Antihyperglycemic effect of Juniperus phoenicea L. on alloxan-induced diabetic rats and diterpenoids isolated from the fruits

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Objective: To explore the traditional use of Juniperus phoenicea L. (J. phoenicea) growing in Egypt as antidiabetic herb.

Methods: The antihyperglycemic activities of the crude 80% ethanol and successive extracts of leaves and fruits of the plant were investigated in alloxan-induced diabetic rats after collecting blood samples through retro-orbital puncture technique. As a consequence of the biological results, phytochemical investigation of the chloroform fraction of fruits was carried out by column chromatography and thin layer chromatography.

Results: Results revealed the reduction in blood glucose levels in rats, which were significantly different from control at 4 and 8 weeks (P < 0.01). The highest antihyperglycemic activity was exhibited by the crude extracts of fruits and leaves of which the potency was 83.6% and 81.9%, respectively, after 8 weeks, comparing to metformin drug (100% potency). Chloroform fractions of leaves and fruits were the most potent fractions (potencies were 70.3%, 71.4%, respectively, along with ethyl acetate fraction of fruits (71.4%). Phytochemical investigating of the chloroform fraction of fruits resulted in the isolation and identification of 5 abietane diterpenoids. Ferruginol, 7-dehydroabietanone, sugiol, 6-α-hydroxy-7-oxoferruginol, totarolone and a labdane diterpenoid, varodiol were isolated for the first time from the fruits of J. phoenicea growing in Egypt. The identification of these compounds was based on spectroscopic analysis: 1hydrogen-nuclear magnetic resonance and electron impact mass spectrometry, comparing the results with the literature.

Conclusions: It has become clear that leaves and fruits of the Egyptian J. phoenicea provide effective antihyperglycemic action in diabetic rats as was reported in folk medicine. The high contents of terpenoids in the non-polar fractions may attribute to the antidiabetic effect of the plant.
that they are rich in essential oil, carbohydrates, and/or glycosides, sterols and/or triterpenes, and flavonoids[8], and they showed significant diuretic action as its use in folk medicine[9].

The aim of the current investigation is to evaluate the efficiency of J. phoenicea extracts for the antihyperglycemic activity in alloxan-induced diabetic rats in order to explore its use in folklore medicine as antidiabetic herb. Also, work continues to isolate and characterize the active compound that is responsible for the activity, providing the rationale behind its use as a useful drug for diabetes.

2. Materials and methods

2.1. Plant materials

Fresh aerial parts of J. phoenicea (family: Cupressaceae) were collected from North Sinai, Maghara Mountain, Egypt and were kindly identified by Prof. Dr. Nabeel El-Hadidi, Botany Department, Faculty of Science, Cairo University. The plant was dried, ground and a voucher specimen (No. 21882) was kept at Pharmacognosy Department, National Research Centre, Cairo, Egypt.

2.2. Preparation of extracts

2.2.1. Crude extracts

About 500 g of air-dried powdered leaves and fruits of J. phoenicea were exhaustively extracted by reflux with 80% ethanol. The combined extract was evaporated under reduced pressure using rotatory evaporator (Heidolph, Germany).

2.2.2. Successive extracts

About 500 g of air-dried powdered leaves and fruits of J. phoenicea were exhaustively and successively extracted in Soxhlet apparatus using petroleum ether, chloroform, ethyl acetate and methanol. These extracts were evaporated to dryness under vacuum at 40 °C.

2.3. Chemicals and drugs

Alloxan (Sigma Co.) was used for the induction of diabetes in rats. Metformin (Cidophag®, Chemical Industries Development Co., Giza, Egypt) was used as standard anti-diabetic drug.

2.4. Experimental animals and diet

Adult albino rats, of Sprague-Dawley strain weighing 130–150 g, were obtained from the animal house colony of National Research Centre, Dokki, Egypt. They were kept under the same hygienic conditions and well-balanced diet and water. Diet was consisted of vitamin mixture (1.0%), mineral mixture (4.0%), corn oil (10.0%), sucrose (20.0%), cellulose (0.2%), casein 95% (10.5%) and starch (54.3%). Samples were administered orally by gastric tube. The experiments were done after taking the permission from the Research Ethics Committee, National Research Centre.

