ANTIDIABETIC ACTIVITY OF ALGERIAN *HYOSCYAMUS ALBUS'S* ON STREPTOZOTOCIN DIABETIC RATS

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Abstract

The aim of the present work is to investigate *in-vivo* the antidiabetic activity of the methanol extract of *Hyoscyamus albus* (HAMeOH) on male Wistar rats. A comparison was made between the action of methanolic extract of *H.albus* and a known antidiabetic drug metformin (500 mg/kg b.w.). The methanolic extract of *H.albus* was obtained by simple maceration and was subjected to phytochemical sreening. Selection of doses 100 and 200 mg / kg b.w. was made on the basis of an acute oral toxicity study organization of economic development cooperation 420 [OECD, 2001] which limits the dose to 2000 mg / Kg b.w.It is clear that our extract significantly decreased the glucose level ($P \leq 0.05$) after 30, 90 and 120 min of glucose overload compared to diabetic control, which means that the HAMeOH extract improved the postprandial glucose level and returned it to its normal value.The antidiabetic activity of extract (HAMeOH) against the diabetes millitus (DM) induced by Streptozotocine (STZ), followed by the oral administration of two doses 100 and 200 mg/Kg b.w of HAMeOH into diabetic rats during 30 days. Our extract significantly decreased ($P \leq 0.0001$) the glucose level and the glycated hemoglobine (HbA1C) level compared to the diabetic control (2,10 ± 0,02 g /L) and (7,73 ± 0,15 %) respectively and also reduced in a significant way the level of lipidic parameters and serum lipid parmeters , and renal function biomarkers and stimulated the secretion of insulin and the rise in the hepatic glycogen content.

Keywords: *Hyoscyamus   albus,   Diabets   millitus,   Insulin,   Wistar   rats*

Introduction

Diabetes mellitus (DM) is the result of low insulin sensitivity and pancreatic beta cell dysfunction. For a few decades, type 2 has become a global health problem . Diabetes millitus begins with a period of insulin resistance yet there is an increase in pancreatic insulin secretion. Thereafter the disease progresses, the pancreatic functions diminish and can no longer meet the peripheral requirements [17]. Currently they are conducting preclinical tests on experimental animal models (DM) that have been useful in understanding the complex pathogenesis of DM. The STZ substance when injected into rats induces diabetes. *H.albus* is a plant which belongs for Solanaceae family, it is used in traditional medicine as a nervous sedative and para sympatholytic [33]. They could isolate some tropane alkaloids such as scopolamine, hyoscyamine, atropine and also with spectral techniques they isolated 2,3 – dimethyl nonacosane. The actual work is toevaluate analgesic and antipyretic activities of the methanol extract of *H.albus* (HAMeOH) as well as acute and subacute toxicity.

Materials and Methods

Chemicals glucose, Acetic acid puriss glacial (SigmaAldrich), Indomethacin and Streptozotocin (Sigma-Aldrich), were used in the present study.
Collection of plant material
The leaves of *H.albus* were collected from their natural habitat around Bouzina, Batna on 2014. This plant was identified by Dr.OUDJHIH, Laboratory of Botanic, Department of Agronomy, University BATNA 1, ALGERIA. The leaves were dried under shade for 40 days at Room temperature, dried leaves parts were blended into fine powder and stored in the dark at a dry place.
Preparation of plant extract
1 Kg of powdered leaves was extracted with petroleum ether three times 5 L for each time. Then, the marc was dried and extracted with chloroform three times 5 L for each time and with methanol three times 5 L for each time and the supernatants were filtered sequentially using cloth filter, cotton wool, and Whatman filter paper. The solvents were then evaporated under reduced pressure (204 mbar) and controlled temperature (30 ∘C) using a vacuum rotary evaporator (Buchi Rotavapor).
Phytochemical screening
The phytochemical screening of HAMeOH was performed using standard method [26]. Phytochemical constituents such as phenolic compounds, terpenoids, saponins, alkaloids, steroids and tannins were qualitatively analyzed.

