Effect of Artemisia herb on induced hyperglycemia in wistar rats

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(Received 4 May 2016, Accepted 21 June 2016)

Abstract

The study was carried out to evaluate the hypoglycemic effect of Artemisia herb aqueous extract on postprandial blood glucose in normoglycemic and dexamethasone-induced hyperglycemic wistar rats. Sixty adult male wistar rats were used; rats were assigned to six equal groups (10 per each). Hyperglycemia had been induced in 40 rats by injection of single dose of dexamethasone (8 mg/2 ml, s.c.). Normal groups drenched the herb extract orally and with drinking water in 100%, and 50% concentration (NA100%, NA50%) respectively. After induction and ensuring of hyperglycemia, distil water (DW), and the (PIA100%, PIA50%) treated groups with herb extract 100%, 50% concentration respectively, finally the (M) group (metformin 500mg/ml) was treated by drenched with metformin 500 mg orally for 10 consecutive days for all group. Results were revealed that drenching of Artemisia herba- alba herb 100% aqueous extract has a highly significant ($p<0.01$) hypoglycemic effect on postprandial blood glucose in both normoglycemic and hyperglycemic rats comparing to normal values and to metformin respectively, whereas hypoglycaemic effect of Ah extract 50% was significant ($p<0.05$) on postprandial blood glucose in normoglycemic rats comparing to normal values and highly significant ($p<0.01$) in hyperglycemic rats comparing to metformin. It can be concluded that drenching of aqueous extract of Ah has potent hypoglycemic effect in experimentally induced hyperglycemic rats.
Key words: Artemisia, dexamethasone, blood glucose, hyperglycemia, rats.

تأثير عشبة الشيح على ارتفاع سكر الدم المستحدث في جرذان الوستر

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الخلاصة

اجريت الدراسة من أجل تقييم فعالية المستخلص المائي لعشبة (الشيح Artemisia) في خفض مستوى سكر الدم بعد الاكل في الجرذان الطبيعية السكر و المستحدث فيها داء السكري تجريبيا" باستخدام الديكساميثازون. تم استخدام 60 جرذا ذكرا" ناضجا من نوع الوستر ، تم توزيع الجرذان على ستة مجموعات متساوية. استحدثت حالة ارتفاع السكر في الدم تجريبياً في 40 جرذاً باستخدام حقنة مفردة من عقار (ديكساميثازون) 8 ملغم / 2مل تحت الجلد). ضمت المجاميع الطبيعية (NA50%, NA100%) حيوانات سليمة وجرعت مستخلص العشبة بتركيز 100% و50% على التوالي بماء الشرب والفم ، بعد استحداث حالة ارتفاع سكر الدم والتأكد منها ، اعتبرت المجموعة (DW) مجموعة الماء المقطر، في حين اعتبرت المجاميع (PIA50% PIA100%) مجاميع المعالجة بالمركز100% و50% من المستخلص المائي للعشبة Ah على التوالي ، واخيرا" عدت مجموعة (M) مجموعة معالجة بعلاج الميتفورمين 500ملغم/ مل بالتجريع بالفم ولمدة 10 ايام متتالية ولجميع المجاميع. كشفت نتائج الدراسة الحالية ان تجريع المستخلص المائي للعشبة Ah بتركيز 100% له تاثير خافض لسكر الدم بعد الاكل عند مستوى معنوي عالي ($p<0.01$) في كلا ذكور الجرذان السليمة والمصابة بحالة ارتفاع سكر الدم المستحدث بالمقارنة مع القيم الطبيعية والميتفورمين على التوالي. بينما كان للمستخلص المائي للعشبة Ah بتركيز50 % تاثير خافض لسكر الدم بعد الاكل تحت مستوى معنوي ($p<0.05$) في الحيوانات السليمة مقارنة مع القيم الطبيعية وتحت مستوى معنوي عالي ($p<0.01$) في الحيوانات المصابة بالسكر بالمقارنة مع الميتفورمين. يستنتج ان تجريع المستخلص المائي لعشبة Ah له تاثير خافض لسكر الدم قوي في الجرذان المستحدث فيها ارتفاع سكر الدم تجريبيا.
الكلمات المفتاحية: الشيح ، الديكساميثازون ، سكر الدم ، ارتفاع السكر في الدم ، الجرذان.

