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Research Article

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Anti-diabetic Activity of Aqueous Extract of Peganum Harmala Roots in Streptozotocin-induced Diabetic Rats

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ABSTRACT

The Peganum harmala plant is commonly used as traditional herbal medicine in many countries including Algeria. The aim of this study was designed to evaluate the anti-diabetic properties of aqueous extract of Peganum harmala roots (AEPHR). The oral acute toxicity was evaluated. Rats were made diabetic by injection of STZ (60 mg/kg body weight, i.p) and diabetes was confirmed 72 h after induction. The AREPH was administered orally at the doses of 200 mg/kg body weight for 21 days. Screening for major classes of phytochemical was done using standard chemical tests. Oral administration of AEPHR did not exhibit toxicity at a dose of 200 mg/kg. AEPHR treated diabetic rats significantly (P <0.001) reduced elevated blood glucose. The body weight were significantly (P <0.001) increased in diabetic rats treated with AEPHR compared to diabetic control rats. Phytochemical screening showed a wealth in compounds: Tannins, saponins, alkaloids, amino acids. However flavonoids, triterpenes and sterols appeared in the form traces. It is concluded that aqueous extract of P. harmala roots are a good natural source of anti-diabetic in streptozotocin-induced diabetic rats.

Keywords: Peganum harmala L; Anti-diabetic activity; Streptozotocin; Blood glucose; aqueous roots extract

INTRODUCTION

Diabetes mellitus is not a single disease but is a group of metabolic disorders affecting a huge number of populations in the world. It is mainly characterized by chronic hyperglycemia, resulting from defects in insulin secretion or insulin action. It is predicated that the number of diabetes person in the world could reach up to 366 million by the year 2030 [1]. It is becoming the third 'killer' of the health of mankind along with cancer, cardiovascular and cerebrovascular diseases [2]. Apart from current available therapeutic options for diabetes like oral hypoglycemic agents and insulin, which have limitations of their own, many herbal medicines have been recommended for the treatment of diabetes [3]. The hypoglycemic effect of many herbs has already been reviewed [4; 5].The present study was carried out to evaluate the anti-diabetic activity of Peganum harmala, which belongs to the family of Zygophylaceae, is distributed mainly in the Mediterranean region, also found in Central Asia, North Africa and also cultivated in America and Australia [6]. In Algeria, it grows spontaneously on the edges of roads, in arid and rocky areas, and sandy soils [7]. It is rich in alkaloids of type β-carboline and contains up to 2 - 7% total alkaloids [8]. Several studies have shown various biological activities and pharmacological characteristics of its seeds such as hypothermia [9], hallucinogen factor [10], antidepressant [11], inhibitor of the enzyme monoamine oxydase (MAO) [8] and myeloperoxidase [12] enzymes, antibacterial, antifungal and anti-virus [13; 14]. It is effective for the treatment of dermatosis disease [15].

MATERIALS AND METHODS

Plant material collection and identification

Roots of P.harmala were collected from the Harmalia region (South east of the town of Ain M'lila, Algeria) in September 2013. The plant was identified by Dr. Y Halis.

Preparation of aqueous extract

Fresh roots were dried at room temperature. Aqueous extract was obtained as follows: 100 g of dried roots powder was soaked in distilled water for 12 h. On the next day, plant material was boiled for 30 min and filtered. The filtrate was dried in the oven at 40°C to make a powder yielding 6.2 % (w/w). The residue was stored at (2-8°C) until the completion of pharmacological studies.

