The hypoglycemic effect of extract of some medicinal plants in diabetes induced rabbits

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Abstract
It is well documented that administration of Alloxan in a dose of 100mg/kg (I.V) resulted diabetes mellitus in experimental animals (p<0.001). the serum glucose levels were measured before and after giving Alloxan and following the administration of insulin or aqueous extract of different plants which had been used in this study. Insulin in dose of 2 I.U/kg (S.C) produced (b=-22.9, P<0.001), in the mean time the aqueous extract of Medicago Sativa (Sgm/kg), Allium sativa (1 gm/kg), phaseolus vulgaris (4gm/kg), Petroselenium sativa (4gm/kg), Teucrium polium (4gm/kg) and trigonella foenum graecum (6 gm/kg) produced a significant reduction in serum glucose level with (b=-12.1, P<0.01), (b=-7.82, p<0.01), (b=-8.62, P<0.01), (b=-6.70, p<0.01), (b=-5.99, p<0.01) and (b=-8.98, p<0.01) respectively. All the result indicated that the aqueous extract of these medicinal plants have beneficial hypoglycemic effect except Myrtus communis

Introduction
A multitude of herbs and other plant materials had been described for treatment of diabetes in different parts of the world. WHO expert committee on diabetes has listed as one of its recommendation those traditional methods of therapy for diabetes should be further investigated (1) Regarding to the classification of WHO (2) to the active part of medicinal plants, this work was carried out to investigate the effect of these active parts on diabetic animals the active parts which had been used include the whole fresh bulb of Allium sativum (garlic), the whole flowering plant
of Medicago sativa (alfalfa), the leaves and branches of Myrtus communis (myrtle), fresh aerial parts of Petroselenium sativum (parsley) the ripe phaseolus vulgaris (Dwarf), the leaves of Teucrium polium (halwort) and the seeds of Trigonella foenum Graecum (fenugreek) in order to evaluate the possible hypoglycemic effect of these extracts and to choose the best to be used as alternative to insulin or as adjunct in therapy.

**Materials and methods**

Sixty New Zealand healthy male rabbits, weighing (1150 - 1250) gm were used in this study. They were supplied by animals house of Al/nahrain College of medicine. Each animal was kept in separate cage, which was provided with a wire mesh floor to avoid coprophagy, they fed standard diet (oxoid) and were given water ad libitum.

The rabbits were separated in to (10) equal groups (each group contains 6 animals):

Group (1): normal rabbits were injected with 1 ml normal saline into the marginal ear vein for 7 days of treatment.

Group (2): diabetic rabbits were injected with 1 ml normal saline into the marginal ear vein for 7 days after induction of diabetes by using alloxan (B.D.H chemical ltd U.K) 100mg/kg.

Group (3): diabetic rabbits were treated with humilin insulin (N.Lilly U.S.A) in a dose of I.U/kg (s.c) for 7 days of treatment.

Group (4): diabetic rabbits were treated with aqueous extract of Medicago sativa in a single dose (5gm/kg) given orally for 7 days of treatment.

Group (5): diabetic rabbits were treated with aqueous extract of Allium sativa in a single dose (1gm/kg) given orally for 7 days of treatment.

Group (6): diabetic rabbits were treated with aqueous extract of Phaseolus vulgaris extract in a single dose (Sgm/kg) given orally for 7 days of treatment.

Group (7): diabetic rabbits were treated with aqueous extract of Petroselenium sativa in a single dose (4gm/kg) given orally for 7 days of treatment.

Group (8): diabetic rabbits were treated with aqueous extract of Myrtus communis in a single dose (2gm/kg) given orally for 7 days of treatment.

Group (9): diabetic rabbits were treated with aqueous extract of Teucrium polium in a single dose (4gm/kg) given orally for 7 days of treatment.

Group (10): diabetic rabbits were treated with aqueous extract of days of treatment Trigonella foenum graecum in a single dose (6gm/kg) given orally for 7 days of treatment.
Some of diabetic rabbits were scarified and histo-pathological examination of the pancreas showed degeneration of beta cell of islets of Langerhans caused by Alloxan.