Introduction

Hyperglycemia is a state characterized by a rapid rise in blood glucose levels, which, is due primarily to increase hydrolysis of starch by pancreatic α-amylase and α-glucosidases leading to enhance absorption of glucose in the small intestine. One of the therapeutic methods for decreasing postprandial hyperglycemia is thus to retard absorption of glucose by the inhibition of carbohydrate hydrolyzing enzymes, mainly α-amylase and α-glucosidase in the digestive organs (1). For hyperglycemia to induce cellular damage, plasma glucose must first be transported across the cell membrane via facilitative glucose transporters. In skeletal muscle and adipose tissue, the rate of this process is drastically increased in the presence of insulin; however, most cell types do not require insulin for glucose uptake. When faced with a hyperglycemic environment the majority of cells decrease the rate of intracellular glucose transport in order to maintain a relatively constant intracellular glucose level. In contrast; certain cell types cannot effectively regulate this process and are more vulnerable to elevate plasma glucose levels (2). Biological actions of the herb products used as alternative medicines to treat diabetes are associated to their chemical composition. Plant products are rich in phenolic compounds, flavonoids, terpenoids, coumarins, and other constituents which show reduction in blood glucose levels (3, 4, 5). Due to their observed effectiveness, fewer side effects in clinical experience and relatively low costs, herbal drugs are prescribed (6). Artemisia herba-alba was known for its therapeutic and medicinal properties. It was used in both traditional and modern medicine (7). Ah is a family of composite and includes compounds such as flavonoids, sesquiterpenes and antioxidants with antidiabetic, analgesic and phytoestrogenic properties. Other studies have been conducted about antioxidants to date and their positive effects have been proven, especially, because the beneficial effects of Ah in reducing diabetes complications in diabetic individuals and physiological changes have been proven (8). The study aims to explore the hypoglycemic effect of Artemisia herba- alba, to find safer treatment to hyperglycemia with less adverse effect.

Materials and methods

Animals and housing

Sixty mature male wistar rats (aged 90 days) with an average weight of 138±8.8g were used. Male rats were allowed to acclimatize to the animal house environment (in the animal house of department of physiology and pharmacology at the College of Veterinary Medicine-University of Al-Qadisiyah) before beginning of the experiment. Animals were housed in polypropylene six cages inside a well-ventilated room for the period from 15th of September 2015 to the 15th of February 2016. Each cage contained of ten rats. Male rats were fed on the standard chow and drinking water *ad libitum* throughout the experiment. Room temperature was maintained at 23±2ºC; the light-dark cycle was on a 12 hrs. light/dark cycle.

Experimental design

The groups of rats were randomly divided into six equal groups.

G1: Normal with Artemisia herba alba 100% aqueous extract group (NA 100%) normal receive 100% of Ah aqueous extract.

G2: Normal with Artemisia herba alba 50% aqueous extract group (NA 50%) normal receive 50% of Ah aqueous extract. The administration of the Ah aqueous extract was drenched by gavage daily for 10 days.

G3: Distilled water group (DW) negative group, the animals of this group were submitted to the induction of hyperglycemia and drenched distilled water only.

G4: Post induction with Ah aqueous extract 100% group (PIA 100%) (Receive 100% of Ah aqueous extract concentration).

G5: Post induction with Ah aqueous extract 50% group (PIA 50%) (Receive 50% of Ah aqueous extract concentration). The oral administration of Ah aqueous extract was begun immediately after induction and ensuring of hyperglycemia. The administration of the Ah aqueous extract was drenched by gavage daily for 10 days.

G6: Metformin group (M). The rats in this group had been drenched immediately with metformin (500 mg/ ml, p.o.) for 10 days after induction and ensuring of hyperglycemia had been established.

Induction of hyperglycemia

Fourty rats were prepared for induction of hyperglycemia, the rats were anesthetized by using chloroform. Hyperglycemia induced by a single subcutaneous injection of dexamethasone at a dose of 8mg/2ml (9). Hyperglycemic rats were confirmed by the elevated glucose levels in plasma after 48 hours from injection by using glucometer. Rats with blood glucose concentration more than 200 mg/dl were considered as hyperglycemic.