Phytochemical analysis

• Detection of Alkaloids: 5mL of the crude extract were added to 2mL of hydrochloric acid. 1mL of Dragendroff s reagent was added to this acidic medium. An orange red precipitation was produced which indicates the presence of alkaloids [16].
• Detection of Saponins: The crude extract solution was diluted with 20mL of distilled water and it was agitated in a graduated cylinder for 15min. The formation of 1cm foam layer showed the presence of saponins [16].
• Detection of Amino acids: 1mL of the crude extract was added a few drops of Ninhydrin reagent. The purple color appearance shows the presence of amino acids [17].
• Detection of Flavonoids: To one milliliter of the crude stock extract, 10mg magnesium turnings were added in to1mL of the filtrate, followed by the addition of 0.5mL concentrated sulphuric acid. The presence of magenta red observed with in 3min confirmed the presence of flavonoids.
• Detection of tannins: To one milliliter of the aqueous solution was treated with 1mL of water and 1-2 drops of dilute solution of ferric chloride. The appearance of a dark green color or blue-green indicates the presence of tannins [18].
• Detection of sterols and triterpenes: The residue of aqueous extract was dissolved in 0.5mL acetic anhydride and then in 0.5mL of chloroform. Then 1mL of concentrated sulphuric acid is added (Liebermann-Burchard reaction). At the contact zone of the two liquids a brownish red ring was formed denoting the presence of sterols and triterpenes [18].

Experimental Animals

Male Wistar albino rats weighing about 170-190 g were obtained from the Pasteur Institute (Algiers, Algeria). Animals were acclimated for two weeks under the same laboratory conditions of photoperiod (12h light/12 h dark) with a relative humidity 70% and room temperature of 22 ± 2◦C, with free access to standard pellets diet provided by National livestock food board (Bejaia, Algeria), and water adlibitum. All experimental procedures were approved by the ethics and regulations of animal experiments of the University.

Behavioral analysis

Animals were observed continuously during the first 30 min after dosing (200 mg/kg body weight of AEPHR) and observed periodically with special attention given during the first 4 hours for the next 24 hours and then daily thereafter, for 14days. All observations were systematically recorded with individual records being maintained for each animal. Observations included changes in skin and fur, eyes, mucous membranes and behavioral pattern. Attention was given for observations of tremors, salivation, diarrhea, lethargy, sleep, coma and mortality.

Induction of diabetes

Diabetes was induced in rats by intravenous injection of STZ at a dose of 60 mg/kg body weight, dissolved in 0.1M cold citrate buff er (pH = 4.5).STZ induces diabetes within 3 days by destroying the beta cells [19]. Blood samples were withdrawn from retro-orbital plexus under light ether anesthesia before. Blood glucose level was measured using GOD-POD (Kit spin react). A fortnight later, diabetes was confirmed.

Experimental protocol

The normal and diabetic rats were divided into four groups of four animals each and treated by per oral as shown below.

• Group I: Normal control given only saline (9 g/L) (NC).
• Group II: Normal treated with AEPHR at dose of 200 mg/Kg (NTAEPHR).
• Group III: Diabetic control given only saline (9 g/L) (DC).
• Group IV: Diabetic rats treated with AEPHR at dose of 200 mg/Kg (DTAEPHR).

Blood samples and body weight were measured at weekly intervals on days 0, 7, 14 and 21, till the end of study. Glucose was measured by the GOD-POD method. At the end of the experiment, an oral glucose tolerance test (OGTT) was practiced. Animals (four groups) were loaded with glucose (3 g/kg). Blood glucose level was determined a t0, 30, 60, 90 and120 min after glucose loading.

Statistical analysis

All data were expressed as mean standard error of the mean (S.E.M). Student's t -test was used to compare between the mean values of paired groups, using Statistica software (Version 5.1, StatSoft France, 1997). Values were considered statistically significant when $P < 0.05$.

RESULTS

Preliminary phytochemical screening

Table 1 indicates the presence different compounds such as tannins, amino acids, alkaloids and saponins. However flavonoids, triterpenes and sterols appeared in the form traces.

Table 1. Phytochemical prospection of aqueous extract roots of P.harmala (AEPHR).

Extract / Metabolites	Aqueous root
Alkaloids	+
Tannins	+
Flavonoids	±
Saponins	+
Triterpenes and Sterols	±
Amino acids	+

+ Presence, ± Trace

Behavioral observation

In this study, all animals were fed the quantity of 200 mg /kg b.w of AEPHR have reacted well after treatment and showed no signs of toxicity or behavioral changes in the dose chosen until the end of the study (Tables 2 and 3).

