

Original Research Article

Antidiabetic effects of ethanol extract of *Uromastix hardwickii* Smith (Agamidae) skin on streptozotocin-induced diabetes mellitus in mice

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Sent for review: 2 September 2020

Revised accepted: 19 February 2021

Abstract

Purpose: To investigate the *in vivo* hypoglycemic potential of the ethanol extract of *Uromastix hardwickii* skin (EEUHS).

Methods: Streptozotocin-induced diabetic mice received graded doses (100 and 200 mg/kg) of EEUHS for oral glucose tolerance and hypoglycemic test. The effects of EEUHS on body weight gain, urea, creatinine, cholesterol and hemoglobin concentration were also investigated.

Result: The blood glucose concentrations of the EEUHS-treated groups were diminished ($p < 0.05$) relative to the diabetic control group. The urea, creatinine and cholesterol levels of the EEUHS-treated groups were lower ($p < 0.05$), but their hemoglobin concentrations were higher ($p < 0.05$) relative to the diabetic control group.

Conclusion: The EEUHS elicits hypoglycemic effects and ameliorates complications of diabetes mellitus in streptozotocin-induced diabetic mice. This suggests that EEUHS can potentially be developed for the management of diabetic patients.

Keywords: Anemia, Antidiabetic, Lizard, Glibenclamide, Streptozotocin, *Uromastix hardwickii*, Weight loss

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INTRODUCTION

Diabetes mellitus (DM) is a chronic non-communicable disease caused by insulin resistance, insulin deficiency and/or combination of both factors [1]. It has affected over 463 million people worldwide [2]. About 75 % of diabetic

patients are within the working age group (24 – 64 years old). The impact of DM on global economy is estimated at over US\$ 827 billion annually [3]. Poorly-managed diabetic conditions predispose to other health complications and about 11.3 % of death are attributed to diabetes globally [2].

The control and treatment of DM involve changes in lifestyle, insulin therapy and oral hypoglycemic agents. Some oral hypoglycemic agents used in the clinical management of DM are sulfonylureas, thiazolidinediones, alpha-glucosidase inhibitors, biguanides and dipeptidyl peptidase-4 inhibitors. The clinical efficacy and patients' compliance with the use of these drugs have been hampered by their side-effects such as anorexia, diarrhea, nausea, vomiting, cholestatic jaundice, weight gain, stomach upset, agranulocytosis, aplastic and hemolytic anemia, increased cardiovascular mortality, generalized hypersensitivity and skin reactions [4]. Aside the aforementioned side-effects, orthodox drugs are expensive and not readily available. Hence, a good proportion of the population use traditional medicines in the care of DM [5].

In ethnomedicine, DM is managed via zootherapy in countries like Brazil, Pakistan, China, India, Argentina etc [6,7]. About 1500 animal species are used in zootherapy [8]. Some of the diseases treated via zootherapy are helminthosis, cancer, asthma, pain, fever, pneumonia, piles, gastric ulcer and DM [8]. In Pakistan, *Uromastyx hardwickii* is used in zootherapy. *Uromastyx hardwickii* is commonly called "Hardwicke's spiny-tailed lizard" or "Indian spiny-tailed lizard" and member of the family Agamidae [9]. It is a diurnal, ground-dwelling and herbivorous lizard that lives in burrows. It is found in Saharo-Sindic region of the Arabian Peninsula [9]. The flesh is consumed by some natives of Pakistan as a supplement for diabetic management [8]. The oil obtained from the body fat is used as embrocation oil and aphrodisiac for the treatment of impotence by the natives of India and Pakistan [9]. The in vitro antidiabetic effects of the ethanol extract of *U. hardwickii* skin have been reported [10]. There is dearth of information on the in vivo hypoglycemic activities of *U. hardwickii* skin extract. This study interrogated the in vivo hypoglycemic activities of the ethanol extract of *U. hardwickii* skin.

EXPERIMENTAL

Capture of *U. hardwickii*

Four (4) mature *U. hardwickii* of both sexes were captured from the desert and semi-desert region of Pakistan. The *U. hardwickii* was authenticated by Dr G. Rehman and stuffed specimen was deposited in the museum of the Department of Zoology, Abdul Wali Khan University. The *U. hardwickii* were managed as recommended by the National Research Council guidelines [11]. The ethical clearance (no. AWKUM/ZOO/2018/1770) for the study was obtained from the

Ethical Committee, of the Department of Zoology, Abdul Wali Khan University.

Preparation of extract of *U. hardwickii* skin

Four adult *U. hardwickii* of both sexes were placed in a box containing diethyl ether (soaked in cotton wool) for few minutes and euthanized via cervical dislocation. The skin was excised, dried laboratory bench, ground into coarse powder, extracted with ethanol in Soxhlet apparatus and evaporated with rotary evaporator [10]. The ethanol extract of *U. hardwickii* skin (EEUHS) was stored at 4 °C in a refrigerator.