2.5. Acute toxicity studies

In a previous study by the authors, the acute toxicity test was carried out in albino mice (25–30 g) and the LD₅₀ of the crude 70% ethanol and successive extracts of leaves and fruits of J. phoenicea were determined[8]. All the extracts were safe showing LD₅₀ more than 5 g/kg body weight. So the effective dose was fixed at 100 mg/kg body weight for evaluation of antihyperglycemic activity.

2.6. Determination of acute antihyperglycemic activity

Rats were injected intraperitoneal with alloxan (150 mg/kg body weight) to induce diabetes mellitus[10]. Hyperglycemia was assessed after 72 h. Animals were divided into seven groups. First group was diabetic rats that served as positive control. Groups from second to sixth were diabetic rats that received 100 mg/kg body weight of crude 70% ethanol, petroleum ether, chloroform, ethyl acetate and methanol extracts of leaves, respectively. The seventh group was diabetic rats that received 150 mg/kg body weight of metformin as reference antidiabetic drug. At the end of each study period, blood samples were collected from the retro-orbital venous plexus through the eye canthus of anesthetized rats after an overnight fast. Serum was isolated by centrifugation and the blood glucose level was measured[11]. The experiment was repeated for fruit extracts.

2.7. Methods for diterpenoids isolation

Half kilogram of the air-dried powdered fruits of J. phoenicea was exhaustively extracted with chloroform several times. The combined extract was evaporated to dryness under vacuum at 40 °C. The chloroform extract (10 g) was fractionated by column chromatography using neutral alumina (aluminium oxide neutral, Sisco Research Laboratories Pvt. Ltd.) and stepwise gradient elution by ether/chloroform/methanol in successive proportions were done. Three main fractions were taken into consideration. Fraction 1 yielded from 75% ether(CHCl₃) and upon further fractionation by preparative thin-layer chromatography (TLC) (silica gel 60 F254 for TLC, Fluka Chemie AG, Switzerland) using chloroform-acetone 9:1 yielded compounds (T1-T3); Fraction 2 yielded from 100% ether and upon further fractionation by preparative TLC/benzene-ethyl acetate 3:1 yielded compound (T4) and Fraction 3 yielded from 75% CHCl₃/MEOH and upon further fractionation by preparative TLC/chloroform-acetone 9:1 yielded compounds (T5, T6). Plates were visualized before and after spraying with 10% H₂SO₄ and heating. The isolated compounds were subjected to spectroscopic analysis techniques: electron impact mass spectrometry (EI-MS) (Finnigan model 3200 at 70 eV) and 1hydrogen-nuclear magnetic resonance (1H-NMR) (Bruker DRX 500 spectrometer, Biospin, Rheinstetten, Germany).

3. Results

3.1. Antihyperglycemic activity

Results listed in Tables 1 and 2 revealed that there was significant antihyperglycemic activities exhibited by all extracts of J. phoenicea leaves and fruits as indicated by the significant reduction in blood glucose levels in alloxan-induced diabetic rats. Effects of extracts were significantly different from control group at 4 and 8 weeks. Fruit crude extract was more potent (83.6%) than leaf crude extract (81.9%) after 8 weeks compared to metformin drug (100.0% potency). Pertaining to the successive extracts of leaves, it was indicated that chloroform extract was the most potent one where its relative potency was 70.3% followed by petroleum ether extract (65.0%), then ethanol extract (54.6%), while ethyl acetate extract showed the lowest activity (38.0%), after 8 weeks compared to metformin drug. Similar experiment was done for fruit successive extracts and it has shown that chloroform and ethyl acetate extracts were the most potent ones where their relative potencies were 71.4% followed by ethanol extract (57.9%), while petroleum ether extract...
showed the lowest activity (52.2%) after 8 weeks compared to metformin drug.

3.2. Identification of the isolated compounds

The isolated compounds were identified based on the spectral analysis of data. EI-MS fragmentation was listed below and while NMR was listed in Table 3 as well as comparing the data with those available in the literature\(^\text{[12,13]}\). Chemical structure of the isolated diterpenoidal compounds are shown in Figure 1.