Table 2. General appearance and behavioral observations for control group

Observation	30mn	4hrs	24hrs	48hrs	1wk	2wks
Skin and fur	Normal	Normal	Normal	Normal	Normal	Normal
Eyes	Normal	Normal	Normal	Normal	Normal	Normal
Mucous membrane	Normal	Normal	Normal	Normal	Normal	Normal
Behavioral pattern	Normal	Normal	Normal	Normal	Normal	Normal
Tremors	Nill	Nill	Nill	Nill	Nill	Nill
Salivation	Normal	Normal	Normal	Normal	Normal	Normal
Diarrheas	Nill	Nill	Nill	Nill	Nill	Nill
Lethargy	Nill	Nill	Nill	Nill	Nill	Nill
Sleep	Normal	Normal	Normal	Normal	Normal	Normal
Coma	Nill	Nill	Nill	Nill	Nill	Nill

Table 3. General appearance and behavioral observation for treated group.

Observation	30mn	4hrs	24hrs	48hrs	1wk	2wks
Skin and fur	Normal	Normal	Normal	Normal	Normal	Normal
Eyes	Normal	Normal	Normal	Normal	Normal	Normal
Mucous membrane	Normal	Normal	Normal	Normal	Normal	Normal
Behavioral pattern	Normal	Normal	Normal	Normal	Normal	Normal
Tremors	Nill	Nill	Nill	Nill	Nill	Nill
Salivation	Normal	Normal	Normal	Normal	Normal	Normal
Diarrheas	Nill	Nill	Nill	Nill	Nill	Nill
Lethargy	Nill	Nill	Nill	Nill	Nill	Nill
Sleep	Normal	Normal	Normal	Normal	Normal	Normal
Coma	Nill	Nill	Nill	Nill	Nill	Nill

Effect of AEPHR on body weight

Figure 1, shown the change in body weight gain in all groups animals, administration of STZ significantly reduced body weight in diabetic rats compared to normal control rats. In diabetic rats, treatment of AEPHR significantly increased body weight compared to diabetic control rats.

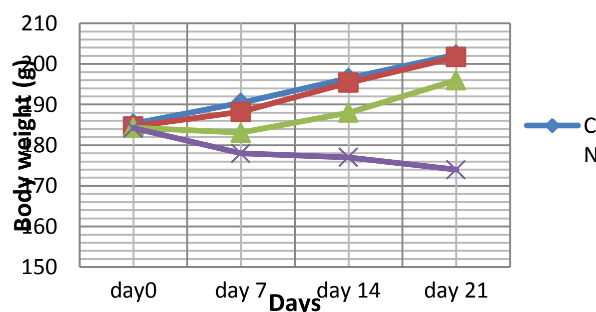

Figure1. Effect of AEPHR on body weight in normal rats and STZ- induced diabetic Rats.

Effect of AEPHR on blood glucose

The effect of AEPHR on the fasting blood glucose levels of both normal and diabetic rats are given in Table 4.The blood glucose levels were significantly (P <0.001) increased after the administration of STZ compared to the normal control rats. The oral treatment of AEPHR(200mg/kg) decreased significantly (P <0.001) blood glucose level of the diabetic rats compared to diabetic control rats. In normal rats, administration of AEPHR showed significant reduction (P <0.05) in glucose level compared to normal control rats after 21days of treatment.

Table 4. Effect of 3-weeks treatment with AEPHR on blood glucose level in normal rats and STZ induced diabetic rats.