Animals
Male Wistar albino rats for (140-170 g) procured for Research institute were housed in separately in plastic cage at temperature of (23±2) °C and 50-55 % relative humidity ,with a 12 light / dark cycle respectively before and during the experiment.Animals were allowed the access to standard pellet diet and water ad libitum.
Study of antidiabetic activity
Study of glucose tolerance
Young male rats were divided into six groups of 6 rats and received the following oral treatments: Group I: received distilled water and serves as a control. Group II and III: received the HAMeOH extract (100 and 200 mg / Kg b.w.) respectively..These treatments were given with a volume of 10 ml / Kg b.w.After 30 min of administration of the extracts, the rats were orally administered a solution of glucose (2 g / Kg b.w.) (20 ml / Kg b.w.) except for the control group. A drop of blood is obtained from the tail vein just before glucose administration and after 30 min, 60 min and 90 min after administration of glucose. The glucose level is measured using blood glucose test strips using an ACCU -CHEK Active Glucometer [42].
Streptozotocin-induced diabetes study
Male rats were divided into six groups of 8 rats, and were fasted 24 hours before the experiment and then diabetes was induced by a single intraperitoneal injection of the streptozotocin solution (55 mg / kg b.w.) in 0.1 mol / L of citrate Buffer (PH = 4.5) with a volume of (1ml / Kgb.w.) [15].After 72 h, blood glucose was measured in all rats, only rats whose blood glucose ≥190 mg / L were selected and considered to be diabetic. These diabetic animals were divided and received the following treatments orally for 30 days as follows: Group I: received distilled water and serves as reference, Group II: control, received the citrate buffer at 0.1 M

at pH = 4.5,Group III: received the HAMeOH extract (100 mg / Kg b.w.),Group IV: received the HAMeOH extract (200 mg / Kg b.w.), Group : received metformin (500 mg / Kg b.w.) as the oral reference standard, Group VI: received insulin (100 IU / ml) (5 ml / Kg b.w.) intravenously via the tail as a reference standard.We measured the body weight of the animals each week.

Determination of biochemical parameters
After 30 days of treatment, rats were fasted and blood was collected from the ocular sinus after anesthesia with chloroform in heparinized tubes to assay glucose, urea, creatinine, PAL, lactate dehydrogenase (LDH), Cholesterol, Triglyceride (TG) and insulin, while for the assay of hemoglobine (HGB) and (HbA1C) were dosed on EDTA tubes.
Urine is collected in metabolic cages to detect the presence of glucose and ketones using specific strips.

Dosage of Glycogen
In order to determine the level of glycogen, the liver is cut into small pieces and then 5 g of liver are boiled in 50 ml of distilled water for 2 min then crushed. To this broth, we added 25 ml of distilled water and the mixture was boiled for 5 min. After a first filtration, 3 ml of Hcl are added to the filtrate and then a second filtration. The 95% ethanol (4 times volume of filtrate) was added to the filtrate and then a third filtration, the filtrate was mixed with 2 ml of distilled water. After, a drop of lugole was added to 3 ml of distilled water and then this mixture was added to the filtrate and the reading was made by spectrophotometry at 470 nm.The glycogen level is calculated from the calibration line established with precise concentrations of pure glycogen (0-4500 µg / ml) [7].

Results
Phytochemical Screening Phytochemical screening of HAMeOH demonstrated the presence of alkaloids, saponins, condensed tannins, and steroids. In addition, the presence of high content of total phenolic compounds in the HAMeOH, we demonstrated the presence of flavonoids (table 1).
For flavonoids, tannins, triterpenes, and steroids , +: weak colour; ++: mild colour; +++: strong colour for akalioids , +:

negligible amount of precipitate; ++: weak precipitate; ++strong precipitate.
Diabetes is a chronic metabolic disorder of carbohydrates, proteins and fats due to the absolute or relative deficiency of insulin secretion with or without insulin resistance. It is characterized by chronic hyperglycaemia which could lead to morbidity and mortality [31, 33].The glucose level in diabetic rats and other groups after administration of glucose is shown in Figure 01.

The glucose level in the control group (diabetic group) reached a maximum level of 3.02 g / L after 30 min of glucose overload and this value was significantly higher compared to the control group (P≤0.0001). It is clear that our extract significantly decreased the glucose level (P≤0.05) after 30, 90 and 120 min of glucose overload compared to diabetic control, which means that the HAMeOH extract improved the postprandial glucose level and returned it to its normal value.

