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## تقييم الفعالية المضادة للأكسدة لكل من الترايجونلين و المستخلص الكحولي لبذور الحلبة العراقية في الأرنب المستحدث فيها داء السكري

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### الملخص:

صممت هذه الدراسة لتقييم الفعالية المضادة للأكسدة لكل من الترايجونلين والمستخلص الكحولي لبذور الحلبة العراقية في الأرنب التي استحدثت فيها داء السكري بفعل الالوكسان. أعطيت جرعة بالفم للأرنب لمدة أربعة أسابيع بمقدار 10 ملغم / 12 ساعة يومياً من الترايجونلين و 7 مل / 12 ساعة من المستخلص الكحولي. تم قياس تركيز كل من المالدلديهايد وغلوتاثيون في البلازما كدليل لجهد الأكسدة في جميع الأرنب قبل العلاج ومن ثم مرة واحدة أسبوعياً بعد العلاج لفترة أربعة أسابيع.

أظهرت النتائج إن المستخلص الكحولي لبذور الحلبة خفض مستوى المالدلديهايد من  $2.5 \pm 0.3$  مايكرومول / لتر قبل العلاج إلى  $1.75 \pm 0.12$  مايكرومول / لتر بعد شهر واحد من العلاج، وأرتفع مستوى غلوتاثيون من  $0.11 \pm 0.02$  مايكرومول / لتر إلى  $0.26 \pm 0.02$  مايكرومول / لتر بعد شهر من العلاج. كانت نسبة الإنخفاض في مستوى المالدلديهايد هي 37%، بينما كانت نسبة الإرتفاع في مستوى غلوتاثيون 136% بعد شهر واحد من العلاج.

أظهرت النتائج إن الترايجونلين كان أقل فعالية من المستخلص الكحولي لبذور الحلبة في خفض مستوى المالدلديهايد أو زيادة مستوى غلوتاثيون، وتشير نتائج هذه الدراسة بوضوح إلى أن الترايجونلين والمستخلص الكحولي لبذور الحلبة العراقية لهما فعالية طبيعية واحدة ضد الأكسدة ويمكن استعمالهما في حالات مرضية عديدة كوقاية.



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ORIGINAL ARTICLE

# Effect of trigonelline and ethanol extract of Iraqi Fenugreek seeds on oxidative stress in alloxan diabetic rabbits

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## KEYWORDS

Fenugreek seeds;  
Trigonelline;  
Antioxidant

**Abstract** This study was designed to evaluate effect of trigonelline and ethanol extract of Iraqi Fenugreek seeds on oxidative stress in alloxan diabetic rabbits.

Oral dose of (10 mg/12 h) isolated trigonelline or (7.7 ml/12 h) of ethanol extract of Iraqi Fenugreek seeds was administered to the rabbits for four weeks. Plasma malondialdehyde (MDA) and glutathione (GSH), as oxidative stress markers were measured in all rabbits before treatment and once weekly for four weeks after the treatment. The results showed a significant improvement in the stress induced oxidation parameters by alloxan-induced diabetes in rabbits.

The ethanol extract of Fenugreek seeds significantly decreases the plasma malondialdehyde (MDA) from  $2.51 \pm 0.34 \mu\text{mol/l}$  in diabetic control rabbits to  $1.57 \pm 0.12 \mu\text{mol/l}$  after four weeks ( $P < 0.05$ ). The plasma glutathione (GSH) level increases significantly from  $0.11 \pm 0.02 \mu\text{mol/l}$  to  $0.26 \pm 0.02 \mu\text{mol/l}$  after four weeks of treatment of the ethanol extract ( $P < 0.05$ ). The percent reduction in plasma MDA level was 37.45%, while the percent elevation in plasma GSH was 136% after four weeks of treatment. The data showed that isolated trigonelline was less effective than the ethanol extract of Fenugreek seeds in lowering plasma MDA or increasing the plasma GSH markers. The results of this study clearly indicate that both ethanol extract of Fenugreek and its major alkaloid, trigonelline are promising natural antioxidants and may be used in the treatment of many diseases, especially diabetes mellitus.

