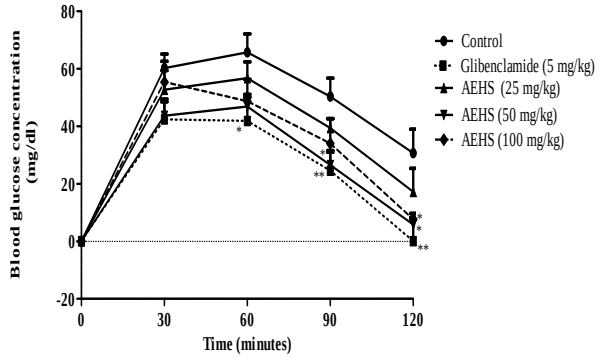

Figure 1: Effect of *H. stipulosa* (HS) on glucose tolerance test in normal rats.

AEHS 25: aqueous extract of HS at dose 25 mg/kg; AEHS 50: aqueous extract of HS at dose 50 mg/kg; AEHS 100: aqueous extract of HS at dose 100 mg/kg. n = 6; data are presented as mean ± SEM. *p < 0.05, **p < 0.01 compared to normal control group.

Effect of AEHS on blood glucose level

Figure 2 showed the hypoglycemic effect of oral administration of HS stem bark on dexamethasone induced insulin resistance in rats. All the doses of AEHS and metformin significantly declined ($p < 0.001$) the blood glucose level at the end of the treatment.

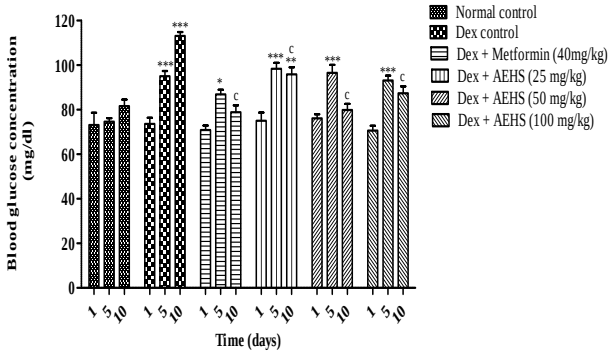

Figure 2: Effect of *H. stipulosa* (HS) on blood glucose level in dexamethasone induces insulin resistance in rats.

AEHS 25: aqueous extract of HS at dose 25 mg/kg; AEHS 50: aqueous extract of HS at dose 50 mg/kg; AEHS 100: aqueous extract of HS at dose 100 mg/kg; Dex: dexamethasone. n = 6; data are presented as mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001 compared to normal control group; $^{c}p < 0.001$ compared to dexamethasone control group.

Effect of AEHS on glucose tolerance test in dexamethasone treated rats

Oral glucose tolerance test after 10 days of dexamethasone administration is showed in figure 3. Dexamethasone control rats's postprandial glucose level increased significantly ($p < 0.05$) compared to normal control rats. Dexamethasone significantly increased blood glucose level at 90 and 120 min compared to normal control rats. Whereas this level was significantly reduced by AEHS (50 and 100 mg/kg) after 10 days of treatment. Metformin reduced significantly ($p < 0.05$, $p < 0.01$, $p < 0.001$) this increase of blood glucose level within 60, 90 and 120 min.

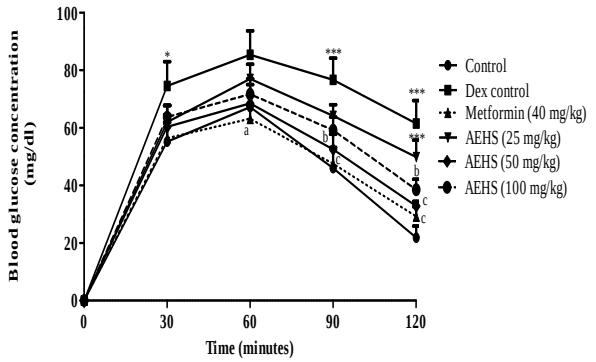

Figure 3: Effect of *H. stipulosa* (HS) on glucose tolerance test in dexamethasone treated rats.

AEHS 25: aqueous extract of HS at dose 25 mg/kg; AEHS 50: aqueous extract of HS at dose 50 mg/kg; AEHS 100: aqueous extract of HS at dose 100 mg/kg; Dex: dexamethasone. n = 6; data are presented as mean ± SEM. *$p < 0.05$, ***$p < 0.01$ compared to normal control group; $^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$ compared to dexamethasone control group.

Effect of AEHS on body weight

Figure 4 showed that, the body weight was significantly decreased ($p < 0.001$) in dexamethasone rats compared to normal rats. Concomitant administration of dexamethasone with AEHS prevented significantly ($p < 0.05$) the reduction of body weight induced by dexamethasone. Metformin also significantly prevented ($p < 0.01$) this body mass reduction.