Blood glucose measurement

The quantitative determination of glucose was done using the glucometer; the blood glucose test-method was used directly with the blood glucose meter Accu-Chek Active®

Plant collection

Artemisia herba alba plant were recognized and collected from the Al-Qadisiyah province. The plant was left under sunlight for one week after cleaned. The plant was chopped after complete drying then grinded using an electrical grinder until herbal powder was obtained**.**

Artemisia herba alba aqueous extraction

The dried Ah powder of about 50g was subjected to hot solvent extraction in a soxhlet extractor by packing the Ah material in a Whitman filter paper. The packing was made wet by polar solvent distilled water as the extractor flask was filled with about 500 ml of distilled water and temperature was set below the boiling point of distilled water (80ºC). The distilled water extractor collected in a round bottom flask was evaporated in a rotary evaporator. The system was set and the heating started. After about 72 hours, Ah extract was obtained. The herb extraction was done in a soxhlet extractor of physiology laboratory of Veterinary College of Al-Qadisiyah University.

Statistical Analysis

The obtained quantitative data were presented as (Mean ± SE) in graphic presentations and tables. Student t-test was used for assessing the effectiveness of employed therapy for the rats in a given group. The differences were accepted as significant if the calculated value for (t) was equal or greater than its tabulated value at (0.05) level of (P) (i.e. $0.01 \leq P < 0.05$) and highly significant if ($P<0.01$). While it considered non-significant if ($P>0.05$) (10).

Results

Post prandial blood glucose (PPBG)

1. Normal with Artemisia herba- alba aqueous extract 100% group (NA 100%)

The normal post prandial blood glucose in 10 included rats, prior the administration of Ah 100% aqueous extract was 135.4± 0.3 mg/dl. After administration of Ah 100% extract, the PPBG decreased to reach to 123.2±0.4 mg/dl at 5th day, and there was significant difference comparing to prior the administration ($P<0.05$), and the continuation with administration of extract, could highly significant decrease in PPBG was occurred ($P<0.01$) Fig.(1).

2. Normal with Artemisia herba alba extract 50% group (NA 50%)

After administration of Ah 50% extract, the normal PPBG decreased to reach to 126.4 ±0.4 mg/dl at 5th day, and there was significant difference comparing to prior the administration ($P<0.05$), the decreasing was persisted for further 7th and 9th day after administration (Fig. 2).

3. Distilled water group (DW) negative group

After induction of hyperglycemia, and with starting of DW administration; the normal PPBG increased to reach 242.5±0.2 mg/dl, and there was a highly significant difference ($P<0.01$) comparing to normal level prior the induction, these elevation of PPBG persisted for further days of trial period (Fig. 3).

4. Metformin group (M)

After treatment of metformin to the rats, metformin caused a significant decrease ($P<0.05$) in PPBG (210.1±0.2 mg/dl) at 1st day post induction. While at 3rd day caused a highly significant decrease ($P<0.01$) in PPBG (195.2±0.3 mg/dl), such reduction persisted

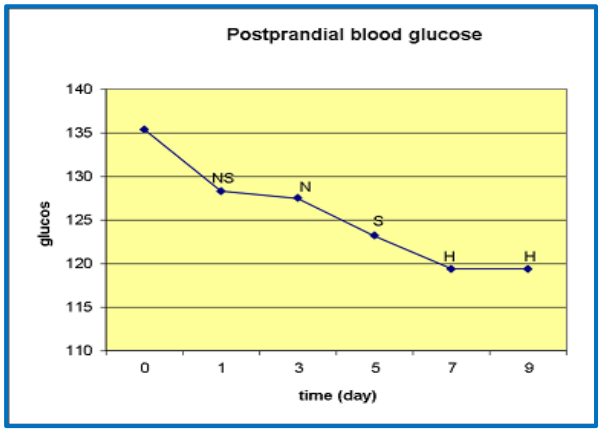

Fig. (1): Effect of Ah 100% extract on PPBG in normal rats. (NS= non-significant (P>0.05), S= significant (P<0.05), HS= high significant difference (P<0.01)