[15] suggested that the improvement in post-prandial blood glucose is due to the use of glucose by the peripheral tissues resulting in increased glucose tolerance. Carbohydrate digestion and glucose absorption are the best control of blood glucose after carbohydrate meals. The enzymes α-amylase and α-glucosidase are the key enzymes responsible for the digestion of dietary carbohydrates into glucose. The released glucose is absorbed by intestinal enterocytes via specific carriers. Thus, inhibition of digestive enzymes or glucose transporters by polyphenols would reduce the rate of glucose release and absorption in the small intestine and hence the inhibition of postprandial hyperglycemia [12]. They have shown that phenolic compounds are also able to regulate postprandial glucose and promote glucose tolerance by facility of insulin response and attenuation of glucose-dependent insulinotropic peptide (GIP) secretion and glucagon -like peptide-1 (GLP-1) [12].

After 72 hours of STZ injection, 9 animals were found dead and we selected only those animals with a blood glucose level of ≥190 mg / L [2] because there are rats that did not trigger diabetes. The body weight of the diabetic control showed a fall in body weight in a proportional manner. On the other hand, the control group, the groups treated with the HAMeOH, metformin and insulin extracts

showed an increase in weight (P≤0.05) starting from the 10th day of treatment until the end of the month (Fig.02).

Several STZ-induced diabetes studies have shown that the latter causes body weight loss due to increased muscle wasting [45] through the use of tissue proteins [5].

The increase in body weight in diabetic rats treated with extract compared to untreated diabetic rats may be due to its protective effect and the fight against muscle wasting, ie the reversal of gluconeogenesis and Glycogenolysis [28].

Our extract significantly decreased (P≤0.0001) the glucose level and the HbA1C level compared to the diabetic control but did not return the values to normal.The STZ is an analogue of nitrosourea, which the N-methyl-N-nitrosourea (MNU) group is attached to the carbon-2 of a hexose. STZ is known for its selective toxicity to β-pancreatic cells because it is selectively accumulated in pancreatic β- cells by inducing low glucose affinity for the GLUT2-type transporter [19]. But the insulin-producing cells that do not express this glucose transporter are resistant to STZ [27].It has been reported that STZ is bound by β-cells through the GLUT-2 glucose transporter and causes the alkylation and methylation of DNA and acts as nitric oxide [8]. In particular, β-cells are sensitive to damage caused by nitric oxide and free radicals due to their low levels of free radical scavenging enzymes [9]. Thus it causes the reduction of adenosine triphosphate and NAD + of the cell [14].

The transfer of the methyl group from STZ to the DNA molecule causes damage [37] among the DNA fragmentation [47] again, glycosylation of proteins [22] although STZ also methylates proteins [1]. DNA methylation is ultimately responsible for the death of β-cells, but protein methylation is likely to contribute to β-cell function defects after exposure to STZ [29].The mechanisms by which oxidative stress is involved in diabetic complications are partially known, including activation of transcription factors, advanced glycation end-products (AGEs) and activation of protein kinase C [23]. STZ-induced diabetes induced anemia in diabetic rats but treatment with HAMeOH extract with both concentrations increased the hemoglobin in a highly significant manner (P≤0.0001) (Table 02), this can be explained

ability of the HAMeOH to decrease the glucose level to a normal level is an essential effect for the liver to return to its normal homeostasis during diabetes.

Metformin and insulin, which are used as reference standards again, significantly decreased (P≤0.0001) glucose level and HbA1C compared to diabetic control (Table 02). And there is no significant difference (P> 0.05) between the effect of extract and the effect of insulin and metformin on glucose and HbA1C levels.

The values expressed as mean ± S.D. (N = 6). The results are compared with the control * P≤0.05, ** P≤0.001, *** P≤0.0001. +++ (300mg / dl), ++ (100mg / dl), + (50mg / dl).

that the extract has the ability to detach glucose from the hemoglobin.