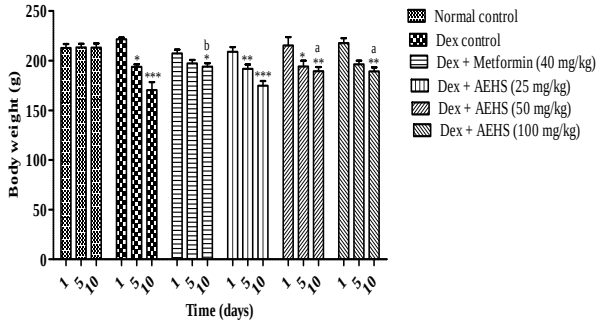

Figure 4: Effect of *H. stipulosa* (HS) on body weight in dexamethasone induces insulin resistance in rats.

AEHS 25: aqueous extract of HS at dose 25 mg/kg; AEHS 50: aqueous extract of HS at dose 50 mg/kg; AEHS 100: aqueous extract of HS at dose 100 mg/kg; Dex: dexamethasone. n = 6; data are presented as mean ± SEM. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared to normal control group; $^{a}p < 0.05$, $^{b}p < 0.01$ compared to dexamethasone control group.

Effect of AEHS on relative organ weight: There was no significant changes on relative weights of heart, pancreas and kidney in all dexamethasone treated groups of rats compared to normal control group. However, the relative liver weight significantly decreased in all these groups compared to normal control group notably in dexamethasone control group and group treated with AEHS at dose 25 mg/kg ($p < 0.001$). Metformin (40 mg/kg) and AEHS (50 mg/kg and 100 mg/kg significantly prevented ($p < 0.001$) this decrease caused by dexamethasone. AEHS at dose 25 mg/kg also significantly reduced ($p < 0.01$) this loss of relative liver weight (Figure 5).

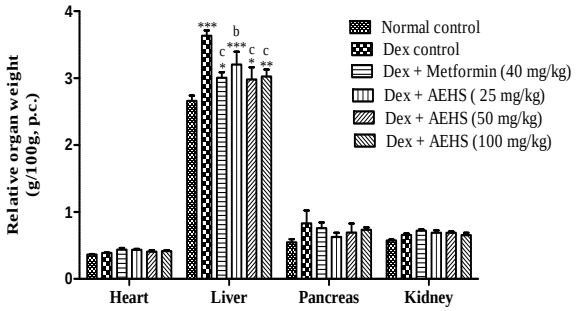

Figure 5: Effect of *H. stipulosa* (HS) on relative organ weight in dexamethasone induces insulin resistance in rats.

AEHS 25: aqueous extract of HS at dose 25 mg/kg; AEHS 50: aqueous extract of HS at dose 50 mg/kg; AEHS 100: aqueous extract of HS at dose 100 mg/kg; Dex: dexamethasone. n = 6; data are presented as mean ± SEM. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared to normal control group; $^{a}p < 0.05$, $^{c}p < 0.001$ compared to dexamethasone control group.

Effect of AEHS on lipid profile: The effect of plant on lipid profile is resumed in Table 2. This reveals that, there is a significant increase ($p < 0.001$) in the level of total cholesterol, triglycerides, LDL-c, TG/HDL-c ratio and significant decrease ($p < 0.001$) in the level of HDL-c in dexamethasone control rats compared to normal control rats. Oral administration of AEHS at doses 50 and 100 mg/kg significantly reduced the increase of TC, TG, LDL-c and TG/HDL-c caused by dexamethasone . At these same doses, the extract significantly increase the reduction of HDL-c induced by dexamethasone. The dose 25mg/kg significantly affected only LDL-c and TG/HDL-c ratio. In the group that received metformin, there were also a significant changes in all studied parameters.

Table 2: Effect of *H. stipulosa* (HS) on lipid profile level in dexamethasone induces insulin resistance in rats.

Biochemical parameters	Normal control	Dex control	Dex + Metformin (40 mg/kg)	Dex + AEHS (25 mg/kg)	Dex + AEHS (50 mg/kg)	Dex + AEHS (100 mg/kg)
Total cholesterol (mg/dl)	58.07 ± 1.946	104.8 ± 9.197***	63.75 ± 4.526c	79.57 ± 6.456	72.15 ± 5.231b	74.06 ± 5.788a
Triglycerides (mg/dl)	85.87 ± 1.282	129.9 ± 4.282***	91.62 ± 3.016c	116.5 ± 3.765***	99.01 ± 3.070c	109.01 ± 3.353***b
HDL cholesterol (mg/dl)	57.37 ± 1.443	36.05 ± 1.754***	48.85 ± 2.754b	40.91 ± 2.390***	47.33 ± 2.144*b	44.99 ± 0.7250**a
LDL cholesterol (mg/dl)	3.838 ± 1.204	85.65 ± 9.702***	19.38 ± 6.294c	53 ± 8.007***a	31.71 ± 3.598 *c	40.75 ± 5.985**c
TG/HDL-c	1.5 ±0.03251	3.652 ± 0.2274***	1.906 ± 0.1279c	2.898 ± 0.1857***b	2.117 ± 0.1277c	2.425 ± 0.06595c

AEHS 25: aqueous extract of HS at dose 25 mg/kg; AEHS 50: aqueous extract of HS at dose 50 mg/kg; AEHS 100: aqueous extract of HS at dose 100 mg/kg; Dex: dexamethasone n = 6; data are presented as mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001 compared to normal control group; [a]p <0.05, [b]p < 0.01, [c]p < 0.001 compared to dexamethasone control group.