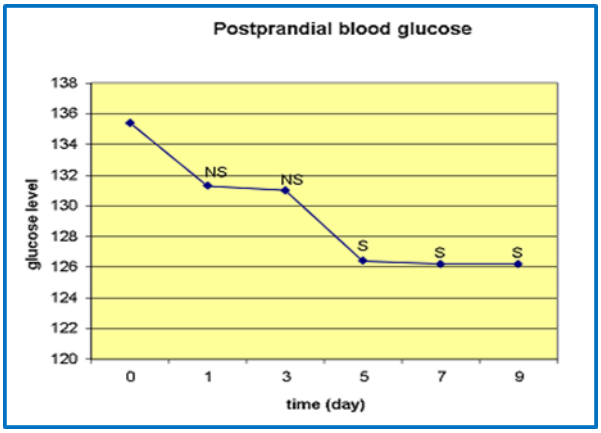

Fig. (2): Effect of Ah 50% extract on PPBG in normal rats. (NS= non-significant difference (P>0.05), S= significant difference (P<0.05).

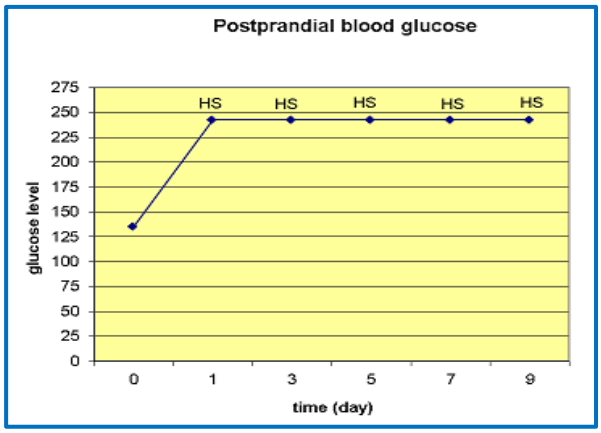

Fig. (3): Effect of DW on PPBG in induced hyperglycemic rats

HS= high significant difference (P<0.01).

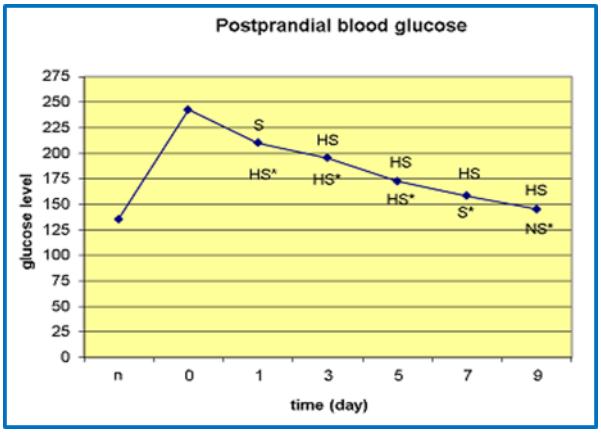

Fig. (4): Effect of metformin on PPBG in induced hyperglycemic rats. (n=normal PPBG prior the induction of hyperglycemia, 0=time of induction of hyperglycemia, NS=non-signifi-cant (P>0.05), S=significant (P<0.05), HS= high signify-cant (P<0.01),*=comparing of PPBG post induction of hyperglycemia to normal level prior the induction).

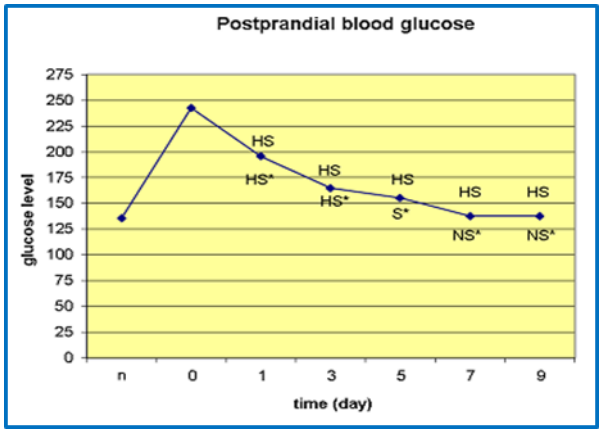

Fig. (5): Effect of Ah 100% extract on PPBG in induced hyperglycemic rat

n= normal PPBG prior the induction of hyperglycemia, 0=time of induction of hyperglycemia, NS= non-significant difference (P>0.05), S=significant difference (P<0.05), HS= high significant difference (P<0.01),* =comparing of PPBG post induction of hyperglycemia to normal level prior the induction.