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## 1. Introduction

Antioxidants are of great importance because of their involvement in decreasing the damages generated from free radicals, that are naturally produced or associated with various diseases such as cardiovascular diseases, diabetes mellitus, acute respiratory distress syndrome, inflammatory diseases and cancer (Young and Woodside, 2001; Maxwell, 1995; Kelly, 1998; Aisling et al., 1996; Rahimi et al., 2005; Manson et al., 1993; Guerrero et al., 2007; Toppo et al., 2009).

Fenugreek seeds are one of the important medicinal plants which are widely used in folk medicine. It is known to have diuretic, hypotensive, hypoglycemic and hypolipidemic effects

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(Newall et al., 1996; Ghosal et al., 1974; Ali and Azad-Khan Hassan, 1995; Bordia et al., 1997). Trigonelline, major alkaloid of Fenugreek seeds was reported to have a hypoglycemic effect in normal and alloxan diabetic rabbits (Adeeb et al., 2002). Shah et al. (2006) showed that the onset of action and maximum decrease in serum glucose were similar in glyburide and trigonelline treated animals, but they differ in respect to the mechanism of action. They proposed that the hypoglycemic effect of trigonelline due to enhanced pancreatic regeneration. Fenugreek extracts were also reported to exhibit antioxidant activity and could be used as a potent source of antioxidants (Syeda et al., 2008).

The aim of this study is to evaluate the antioxidant effect of isolated trigonelline and the ethanolic extract of Iraqi Fenugreek seeds in alloxan-induced diabetic rabbits.

## 2. Material and methods

### 2.1. Materials

In this investigation, the ethanol extract of Fenugreek seeds was prepared in our laboratories by refluxing the defatted Fenugreek seeds for 2 h and Trigonelline was isolated from Fenugreek seeds according to the method of Adeeb et al. (2002).

### 2.2. Experimental animals

#### 2.2.1. Preparation of alloxan-diabetic rabbits

Fasting rabbits were made diabetic by a single intraperitoneal dose of 75 mg/kg alloxan monohydrate (Hopkins and Williams, England) dissolved in 1 ml normal saline. After 10 h, animals received glucose water to avoid hypoglycemia. Diabetes was confirmed (after 24 h of alloxan injection) by the presence of glucosuria using urine test tap (Glukotest)<sup>®</sup> and by hyperglycemia using Lab kit for glucose. Animals were left for four weeks before starting the treatment. Experimental methods were approved by the graduate study committee at the college of pharmacy/University of Baghdad.

#### 2.2.2. Experimental design

Thirty male albino rabbits weighing  $1.36 \text{ kg} \pm 45\text{g}$  were used in this study. They were classified into: group 1 ( $N = 10$ ) normal (non-diabetic) untreated rabbits; group 2 ( $N = 10$ ) diabetic rabbits received 10 mg/12 h of isolated trigonelline; group 3 ( $N = 10$ ) diabetic rabbits received 7.7 ml/12 h of ethanol extract of Fenugreek seeds. Before treatment with ethanol extract or isolated trigonelline, diabetic rabbits (groups 2 & 3) received 7.7 ml distilled water orally twice daily for four weeks and were considered as diabetic control. Fasting blood samples were collected from the middle ear of these animals once weekly for four weeks and analyzed for the determination of glucose, malondialdehyde (MDA) and glutathione (GSH). Then diabetic rabbits, groups 2 & 3 received the mentioned dose of isolated trigonelline & ethanol extract of Fenugreek seeds, respectively. Both types of treatment were administered orally twice daily (every 12 h) for four weeks via oro-gastric tube to avoid any rejection of the dose. Fasting blood samples were collected from the middle ear artery at zero time (just before starting the treatment), and once weekly for four weeks

and analyzed for the determination of glucose, malondialdehyde (MDA) and glutathione (GSH). The treatment period with ethanol extract or trigonelline was four weeks. Xylol was used to facilitate blood sampling.