Animals

Fifty (50) mature male healthy albino mice (6-8 weeks of age) weighing 28 - 35 g were used for this study. The animals were kept in clean and dry plastic cages, with natural light/dark cycle at 25 ± 2 °C environmental temperature and 45 - 55% relative humidity. The animals were fed with standard pellet diet and water *ad libitum*. The mice were handled with minimal pain [11].

Acute toxicity test

The "Up and Down" method of oral acute toxicity test was used evaluate the toxicity of EEUHS. The upper dose limit was 2000 mg/kg.

Blood glucose concentration (BGC) assay

The BGC of the mice were assay using Accu-Chek glucometer test kit (Roche, Germany) using blood samples drawn from the tail vein upon a tail snip.

Induction of experimental DM

Streptozotocin (STZ) (Fine Chemicals Pvt Ltd, Biosar) was dissolved in citrate buffer and injected intraperitoneal once at dose of 50 mg/kg to fasted mice. The fasting BGC was evaluated 48 hours post STZ administration with Accu-Chek glucometer test kit (Roche, Germany) and mice with BGC above 150 mg/dL were recruited for the experiment [12].

Oral glucose tolerance test (OGTT)

Twenty (20) STZ-induced hyperglycemic mice were used for the experiment. The mice were randomly assigned to 4 groups (A - D) of 5 mice each. Group A (diabetic control) received normal saline, groups B and C received EEUHS 100 and 200 mg/kg, respectively, while group D received glibenclamide 2 mg/kg (positive control). All treatments were given via the oral route with the

aid of a gastric gavage. Thirty (30) minutes post-treatment, glucose load (2000 mg/kg) was administered to each mouse. Thereafter, BGC was evaluated at 30, 60, 90- and 120-minutes post-glucose load.

Evaluation of *in vivo* hypoglycemic activity of EEUHS

We used 25 mice (5 normoglycemic and 20 hyperglycemic mice) for this experiment. The 5 normoglycemic mice were assigned to Group A (normal control) which received normal saline, 5 ml/kg. The 20 hyperglycemic mice were randomly assigned to 4 groups (n = 5, B - E). Group B (diabetic control) received normal saline; groups C and D received EEUHS 100 and 200 mg/kg respectively, while group E received glibenclamide 2 mg/kg. The treatment were given once daily for 17 consecutive days via gastric gavage. Fasting BGC was assayed on days 1, 4, 10 and 17 with Accu-Chek active glucometer test kit (Roche, Germany). The mice were weighed on days 1, 4, 10 and 17. Twenty-four (24) hours after the last treatment on day 17, the mice were fasted for 16 hours and blood samples were drawn via the retro-orbital plexus into EDTA bottles for hemoglobin concentration determination and also into plain bottles for serum biochemical (blood urea nitrogen (BUN), creatinine and cholesterol) analysis.

Determination of hemoglobin, BUN, creatinine and cholesterol levels

Cyanomethemoglobin method was used in the determination of hemoglobin concentration [13]. The serum concentration of BUN, creatinine and cholesterol were assayed spectrophotometrically using commercial reagent kits (Randox Laboratories, United Kingdom).

Statistical analysis

Data generated were analyzed using one-way analysis of variance (ANOVA). Mean differences were separated using Tukey's test (SPSS 22.0 software) and significance difference was accepted at $p < 0.05$.

RESULTS

Effect of EEUHS on OGTT of STZ-induced diabetic mice

The effects of EEUHS on the OGTT on STZ-induced diabetic mice are presented in Figure 1. At 120 minutes, the BGC of the EEUHS and glibenclamide-treated groups were lower ($p < 0.05$) than the BGC of the diabetic control group.

The EEUHS produced dose-dependent decrease in the BGC.

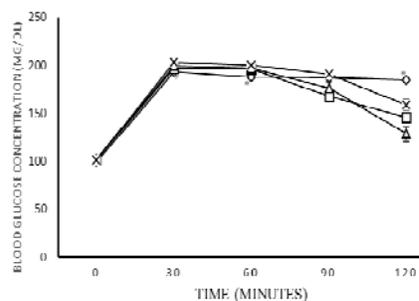


Figure 1: Effect of EEUHS on OGTT of STZ-induced diabetic mice; * $p < 0.05$ relative to other treatment groups; EEUHS = ethanol extract of *Uromastix hardwickii* skin; -◇- = diabetic control; -□- = EEUHS 100 mg/kg; -△- = EEUHS 200 mg/kg; -x- = glibenclamide 2 mg/kg

Effect of EEUHS on fasting BGC of STZ-induced diabetic mice

On days 10 and 17, the BGC of EEUHS-treated groups were diminished ($p < 0.05$) relative to the BGC of diabetic control group. The EEUHS produced time-dependent decline in the BGC of the treated groups.