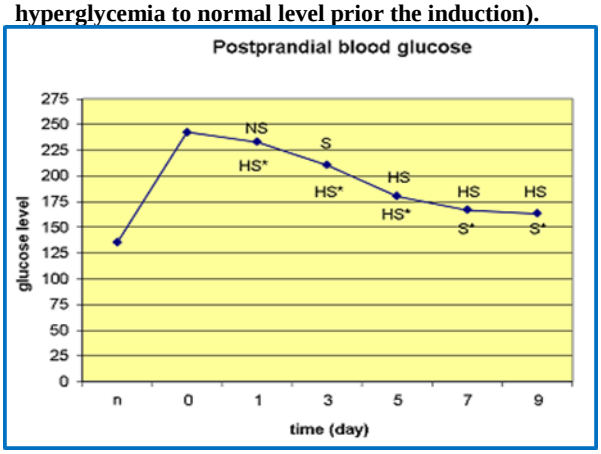

Fig. (6): Effect of Ah 50% extract on PPBG in induced hyperglycemic rats.

n=normal PPBG prior the induction of hyperglycemia. 0=time of induction of hyperglycemia, NS=non-significant difference (P>0.05), S=significant difference (P<0.05), HS=high significant difference (P<0.01),*=comparing of PPBG post induction of hyperglycemia to normal level prior the induction.

Table (1): Significance of differences between Ah 100% and each of metformin and DW groups regarding the reduction of PPBG in hyperglycemic rats.

Group	1st day	3rd day	5th day	7th day	9th day
DW group	HS (A)	HS (A)	HS (A)	HS (A)	HS (A)
M group	S (A)	HS (A)	HS (A)	HS (A)	NS

S=significant difference (P <0.05), HS= highly significant difference (P<0.01), NS=non-significant difference (P>0.05), A= the difference belong Artemisia group.

Table (2): Significance of differences between Ah 50% and each of Metformin and DW groups regarding the reduction of PPBG in hyperglycemic rats.

Group	1st day	3rd day	5th day	7th day	9th day
DW group	HS (A)	HS (A)	HS (A)	HS (A)	HS (A)
M group	S (M)	S (M)	NS	NS	HS (M)

S=significant difference (P <0.05), HS= highly significant difference (P<0.01), NS=non-significant difference (P>0.05), A= the difference belong Artemisia group M= the difference belong metformin group.

to the last of treatment period. In comparison to normal level of PPBG (prior the induction of hyperglycemia); there was a highly significant difference (P<0.01), and the level of glucose was higher than the normal and such difference was persisted to the 7th day. But at 9th day metformin was able to cause a non-significant difference (P>0.05) in PPBG comparing to normal level prior the induction of hyperglycemia. (Fig. 4).

5. Post induction with Artemisia herba alba extract 100% group (PIA 100%)

After treatment with Ah 100% extract to the included rats, it caused a highly significant decrease (P<0.01) in PPBG and it was 195.5±0.4 mg/dl at 1st day post induction, such decreasing persisted for further days and the PPBG was 137.5±0.2 mg/dl at the 9th day post induction. In comparison to normal level of PPBG (prior the induction of hyperglycemia); there was a highly significant difference (P<0.01), and the level of glucose was higher than the normal and such difference was persisted to the 5th day. But at 7th day Ah 100% extract was able to cause a non-significant difference (P>0.05) in PPBG comparing to normal level prior the induction of hyperglycemia (Fig.5). The comparison of effect of Ah 100% extract group to DW and metformin groups was presented in table (1).