#### 2.2.3. Measurement of glucose levels

Glucose level was determined using the Lab kit for glucose. According to the method of Barham and Trindoer, 1972, glucose was measured after the enzymatic oxidation of glucose by glucose oxidase to form hydrogen peroxide and gluconate. Hydrogen peroxide then reacts with phenol and 4-aminophenazone in the presence of peroxidase to form quinonimine. The absorbance at 505 nm was determined against blank reagent and results expressed as mg/dl, based on the standard glucose solution treated in the same manner. The test involved mixing 1 ml of the working reagent with 10  $\mu\text{l}$  sample (or standard), incubating the mixture for 10 min. at 37 °C and the absorbance was measured at 505 nm.

#### 2.2.4. Measurement of plasma malondialdehyde (MDA) level

1.75 ml of saline azide was added to 0.25 ml of plasma and mixed with 0.5 ml of  $\text{H}_2\text{O}_2$  and 0.5 ml of 1% thiobarbituric acid (TBA) in 0.05 M sodium hydroxide. The mixture was incubated in a boiling water bath for 15 min to achieve color development. The tubes were cooled under tap water and the extent of MDA production was estimated from the absorbency at 532 and 543 nm.

MDA concentration was calculated using a molar absorptivity coefficient of  $1.56 \times 10^{-5} \text{ M}^{-1} \text{ cm}^{-1}$  and the results were expressed as micromole MDA/L ( $\mu\text{mol/l}$ ) (Stocks and Dormandy, 1971; Gilbert et al., 1984).

#### 2.2.5. Measurement of plasma glutathione (GSH) level

0.5 ml of plasma was mixed with 0.5 ml of 3 mM 5,5-Dithiobis (2-nitrobenzoic acid (DTNB) in phosphate buffer and 2.6 ml of 0.1 M phosphate buffer (pH 8)). The absorbency of the solution was measured at 412 nm during 2 min and the concentration of GSH was calculated by comparison with a standard curve prepared for this purpose (Goldin et al., 1988).

#### 2.2.6. Statistical analysis

The data were statistically analyzed using the analysis of variance (ANOVA). Results were expressed as mean  $\pm$  standard error of mean (SEM). The results were considered significant with  $P < 0.05$ .

## 3. Results and discussion

Before treatment with ethanol extract of Fenugreek seeds or isolated trigonelline, the results showed that the mean blood glucose level in normal rabbits (group 1) was  $98 \pm 3.08 \text{ mg/dl}$  ( $5.44 \pm 0.17 \text{ mmol/l}$ ) while in groups 2 & 3 (before treatment, diabetic control) was  $390 \pm 15.41 \text{ mg/dl}$  ( $21.66 \pm 0.85 \text{ mmol/l}$ ) &  $381 \pm 22 \text{ mg/dl}$  ( $21.16 \pm 1.22 \text{ mmol/l}$ ), respectively. Statistical analysis showed that the mean blood glucose was increased significantly in diabetic control compared to group 1 ( $p < 0.05$ ) which confirmed the state of diabetes that was induced by alloxan dose. The data also showed that the mean  $\pm$  SEM plasma MDA and GSH in normal rabbits (group 1) were  $1.13 \pm 0.2$  and  $0.28 \pm 0.03 \mu\text{mol/L}$ , respectively. The mean  $\pm$  SEM plas-

**Table 1** Effect of ethanol extract of Fenugreek seeds (7.7 ml/12 h) on plasma MDA and GSH levels in alloxan diabetic rabbits.