**Table 1**

<table>
<thead>
<tr>
<th>Group</th>
<th>Value</th>
<th>Time</th>
<th>RP (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M ± SE</td>
<td>Zero</td>
<td>4 Weeks</td>
</tr>
<tr>
<td>Group 1</td>
<td>246.8 ± 9.2</td>
<td>257.9 ± 10.3</td>
<td>262.4 ± 10.8</td>
</tr>
<tr>
<td>Group 2</td>
<td>257.6 ± 8.2</td>
<td>126.9 ± 4.5</td>
<td>112.4 ± 3.9</td>
</tr>
</tbody>
</table>

\(\%\) Change\(^a\): % Of change calculated as regard the control group; RP: Relative potency; Potencies are calculated relative to metformin after 8 weeks. \(^*\) Significantly different from zero time at \(P < 0.01\).

**Figure 1.** Chemical structures of the isolated diterpenoids.

**Table 2**

<table>
<thead>
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<th>RP (%)</th>
</tr>
</thead>
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<tr>
<td></td>
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<td>246.8 ± 9.2</td>
<td>257.9 ± 10.3</td>
<td>262.4 ± 10.8</td>
</tr>
<tr>
<td>Group 2</td>
<td>251.5 ± 9.6</td>
<td>131.2 ± 5.1</td>
<td>106.4 ± 4.7</td>
</tr>
</tbody>
</table>

\(\%\) Change\(^a\): % Of change calculated as regard the control group; RP: Relative potency; Potencies are calculated relative to metformin after 8 weeks. \(^*\) Significantly different from zero time at \(P < 0.01\).
were tricyclic diterpenoid with abietane skeleton and identified as ferruginol (T1), 7-dehydroabietanone (T2), sugiol (T3), 6α-hydroxy-7-oxoferruginol (T4), totarolone (T5), in addition to a bicyclic diterpenoid with labdane skeleton, varodiol (T6) (ent-13-hydroxy-7-oxoferruginol (T4), totarolone (T5), in addition to a bicyclic diterpenoid with labdane skeleton; varodiol (T6) (ent-13-hydroxy-7-oxoferruginol (T4), totarolone (T5), in addition to a bicyclic diterpenoid with labdane skeleton; varodiol (T6)

### 4. Discussion

Diabetes mellitus, a metabolic disorder is characterized by increase in blood glucose level. The main objective of the drugs acting as antihyperglycemic is to reduce the blood glucose level to normal so as to reduce the diabetes related complications. In the current study, diabetes was induced using alloxan. The results revealed that the crude and successive extracts of the current study, diabetes was induced using alloxan. The results revealed that the crude and successive extracts of J. phoenicea have the potential to reduce the elevated blood glucose level in the tested rats at doses of 100 mg/kg body weight.

Following the bio-guided fractionation, the most promising fraction as antihyperglycemic extract was the chloroform fraction. The phytochemical investigation of that fraction revealed the isolation and characterization of 6 terpenoidal compounds, five compounds of the phytochemical contents of fruit chloroform extract revealed in diabetic rats as was reported in folk medicine. Investigating the phytochemical contents of fruit chloroform extract revealed the isolation of 6 diterpenoidal compounds, five compounds of abietane skeleton identified as ferruginol, 7-dehydroabietanone, sugiol, 6α-hydroxy-7-oxoferruginol, totarolone in addition to a bicyclic diterpenoid with labdane skeleton, varodiol. To support this work.

In conclusion, it has become clear that leaves and fruits of Juniperus phoenicea provide effective antihyperglycemic action in diabetic rats as was reported in folk medicine. Investigating the phytochemical contents of fruit chloroform extract revealed the isolation of 6 diterpenoidal compounds, five compounds of abietane skeleton identified as ferruginol, 7-dehydroabietanone, sugiol, 6α-hydroxy-7-oxoferruginol, totarolone in addition to a bicyclic diterpenoid with labdane skeleton, varodiol. To support this work.

### Conflict of interest statement

We declare that we have no conflict of interest.

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### References