HbA1c values increased proportionally with increasing blood glucose levels at younger ages. Therefore, the HbA1c measurement is a very sensitive index for glycemic control [34] (Table 02).The observed increase in the level of HbA1C in the diabetic group (control) is due to the presence of excessive amounts of glucose in the blood and the excess glucose combining with hemoglobin and the low levels of total hemoglobin observed in diabetic rats may be due to the increased formation of HbA1c [43].

In addition, the antihyperglycaemic activity of HAMeOH extract was associated with a significant increase in plasma insulin level (P≤0.0001) compared with diabetic control, which showed a low level of insulin Insulin, we have suggested that the extract have insulinogenic activity by stimulating insulin secretion from the rest of the β-cells [6].The possible mechanism by which extract provide their antihyperglycaemic action may be through the induction of pancreatic insulin secretion from islet cells of Langerhans or through increased transport of blood glucose to peripheral tissues [34].After STZ was administered at a single dose of (55 mg / kg b.w.), the level of urine in the diabetic control was grade +++ (300 mg / dl) and that of ketone bodies (+++) .The extract HAMeOH (100 mg / kg b.w.) was able to reduce the level of urine sugar to a grade of ++ (100 mg / dl) and ketone bodies (++),. Also, treatment with metformin resulted in urine glucose levels of + 50 mg / (+) But the insulin-treated group did not show either glucose or

ketones in the urine. The results are summarized in Table 03. It has been shown that most of the most common lipid abnormalities in diabetes are hypertriglyceridaemia and hypercholesterolemia [20].The potential for lipid reduction is significantly beneficial for people with diabetes and cardiovascular disease.These changes may be beneficial in preventing diabetes complications and improving lipid metabolism in diabetics [11].Reduction of serum lipid levels with dietary therapy or a drug appears to be associated with decreased risk of vascular disease in humans.It has also been found that flavonoids, phenolic acids and tannins inhibit α-amylase and β-glucosidase enzymes, which are key enzymes in carbohydrate metabolism [12].Polyphenols can lower the level of glucose in the blood with different mechanisms, including inhibition of carbohydrate digestion and inhibition of glucose uptake by the intestine, stimulate insulin secretion by the cells β of the pancreas, modulation of glucose release by the liver, absorption of glucose from insulin-sensitive tissues, and modulation of intracellular signaling pathways [12].Diabetes caused a significant increase in PAL and LDH activity (P≤0.0001) compared to the reference group, but treatment with HAMeOH (100 and 200 mg / kg b.w.), metformin and insulin lowered their activities significantly (P≤0.0001).Still a decrease in glycogen levels was observed in the significantly untreated diabetic batch (P≤0.0001) compared to the control batch, but treatment with reference standards and extracts increased glycogen levels (P≤0.0001) and the increase in LDH activity in diabetes millitus.From the results obtained, lots of rats treated with the extracts HAMeOH and metformin and insulin showed a highly significant increase (P≤0.0001) in the level of glycogen (Table 04).

Low glycogen content in skeletal muscle and live ris due to decreased glycogen synthtase [10,44] and increased glycogen phosphorylase activity [40].It was suggested that diabetic rats treated with HAMeOH metformin, and the insulin could restore the level of hepatic glycogen by decreasing glycogen phosphorylase activity and increasing of glycogen synthase activity [4].Our extracts are rich in polyphenols, terpenoids and phenolic acids which are

known for their antidiabetic effect [39, 30].Some polyphenols, including catechins and epicatechins, chlorogenic acids, ferulic acid, caffeic acid and tannic acid, quercetin and naringenin, may alter glucose uptake by entherocytes by inhibition of glucose carriers (Sodium glucose transporter-1) and SGLT2 (sodium glucose transporter-2) [21,18]. *In vitro* studies have shown that certain phenolic compounds such as quercetin and resveratrol improve insulin-dependent glucose uptake by muscle cells and adipocytes by the translocation of the glucose transporter GLUT4 (Glucose transporter type 4) to the plasma membrane primarily through the induction of the AMP-activating protein kinase (AMPK) [35,49].It has been found that some polyphenols are also capable of inducing phosphatidylinositide 3-kinase (PI3k) as the primary signaling pathway for regulation for glucose uptake [24].