Parameter	Control <sup>c</sup> group	Diabetic <sup>d</sup> control	Diabetic after treatment with ethanol extract of Fenugreek seed			
			1st week	2nd week	3rd week	4th week
MDA <sup>a</sup>	1.13 ± 0.2	2.51 ± 0.34*	2.52 ± 0.19	2.17 ± 0.23	1.96 ± 0.26	1.57 ± 0.12**
GSH <sup>b</sup>	0.28 ± 0.03	0.11 ± 0.02*	0.11 ± 0.05	0.18 ± 0.02	0.22 ± 0.03	0.26 ± 0.02**

<sup>a</sup> Plasma MDA (μmol/L) ± SEM.

<sup>b</sup> Plasma GSH (μmol/L) ± SEM.

<sup>c</sup> Control: normal rabbits (non-diabetic, group 1).

<sup>d</sup> Diabetic control (diabetic rabbits received distilled water).

\* Significant difference with respect to control group ( $P < 0.05$ ).

\*\* Significant difference with respect to diabetic control ( $P < 0.05$ ).

**Table 2** Effect of isolated trigonelline (10 mg/12 h) on plasma MDA and GSH levels in alloxan diabetic rabbits.

Parameter	Control <sup>c</sup> group	Diabetic <sup>d</sup> control	Diabetic after treatment with isolated trigonelline			
			1st week	2nd week	3rd week	4th week
MDA <sup>a</sup>	1.13 ± 0.2	2.28 ± 0.22*	2.26 ± 0.19	2.11 ± 0.25	1.35 ± 0.27	1.75 ± 0.15
GSH <sup>b</sup>	0.28 ± 0.03	0.16 ± 0.05*	0.16 ± 0.04	0.17 ± 0.03	0.17 ± 0.02	0.19 ± 0.01

<sup>a</sup> Plasma MDA (μmol/L) ± SEM.

<sup>b</sup> Plasma GSH (μmol/L) ± SEM.

<sup>c</sup> Control: normal rabbits (non-diabetic, untreated rabbits).

<sup>d</sup> Diabetic control (diabetic rabbits received distilled water).

\* Significant difference with respect to control group ( $P < 0.05$ ).

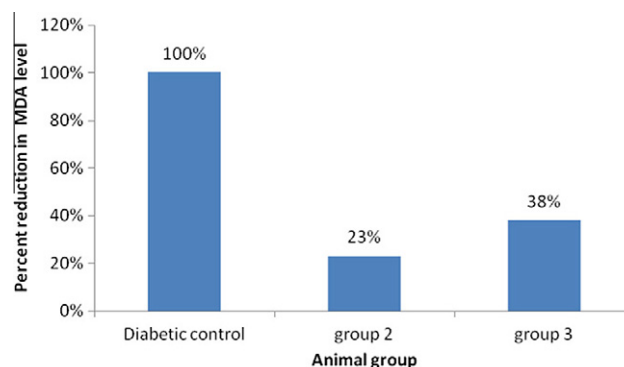
ma MDA, GSH in diabetic control rabbits were  $2.51 \pm 0.34$ ,  $0.11 \pm 0.02$  and  $2.28 \pm 0.22$ ,  $0.16 \pm 0.05$  μmol/L, respectively. Statistical analysis showed that there was a significant increase in MDA and decrease in GSH level in diabetic control rabbits compared to the normal rabbits ( $P < 0.05$ ) which confirmed the state of oxidative stress in alloxan-induced rabbits as shown in Tables 1 and 2.