Plants were collected crushed, minced thoroughly and dissolved in distilled water at the dose, which mentioned before. They were given orally per os via stainless tube for 7 successive days. Blood samples were collected from marginal ear vein of each rabbit before giving Alloxan(LV) and following the administration of aqueous extract (orally) or injection of insulin (s.c). serum glucose was determined by Trinder method (3). The analysis of variance and F-test of significance was conducted to compare mean of all treated groups and days. The association between means of glucose levels and days of treatment was presented as regression coefficient (b). All statistical manipulation was checked according to Snederos and Cochran method (4).

**Results**
The results of this study revealed that the serum glucose levels of normal rabbits in group (1) were approximately with in the normal ranges of the serum glucose levels of the other groups before administration Alloxan. Using Alloxan resulted in significant elevation \((p< 0.0001)\) of the serum glucose levels of the rabbits in group (2) as compared with serum glucose levels of the normal rabbits in group (1). The difference was highly significant between the 1\(^{st}\) and 7\(^{th}\) days of the experiment as show in table I. The diabetic rabbits in group (3) were treated with daily insulin 2 I.U/kg (s.c) showed a high significant reduction in serum glucose levels \((p<0.001)\). The reduction was started with in the first day of treatment in comparison with serum glucose levels of diabetic rabbits in group (2). The treatment of diabetic rabbits with different extracts of *Medicago sativa* group (4), *Allium sativa* group (5), *Phaseolus vulgaris* group (6), *Petroselenium sativum* group (7), *Teucrium polium* group (9) and *Trigonell foenum graecem* group (10) revealed a significant reduction in serum glucose levels as compared with serum glucose levels of diabetic rabbits in group (2) \(P< 0.01\) as shown in table I. The change in serum glucose levels with extract was slowly reduced with comparison to the effect of insulin on serum glucose levels of diabetic rabbits group(3). There is no significant reduction in serum glucose levels of diabetic rabbits in group(8) after treatment with *mytrus communis* for 7 days \((p>0.05)\) as compared with serum glucose levels in diabetic rabbits in group(2). Indeed there is marked fluctuation of serum glucose levels along all days of treatment table I.
**Discussion:** Alloxan monohydrate is a diabetogenic agent that causes irreversible inhibition of pancreatic beta cells \(^5\). The dose of insulin that has been used was relevant to control hyperglycemia and to maintain the serum glucose levels within the normal range \(^6\).

The hypoglycemic effect of *Medicago sativa* which had been seen in this study, may be in part contributed to the manganese content of this plant (4.5-5mg/kg) which reported to be active principle responsible for this effect \(^7\).

Manganese is now recognized as necessary factor for adenine triphosphate (ATP) phosphorylation of beta-subunit of insulin receptors \(^{7,8}\). *Medicago sativa* contains saponine, which may be in part contributed as additional hypoglycemic agent \(^9\) also, presence of insulin releasing and insulin like activity in this plant \(^10\). An aqueous extract of *medicago sativa* stimulate 2-deoxy-glucose transport, glucose oxidation and in incorporation of glucose into glycogen in mouse abdominal muscle \(^10\).

The hypoglycemic effect of *Allium sativa* has been attributed to the volatile oils allyl-propyl disulfide oxide and di allyl-disulfide oxide \(^11\). Another study mentioned that garlic sulfoxide amino acid 5-methyl cystein sulfoxide and 5-allyl cysteine sulfoxide are associated with hypoglycemic effect of this plant \(^2\). Recent study demonstrated that aged garlic extract may prevent stress induced hyperglycemia and the risk of suffering from diabetes mellitus \(^13\).

The hypoglycemic effect of *Phaseolus vulgaris* may be in part related to the alkaloid trigonelline which known to have hypoglycemic effect \(^14\). The plant was significantly decreased the area under the glucose tolerance curve and the hyperglycemic peak in rabbit \(^15\). The chromium salts that present in *phaseolus vulgaris* may produce anti diabetic activity \(^16\).