Conclusion

It can be concluded that traditional use of *Hyoscyamus albus* as a hypoglycaemic agent have been shoowed by the extracts from the leaves of this plant which show a significant activity and which is comparable to the standard hypoglycemic drug metformin and insulin and that's gives more importance to hyoscymaus albus

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| Sample	Phytochemical constituents	Result
HAMeOH	Alkaloid	+++
	Saponin	+++
	Flavonoid	++
	Tannins and polyphenolic compounds	++
	Terpenoids	+++
	Steroid	++

Table 1: Phytochemical constituents of methanolic extract from *H.albus*'s leaves.
For saponins , +: 1-2 cm froth; ++: 2-3 cm froth; +++ : >3 cm froth.
For flavonoids, tannins, triterpenes, and steroids , +: weak colour; ++: mild colour; +++:
strong colour for akalioids , +: negligible amount of precipitate; ++: weak precipitate; +++:
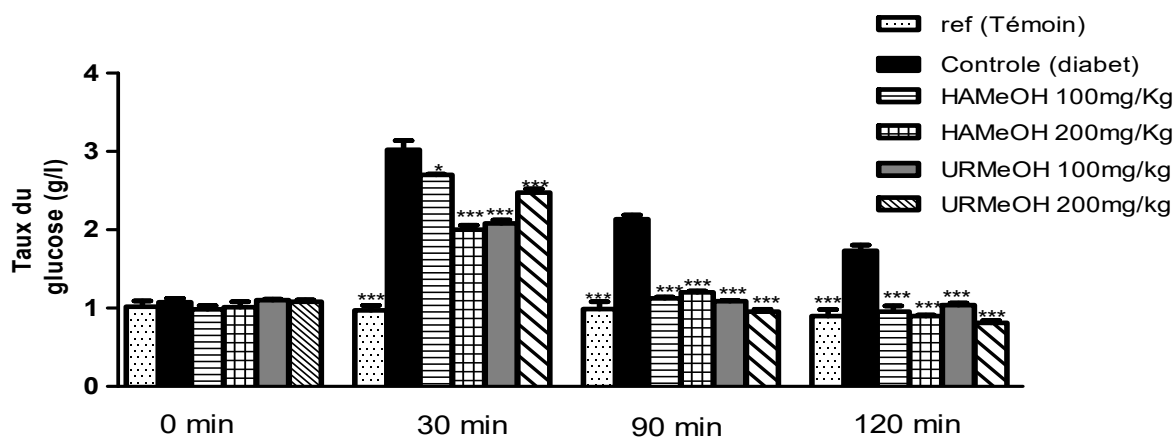
Figure 01. Glucose tolerance for the control group and other groups. Values expressed on average ± S.D. (N = 6). The results are compared with the control * P≤0.05, ** P≤0.001, *** P≤0.0001.
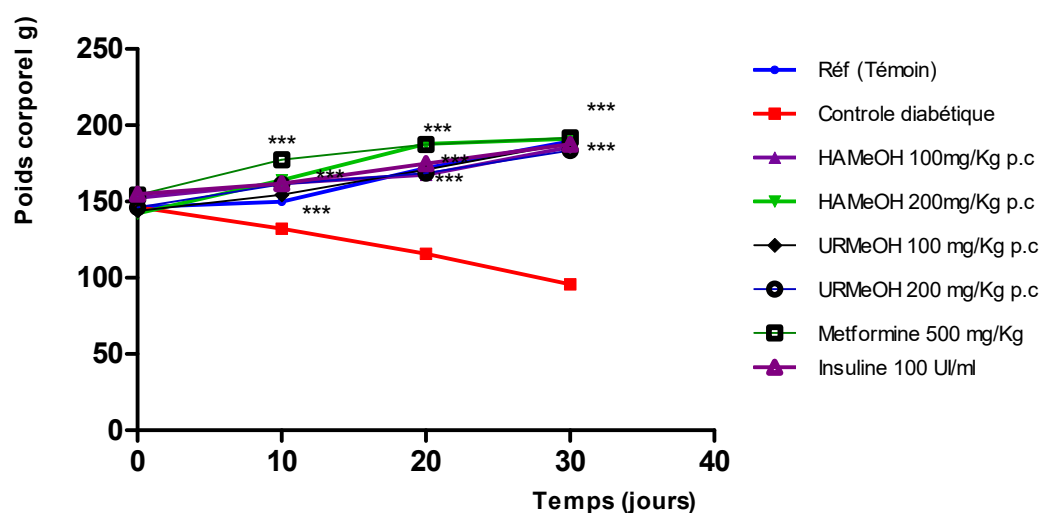
Figure 02. Changes in body weight of different groups over 30 days. Values are mean ± S.D. (n = 6). The results are compared with the control * P≤0.05, ** P≤0.001, *** P≤0.0001.