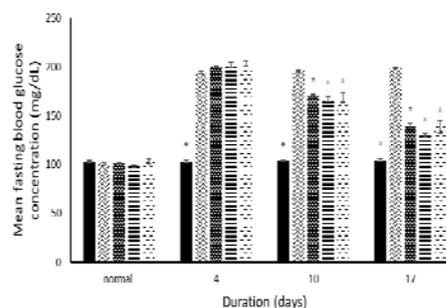


Figure 2: Effect of EEUHS on fasting BGC of STZ-induced diabetic mice. * $p < 0.05$ relative to the diabetic control group; EEUHS = ethanol extract of *Uromastix hardwickii* skin; ■ = normal control; ◻ = diabetic control; ▨ = EEUHS 100 mg/kg; ▩ = EEUHS 200 mg/kg; ▤ = glibenclamide 2 mg/kg

Effect of EEUHS on body weight of STZ-induced diabetic mice

The normal control, EEUHS (100 and 200 mg/kg) and glibenclamide-treated groups gained weight while the diabetic control group lost weight throughout the period of the experiment (Figure 3). The percentage weight gain of the EEUHS and glibenclamide-treated groups on days 10 and 17 were higher ($p < 0.05$) relative to the diabetic control group.

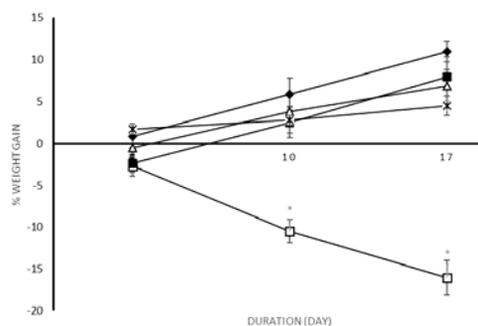


Figure 3: Effect of EEUHS on body weight of STZ-induced diabetic mice. * $p < 0.05$, relative to other treatment groups; EEUHS = ethanol extract of *Uromastix hardwickii* skin; \blacklozenge = normal control; \square = diabetic control; \triangle = EEUHS 100 mg/kg; \blacksquare = EEUHS 200 mg/kg; \circ = glibenclamide 2 mg/kg

Effect of EEUHS on serum urea, creatinine and cholesterol, and hemoglobin concentrations of STZ-induced diabetic mice

The BUN, creatinine and cholesterol concentrations of the normal control, EEUHS (100 and 200 mg/kg) and glibenclamide-treated groups were diminished ($p < 0.05$) when related to the diabetic control group (Table 1). The hemoglobin concentration of the normal control, EEUHS (100 and 200 mg/kg) and glibenclamide-treated were elevated ($p < 0.05$) when related to the diabetic control group.

DISCUSSION

We investigated the acute toxicity and hypoglycemic effects of EEUHS on STZ-induced diabetic mice. The EEUHS was well tolerated by the mice and the LD_{50} was greater than 2000 mg/kg. The EEUHS reduced BGC, BUN, creatinine and cholesterol levels, and increased hemoglobin concentration and weight gain of STZ-induced diabetic mice.

Oral glucose tolerant test evaluated the influence of EEUHS on the post-prandial glucose metabolism [14]. The first 30 min post glucose load was the absorptive phase while the 90 and

120 min was the assimilation or the storage phase. The elevated BGC in the diabetic control group at 120th minutes is linked to glucagon secretion. Non-inhibition of glucagon secretion accompanies insulin deficiency and is incriminated in post-prandial hyperglycemia in DM [15]. The diminished BGC of the EEUHS- and glibenclamide-treated groups might be linked to enhanced incretin effect [15]. This finding indicates that the EEUHS can effectively manage post-prandial hyperglycemia in a diabetic patient. STZ is a nitrosourea analogue with alkylating and antimicrobial properties that cause necrosis of beta cells of the pancreas. It causes insulinopenia and DM in rodents [16]. It accumulates in beta cells through GLUT-2 transporters. Treatment of mice with STZ caused hyperglycemia which indicates insulinopenia and diabetic condition. The EEUHS administration caused mild dose-dependent decline in BGC of the treated mice on days 10 and 17 (Figure 2). The mechanism of the hypoglycemic effects of EEUHS could be via reduction in gut glucose absorption, enhanced hepatic glucose uptake and increased insulin secretion or sensitivity. The *in vitro* anti-glucosidase and anti-amylase activities of EEUHS have been reported [10]. Glucosidase and amylase catalyze carbohydrate digestion in the gut. Agents that inhibit the activities of these enzymes are known to reduce glucose absorption and post-prandial hyperglycemia, and hence exploited in the care of DM. The hypoglycemic potential of acarbose is based on its inhibition of glucosidase and amylase [17]. Another possible mechanism of the hypoglycemic effects of EEUHS, is the enhanced hepatic and muscular uptake of glucose. Liver and partly muscles are the major storage organs of glucose in the form of glycogen.