The hypoglycemic effect of *Petroselenium sativum* may be related to unknown alkaloid which is present in this plant \(^2\) which employed for hypertension and diabetes therapy \(^17\).

The active compounds of *Teucrium polium* which posses hypoglycemic effect, are not determined yet, and may be related to unknown alkaloid \(^2\) or flavonoids and saponins which are present in this plant \(^9\).

The hypoglycemic effect of *Trigonella foenum graecum* seeds may be related to alkaloid trigonelline which present in the seeds \(^14\) or to the nicotinic acid which had been isolated from the seeds \(^8\) but chronic administration of defatted fraction of these seeds reduced glucagon and somatostatin concentration in healthy dogs \(^19\). A recent study demonstrates that the enzymes of these seeds are implicated in biosynthesis of 4-hydroxyl isoleucine which has insulin stimulating effect \(^2\). Trigonella foenum graecum plays a role in the modulation of some
gluconeogenic enzymes activities in diabetic rat \textsuperscript{(21)} also on glyoxalase activity \textsuperscript{(22)}. Administration of \textit{trigonella foenum graecum} significantly reduced the blood sugar, both the fasting and postprandial in NIDDM patients \textsuperscript{(23)}.
References


Means & standard errors of serum glucose levels (mg/100 ml) in normal rabbits (Group 1), diabetic rabbits (Group 2), diabetic rabbits treated with insulin (Group 3), diabetic rabbits treated with aqueous extract of *Medicago sativa* (Group 4), diabetic rabbits treated with aqueous extract of *Allium sativum* (Group 5), diabetic rabbits treated with aqueous extract of *Phaseolus vulgaris* (Group 6), diabetic rabbits treated with aqueous extract of *Petroselinum crispum* (Group 7), diabetic rabbits treated with aqueous extract of *Myrtus communis* (Group 8), diabetic rabbits treated with aqueous extract of *Teucrium polium* (Group 9), & diabetic rabbits treated with aqueous extract of *Trigonella foenum graecum* (Group 10).

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<td>175.83 ± 0.54</td>
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Values represent means of serum glucose levels (mg/100 ml) ± SE.

Pt- represent levels of serum glucose pretreatment.

**LSD 5%** = 1.72

**LSD 1%** = 2.26

**P < 0.0001**
Regression coefficient (b), of the serum glucose levels (mg /100 ml) within each treatment throughout the seven days of experiment.

<table>
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<th>Effect on serum sugar levels</th>
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<td><em>Teucrium polium</em></td>
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<td><em>Trigonella foenum graecum</em></td>
<td>Decrease</td>
<td>-8.98</td>
<td>I’ &lt; 0.01</td>
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<td>Decrease</td>
<td>-8.62</td>
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<td><em>Allium Sativum</em></td>
<td>Decrease</td>
<td>-7.82</td>
<td>P &lt; 0.01</td>
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<td><em>Petroselinum crispum</em></td>
<td>Decrease</td>
<td>-6.70</td>
<td>P &lt; 0.01</td>
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<td><em>Myrtus communis</em></td>
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NS = Not significant.
تأثيرا لخلاصة المائية لبعض النباتات الطبية في خفض سكر الدم بالأرانب

الخلاصة:

*حَن حوريق الالوكساث (100 ملغم/ كغم) لمكافحة داء السكر في الحيوانات الخاضعة ولقذ قيس هناوى السكر، والذم قبل وبعد أعطاء الالوكساث وكذلك بعذ أعطاء الأنسولين أو الخلاصت الوائيت للنباحاث الطبيت حيذ سبب الأنسولين (2 وحدة قياسية /كغم) تحت الجلد انخفاضا معتمدا بمستوى سكر الدم وكذلك ألجج (1 غم/كغم) والمعدوس (5 غم/كغم) والحلبة (6 غم/كغم) والفصوليا (4 غم/كغم) وغيرها تسبب انخفاضا معتمدا بمستوى السكر بالدم ما عدا الياس حيث يمكن الاستفادة منه في معالجة داء السكري.

الدكتور
فلاروق حسن الجواد