Table 02. Glucose, insulin, hemoglobin and HbA1C levels and presence of glucose and ketones in urine.

Groupes	Glucose (g/l)	Insulin (µU/ml)	HGB (g/dl)	HbA$_1$C (%)	Glucose urine	Ketones
Ref (distilled water)	0.89±0.08****	19,93 ± 1,99***	12,80 ± 0,89***	4,66 ± 0,72***	-	-
Control	3,46 ± 0,31	2,13 ± 0,17	8,64 ± 0,45	11,52 ± 1,61	+++	+++
HAMeOH 100 mg/Kg b.w.	3,20 ± 0,04***	3,16 ± 2,67***	11,87 ± 0,06***	9,04 ± 0,21***	++	++
HAMeOH 200 mg/Kg b.w.	2,10 ± 0,02***	3,51 ± 2,10***	12,57 ± 0,39***	7,73 ± 0,15***	++	+
Metformin (500mg/Kg b.w.)	1.60± 0,05***	4,35 ± 0,32***	12,88 ± 0,20***	7,13 ± 0,20***	+	+
Insulin (100UI/ml)	0,95 ± 0,05***	20,35 ± 0,32***	12,88 ± 0,20***	5,13 ± 0,20***	-	-

Table 03. Urea, creatinine, cholesterol and tg parameter in urine.

Groupes	Urea (g/l)	Creatinine (mg/l)	Cholesterol (g/l)	TG (g/l)
Ref (distilled water)	0,39±0.0650***	7,55 ± 1.74***	1,49 ±0.25***	1,15±0.037***
Diabetic control	0,7± 0,045	18,82 ± 0,22	2,27± 0,02	1,86 ± 0,05
HAMeOH (100 mg/Kg b.w.)	0,26 ± 0,04***	10,40 ± 0,57***	1,740 ± 0,05***	1,067 ± 0,15***
HAMeOH (200mg/Kg b.w.)	0,45 ± 0,07***	11,58 ± 0,35***	1,49 ± 0,06***	1,35 ± 0,05***
Metformin (500 mg/Kg b.w.)	0,40 ± 0,02***	12,59 ± 0,49***	1,25 ± 0,14***	0,91 ± 0,05***
Insulin (100 UI /ml)	0,49 ± 0,30***	14,59 ± 0,79***	1.23 ± 0,21***	0,74 ± 0,09***

Table 04. Rate of LDH, plasma PAL and the hepatic glycogen level after 30 days of treatment.

Groupes	Glycogen (mg of glucose/g wet tissue)	PAL (UI/L)	LDH (UI/L)
Distilled water (Ref)	69,48 ± 3,03***	81.38± 6.55***	191.8 ±1.552***
Diabetic control	21,49 ± 2,12	284,4 ±12,48	370,0 ± 27,84
HAMeOH (100 mg/Kg b.w.)	59,54 ±1,07***	127,5 ± 15,15***	209,5 ±4,875***
HAMeOH (200 mg/Kg b.w.)	57,15 ± 0,28***	75,81 ± 6,47***	217,1±3,561***
Metformin (500 mg/Kg p.c)	48,48 ± 1,43***	94,09 ± 5,20***	214,1 ± 9,459***
Insulin (100 UI/ml)	66,56 ± 1,43***	81,81± 4,34***	188,54 ± 5,15***

Values on average ± S.D. (N = 6). The results are compared with the diabetic control * P≤0.05, ** P≤0.001, *** P≤0.0001.