6. Post induction with Artemisia herba alba extract 50% group (PIA 50%)

After treatment with a 50% extract to the included rats, it caused a significant decrease (P<0.05) in PPBG at the 3rd day post induction and the level was 210.5±0.3 mg/dl. Such decreasing persisted to the last of experimental period and there was a highly significant difference (P<0.01) and the PPBG was 163.5±0.2mg/dl at the 9th day post induction. In comparison to normal level of PPBG (prior the induction of hyperglycemia); there was a highly significant difference (P<0.01) at 1st, 3rd, and 5th day post induction, and a significant difference (P<0.05) at 7th, 9th day post induction, and the level of glucose was higher than the normal (Fig. 6). The comparison of effect of Ah 50% extract group to DW and metformin groups were presented in table (2).

Discussion

After administration Ah 100% extract to normoglycemic rats, the PPBG decrease at 5th day, at 7th day and persist like this for 9th day. The normal PPBG has a (HS) decrease (P<0.01). Whereas in NA 50% group, the normal PPBG decrease, and there is a (S) difference comparing to prior the administration (P<0.05). In DW group, after induction of hyperglycemia, and with starting of DW administration; the PPBG increase to reach 242.5 ± 0.2 mg/dl, and there is a (HS) difference (P<0.01) at the 1st day comparing to normal level prior the induction, these elevation of PPBG persist for further days of trial period. The mechanism by which dexamethasone induces peripheral insulin resistance is, by inhibiting GLUT-4 (glucose transporters) translocation from intracellular compartments to the plasma membrane mainly of skeletal muscles. The extracts could act by reversing the glucocorticoid mediated translocation of the glucose

transporters from the plasma membrane to the intracellular compartment (11). In (M) group after treatment the hyperglycemic rats with metformin, it causes a (HS) decrease ($P<0.01$) in PPBG at 3rd day. In comparison to normal level of PPBG (prior the induction of hyper-glycemia). Whereas in PIA 100% group, after treatment to the hyperglycemic rats with Ah 100% extract, it causes a (HS) decrease ($P<0.01$) in PPBG at 1st day post induction, such decreasing persisted for further days. In comparison to normal level of PPBG (prior the induction of hyperglycemia).The comparison of effect of Ah 100% extract group to DW and metformin groups is presented in table (1), in the 1st day there is a (HS) difference belong Artemisia group in DW group. Where as in PIA 50% group, after treatment with Ah 50% extract to the included rats, it causes a (S) decrease ($P<0.05$) in PPBG at the 3rd day post induction, and there is a (HS) difference ($P<0.01$) at the 5th day post induction to the 9th day. In comparison to normal level of PPBG (prior the induction of hyperglycemia). The comparison of effect of Ah 50% extract group to DW and metformin groups is presented in table (2). Results of the present study clearly indicate that aqueous extract from Ah100% produced a (HS) hypoglycaemic effects in both normglycemic and hyperglycemic rats. Results of the present study are agreed to those of Ibrahim (12). Tastekin (13) came in agreement with Twaij (14) studies. Bennani-Kabchi (15) reported that Ah was a hypoglycemic, antihyperglycemic plant in diabetic sand rat with hypolipemic action in case of severe obesity. Also recently agreed with Twaij and Al-Dujaili (16) who reported that the aqueous extract of the aerial parts of Ah produced a significant hypoglycemic activity when administered orally to normoglycemic and alloxan diabetic rabbits and rats. Results of the present study expect that the mechanism of action agreed with previous studies 1-hypoglycemic effects of Ah could be due to improved peripheral glucose utilization (17). 2-The hypoglycemic activity may be due to inhibition of the activity of α-amylase. One therapeutic approach for treating diabetes is, to decrease the post-prandial hyperglycemia. This is done by retarding the absorption of glucose through the inhibition of the carbohydrate hydrolyzing enzymes, α-amylase and α-glucosidase in the digestive tract. Inhibitors of enzymes delay carbohydrate digestion and prolong overall carbohydrate digestion time causing a reduction in the rate of glucose absorption and consequently blunting the postprandial plasma glucose rise (13,18). (13) suggested that Ah inhibition of the proximal tubular reabsorption mechanism for glucose in the kidneys can also contribute towards blood lowering effect. The Ah hypoglycemic effects may be due to its phytoconstituent from flavonoids (19), and main essential oil components β-thujone and α-thujone,1,8-cineole, camphor, chrysanthen-one, trans-sabinyl acetate, trans-pinocarveol and borneol (20).

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