Elevated uptake of glucose by muscle and liver lead to hypoglycemia and some antidiabetic drugs such as metformin exhibit this mechanism of action [18]. The EEUHS might have also elicited its antihyperglycemic potential via the enhanced insulin secretion and/or increased

Table 1: Effect of EEUHS on BUN, creatinine, cholesterol, and hemoglobin concentration of STZ-induced diabetic mice (mean \pm SD, n = 5)

Treatment	BUN (mg/dL)	Creatinine (mg/dL)	Cholesterol (mg/dL)	Hemoglobin (g/dL)
Normal control	22.70 \pm 0.61*	0.47 \pm 0.01*	66.99 \pm 0.86*	14.19 \pm 0.20*
Diabetic control	35.45 \pm 0.51	1.50 \pm 0.09	122.26 \pm 2.16	8.83 \pm 0.35
EEUHS (100 mg/kg)	26.20 \pm 0.78*	0.43 \pm 0.02*	68.65 \pm 0.58*	11.61 \pm 0.22*
EEUHS (200 mg/kg)	26.71 \pm 1.06*	0.51 \pm 0.02*	64.25 \pm 1.00*	12.61 \pm 0.23*
Glibenclamide (2 mg/kg)	25.72 \pm 0.91*	0.43 \pm 0.01*	73.75 \pm 0.83*	11.84 \pm 0.41*

* $P < 0.05$ when related to the diabetic control; EEUHS = ethanol extract of *Uromastix hardwickii* skin; BUN = blood urea nitrogen

insulin sensitivity. The EEUHS may have stimulated insulin secretion, since the result produced was similar to the effects of glibenclamide [4]. This finding corroborated the report on the hypoglycemic effects of *Uromastix acanthinura* [19].

Treatment of the mice with STZ caused a consistent weight loss (Figure 3) which could be linked to polyuria, dehydration and muscle wasting [20]. STZ-induced DM is often accompanied by weight loss, linked to impaired glucose utilization and increased gluconeogenesis [20]. The gluconeogenesis involves the mobilization of muscular protein and fat from adipose tissue which predispose to weight loss. The findings indicate that EEUHS like glibenclamide reversed weight loss in the treated groups which could be via amelioration of negative energy balance.

Chronic hyperglycemia elevates advanced glycation end products (AGEP) and causes vascular complications such as nephropathy, neuropathy, stroke, myocardial infarction etc [20]. AGEP generate free radicals which cause protein fragmentation and lipid peroxidation. The product of lipid peroxidation, malondialdehyde, cause cell membrane damage and predispose to anaemia [21]. Anaemia is a common complication of DM and its effective management will improve the quality of life in affected patients [22]. Our findings indicate that EEUHS reversed anaemia in the treated groups (Table 1). This corroborated the report on the ameliorative effects of *Helianthus annuus* on haematological disorder in alloxan-induced hyperglycemia. The EEUHS reduced BUN and creatinine in the treated groups (Table 1). This indicates that EEUHS restored the deranged kidney function marked by elevated BUN and creatinine to near normal [23]. The mechanism of action of the nephroprotective effects of EEUHS could be via improved glucose metabolism, which reduced the liberation of AGEP that predispose to nephropathy. The improved kidney function may have elicited the reversal of the anaemia. The kidney secretes erythropoietin which stimulates erythropoiesis [22]. Dyslipidemia, characterized by hypercholesterolemia, is a complication of DM and a major risk factor of cardiovascular disease [20]. The EEUHS- and glibenclamide-treatment diminished serum cholesterol concentration in the treated groups (Table 1). This implies that EEUHS has the potential to ameliorate dyslipidemia and prevent its related complication in DM patients. The anti-dyslipidemic effects of EEUHS may be via inhibition of cholesterol absorption or biosynthesis and/or improved glucose metabolism [20].

CONCLUSION

The EEUHS possesses the potential to manage DM and its related complications (anaemia, dyslipidemia and nephropathy) in patients. However, further studies aimed to isolate the active hypoglycemic principle(s) are required.

DECLARATIONS

Acknowledgement

We are grateful to Prof IU Asuzu for proof-reading the manuscript. This research was not supported by any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. All authors read and approved the final version of the manuscript.

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