Group	Treatment Dose	Blood glucose concentration (mg/dl)			
(n=4)	200 (mg/kg)	0 Day	7 Day	14 Day	21 Day
1	NC	112,5 ± 4,1	112,97 ± 3,11	106 ± 7,0	106 ±5,5
2	NTAEPHR	104,1 ± 6,75	103,56 ± 5,57	96,8 ± 7,4	87,28^{b} ± 5,86
3	DC	219, 26^{a} ± 6,00	290,7^{a} ± 2,8	297,4^{a} ± 3,3	354^{a} ±1.88
4	DTAEPHR	203,46^{c,d}± 0,68	113,7^{c} ± 1,8	107,7^{c} ± 1,2	85,34^{c,d} ±2,20
P-Value		a, d (P<0,001)	a, c (P<0,001)	a, c (P<0,001)	a, c (P<0,001)
		c (p<0.01)			b (P<0,05)
					d (P<0,01)

Values were represented as the mean ± SEM; the data were statistically analyzed using (T-test).
a: normal control vs. diabetic control, b: normal control vs. normal treated, c: diabetic control vs. diabetic treated, d: normal control vs. diabetic treated.

Oral glucose tolerance test (OGTT)

Oral glucose tolerance test in diabetic and normal rats showed an increasing in blood glucose level one hour after glucose administration. A significant reduction (P< 0.001) was marked in diabetic treated with AEPHR 2 h after treatment compared with diabetic group. OGTT does not show significant effect in normal and diabetic rats treatment with AEPHR compared with normal control rats (Figure 2).

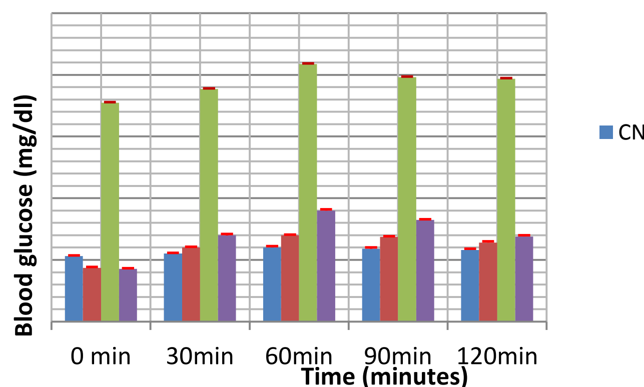

Figure 2. Effects of AEPH on OGTT in normal and diabetic rats (Mean±SEM).

DISCUSSION

P.harmala on normal and STZ-induced-diabetic rats. Acute toxicity studies revealed the non-toxic nature of the aqueous root extract of P.harmala. Oral administration of AEPHR 200 mg/kg to the diabetic rat's significantly reduced blood glucose level from the first week to the three week compared to diabetic control rats. Hence, the hypoglycemic activity of AEPHR may be due to its protective action against STZ-mediated damage to the pancreatic beta cells and also possibly because of regeneration of damaged beta cell or increased insulin release or secretion. Other studies, reported that the oil extract of P.harmala seed is effective in reducing blood glucose levels in diabetic rats [20] and the ethanolic extract of P.harmala seed has good anti-diabetic activity in streptozotocin-

induced diabetic rats [21; 22]. Another observation arising from this study is the eff ect of the AEPHR on the body weight in the treated rats. The improvement in body weight in diabetic rats treated may be due to improvement in metabolic activity of the system o maintain glucose homeostasis. These results suggested that root contained some bioactive principles that possess insulin protective or insulin-like activity [23]. The phytochemical study of root revealed the presence saponins, tannins, alkaloids, amino acids, flavonoids, triterpenes and Sterols that are known to be bio active anti-diabetic principles [24]. Amino acids have been reported as anti hyperglycemic agents. This eff ect may be due to a direct action of amino acids on the sensitivity of cells to insulin or increase the anabolism of muscle cells [25]. Tannins have been reported to possess hypoglycemic activity [26]. Alkaloids inhibit alpha-glucosidase and decrease glucose transport through the intestinal epithelium. Flavonoids suppress the glucose level, reduce plasma cholesterol and triglycerides significantly and increase hepatic glucokinase activity probably by enhancing the insulin release from pancreatic islets. Saponins stimulate the release of insulin and block the formation of glucose in the bloodstream [24]. It is concluded that Peganum harmala has significant anti-diabetic activity as it lowers the fasting blood sugar level in diabetic rats and increases the glucose tolerance. However, further studies are required for the isolation and structural elucidation of the active components of the plant material.

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