DISCUSSION

Dexamethasone is a synthetic glucocorticoid that is commonly used in the treatment of numerous medical conditions due to its anti-inflammatory and immuno-suppressive properties.[22] However, chronic exposure to high levels of circulating exogenous and endogenous glucocorticoids is associated with several sides effects, including insulin resistance.[23]

Insulin resistance in human has been shown to be present in conditions like noninsulin-dependant diabetes mellitus (NIDDM), obesity and dyslipidemia. Thus, intervention to decrease insulin resistance may postpone the developpement of NIDDM and its complications.[24]

In this study, administration of dexamethasone at a dose 1 mg/kg continously for 10 days results in increase in serum glucose levels, TC, TG, LDL-c, ratio TG/HDL-c and decrease in HDL-c and body body weight in dexamethasone control groups rats. These results suggest that ther was induction of insulin resistance.

Reduction of body weight and increase in fasting blood glucose levels observed in dexamethasone group were inhibited in treated groups. It is well known that in the peripheral tissues, glucocorticoids diminish glucose utilization, increase protein breakdow and lipolysis, their by providing amino acids for gluconeogenesis.[25] Moreover, they cause insulin resistance by decreasing hepatic glucose utilization and decreasing glycogen synthesis.[26] It then results the decline of body weight and an increase in blood glucose levels. Thus, the effect of the AEHS on the body weight and serum glucose levels may be attributed to the increase in the sensitivity of peripheral tissues to insulin, and subsequently the increase in glucose utilization and stimulation of lipogenesis and protein synthesis; reducing therefore weight loss and serum glucose levels. The extract may also reduce blood glucose levels by increasing hepatic glucose utilization and reducing glycogen synthesis.

Dexamethasone also provoks the increase of relative liver weight. According to Kneeman et al.[27], corticosteroids contribute to the development of insulin resistance and hyperinsulinemia, leading to lipogenesis in liver. Thus, the increase in liver weight could be attributed to a rapid mobilization of fat under the glucocorticoids rendering more fatty acids available for the laying up in the form of triglycerides .[28] The inhibitory effect of the AEHS on the increase liver weight could be due to the increase effect of the extract on the sensitivity of adipose tissues which promotes lipogenesis and therefore decreases avaibility of free fatty acids; thus reducing lipogenesis in liver.

This study reveals that, treatment with AEHS significantly improved the altered lipid profile and reduced TG/HDL-c ratio. Glucocorticoids are well known to induce dyslipidemia which is likely mediated by an increase of plasma insulin levels, impaired lipid catabolism and an increase of lipid production in liver.[29] The alterations of lipid profile contribute to the development of coronary artery desease.[30,31] Besides, elevated TG/HDL-c ratio is associated with the presence of insulin resistance.[32,33] Moreover, Yuji et al.[34] showed that diabetes combined with high TG/HDL-c ratio constitute a risk for atherosclerosis. Thus, potential effect of the extract to improve different cholesterols and triglyeride levels could be helpful in the prevention of diabetic complications and in the traitment of insulin resistance.

As the insulin resistance is characterized by increase and prolongate in postprandial glucose, hence the tolerance test was performed in rats. Kumar et al.[35] mentioned that improvement of glucose tolerance by extracts indicates that, these extracts may show insulin mimetic activity or improve glucose utilization mechanism. AEHS significantly reduced the postprandial glucose in both normal and dexamethasone treated rats. This result therefore indicates that *H. stipulosa* may show insulin

mimetic activity, improves glucose utilization or may increase insulin sensivity.

Alpha-glucosidases are the enzymes involved in the digestion of carbohydrates. Inhibition of theses enzymes can significantly decrease the postprandial increase blood glucose after a mixed carbohydrate diet.[36] This study reveals that AEHS showed a significant α-glucosidase inhibitory effect. Fatima et al.[17] isolated ursolic acid from *H. stipulosa*. It has been recently shown that ursolic acid reduces at least 74 % the sodium-dependant glucose uptake of Caco-2 cells and do not have a significant effect on sodium-independent glucose uptake of these cells.[37] This suggests that α-glucosidase inhibitory effect of *H. stipulosa* could be associated with it inhibitory action on sodium glucose transporter (SGLT) in intestine.

CONCLUSION

In conclusion, our investigations clearly demonstrated that stem bark of *H. stipulosa* showed a remarkable antihyperglycemic and hypolipidemic activities on dexamethasone induce insulin resistance in rats. These effects can be due to the increase action of this plant extract on the insulin sensitivity of peripheral tissues. The study also revealed that antidiabetic effect of the plant can be also attributed to it inhibitory effetcs on α-glucosidase enzyme.

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