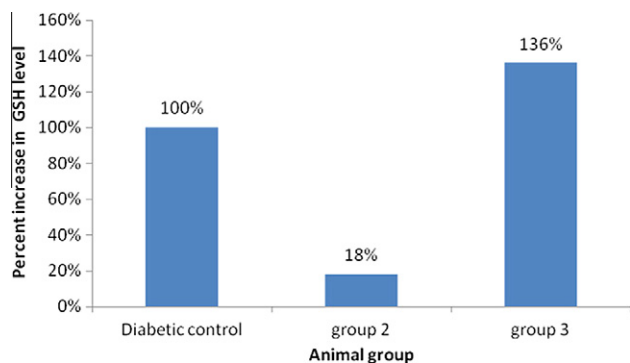
The data showed that treatment with the ethanol extract of Fenugreek seeds exhibited gradual reduction in plasma MDA level during three weeks, while plasma GSH was gradually elevated but it was not significant during the three weeks of treatment (Table 1). However, there was a significant reduction ( $p < 0.05$ ) in plasma MDA and elevation ( $p < 0.05$ ) in plasma GSH level after four weeks of treatment compared to the diabetic control (pretreatment) as shown in Table 1. Trigonelline, on the other hand gradually, reduced plasma MDA and elevated plasma GSH after four weeks of treatment and these changes in plasma MDA & GSH were not significant ( $p > 0.05$ ) compared to diabetic control as shown in Table 2.

**Table 3** Percent of reduction in plasma MDA and elevation in plasma GSH after four weeks of treatment with trigonelline and ethanol extract of fenugreek seeds.

Parameter	Four weeks treatment with isolated trigonelline (group 2)	Four weeks treatment with ethanol extract (group 3) (%)
% of Reduction in plasma MDA	23.24%	37.69
% of Elevation in plasma GSH	18.36%	136.36

The changes in plasma level of MDA and GSH (increase in MDA & decrease in GSH) represent 100% in diabetic control rabbits due to the oxidative stress state that was induced by alloxan dose. Therefore, the percent of reduction in plasma MDA after four weeks of treatment with isolated trigonelline or ethanol extract was calculated and presented in Table 3 and Fig. 1. Similarly, the percent of elevation in plasma GSH after four weeks of treatment with isolated trigonelline or ethanol extract is illustrated in Table 3 and Fig. 2. The data in Table 3, Figs. 1 and 2, clearly showed that both isolated trigonelline and ethanol extracts of Fenugreek seeds affect the plasma level of MDA and GSH but the magnitude of the effect is different. The effect of the ethanol extract of Fenugreek seeds was more (reduced plasma MDA level by 37.67%

**Figure 1** Percent of plasma MDA level decreased after four weeks of treatment with 10 mg/12 h of isolated trigonelline (group 2) and 7.7 ml/12 h of ethanol extract of Fenugreek seeds (group 3) in alloxan diabetic rabbits. Diabetic control: diabetic rabbits without treatment.



**Figure 2** Percent of plasma GSH level increased after four weeks of treatment with 10 mg/12 h of isolated trigonelline (group 2) and 7.7 ml/12 h of ethanol extract of Fenugreek seeds (group 3) in alloxan diabetic rabbits. Diabetic control: diabetic rabbits without treatment.

and elevated GSH level by 136.36%) than that of isolated trigonelline, which only reduced plasma level of MDA by 23.24% & elevated plasma GSH level by 18.36.

The results of this study revealed that ethanol extract of Fenugreek seeds had a significant improvement ( $p < 0.05$ ) in the oxidative stress parameters which could be attributed to the flavonoid content of Fenugreek such as vexitin, isovexitin and orientin.

Although, trigonelline also reduced oxidative stress markers this improvement was not significant ( $p > 0.05$ ). This may be due to the dose given or due to the short period of the study. Fenugreek seeds have been reported to have antioxidant activity in vitro (Anuradha and Ravikumar, 1998; Hettiarachchy et al., 1996)

The ethanol extract of Fenugreek seeds & isolated trigonelline reduced blood glucose and lipid profile in alloxan-diabetic rabbits (Adeeb et al., 2002). This effect may be explained in part due to the antioxidant property of trigonelline because of its structural similarity to nicotinamide which has an antioxidant effect (Velikii et al., 1992). Furthermore, nicotinic acid, one of the metabolite of trigonelline, also had an antioxidant effect.

In conclusion both, ethanol extract of Fenugreek and its major alkaloid, trigonelline are promising natural antioxidants and long trial period; different dose and total antioxidant status (TAS) measurement may be needed in the future.

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