Anti-hyperglycemic Effects of Saffron and its Active Constituents, Crocin and Safranal, in Alloxan-Induced Diabetic Rats

Kianbakht S (Ph.D.)[1]*, Hajiaghaee R (Ph.D.)[2]

1- Department of Pharmacology and Applied Medicine, Institute of Medicinal Plants, ACECR, Karaj, Iran
2- Department of Pharmacognosy and Pharmaceutics, Institute of Medicinal Plants, ACECR, Karaj, Iran
*Corresponding author: Iranian Academic Center for Education, Culture and Research (ACECR), Institute of Medicinal Plants, Karaj, Iran
P.O.Box: 33651/66591
Tel: +98 - 261 - 4764010 - 9, Fax: +98 - 261 - 4764021
Email: skianbakht@yahoo.com

Receive: 25 June 2011 **Acceptance:** 23 Aug. 2011

Abstract

Background: Saffron is the dried stigmata of the flowers of saffron (*Crocus sativus* L., Iridaceae). Saffron and its major active constituents, crocin (crocetin glycoside), crocetin and safranal, have a variety of pharmacological effects including high antioxidant properties and crocetin has insulin sensitizing effect possibly due to its antioxidant activity. Oral crocin is not absorbed and is hydrolyzed and absorbed as the active metabolite crocetin in the rat intestines.

Objective: Study of the saffron, crocin and safranal effects on the blood levels of fasting glucose, HbA1c (glycosylated hemoglobin) and liver/kidney function tests in alloxan-diabetic rats.

Methods: The effects of 6 weeks' daily oral administration of saffron methanolic extract, crocin and safranal on the fasting blood glucose, HbA1c, insulin, creatinine and SGOT and SGPT levels after single alloxan intraperitoneal injection at a dose of 125 mg/kg in rats were evaluated.

Results: Alloxan injection resulted in significant increase of fasting blood glucose and HbA1c levels but decreased blood insulin levels significantly. Saffron methanolic extract (80 and 240 mg/kg), crocin (50 and 150 mg/kg) and safranal (0.25 and 0.5 ml/kg) significantly reduced the fasting blood glucose and HbA1c levels but significantly increased the blood insulin levels without any significant effects on the blood SGOT, SGPT and creatinine levels in the diabetic rats compared with the control diabetic rats.

Conclusion: The results suggest that saffron may have anti-hyperglycemic and blood insulin level elevating effects without hepatic and renal toxicities in the alloxan - diabetic rats. Further, crocin, crocetin and safranal may be involved in these effects of saffron.

Keywords: Anti-hyperglycemic, *Crocus sativus*, Saffron, Crocin, Safranal, Alloxan

[Downloaded from jmp.ir on 2026-11-03]

Introduction

Diabetes mellitus is the most prevalent metabolic disorder. The prevalence of diabetes is nearly 6% of the population and the diabetes type 2 constitutes 90 to 95% of it. If diabetes is not duly treated, it will lead to serious complications such as atherosclerosis, retinopathy, nephropathy, neuropathy and limbs gangrene. The complications are the main causes of morbidities and mortalities due to diabetes [1, 2]. While insulin and oral anti-hyperglycemic drugs such as biguanides, sulfonylureas, thiazolidinediones and alpha-glucosidase inhibitors are the cornerstone of the diabetes treatment, they have important adverse effects and can not always maintain euglycemia and prevent diabetes complications significantly [3,4]. Thus there is a continuing need for alternative anti-diabetic remedies with better risk-benefit ratios and greater patient acceptability [1, 2, 4]. Plants have always been sources of drugs and many of the existing drugs have originated from plants directly or indirectly. Ethnobotanical studies have reported more than 1200 plant species with potential anti-diabetic effects [5].

Saffron is the dried stigmata of the flowers of saffron (*Crocus sativus* L., Iridaceae) and is cultivated principally in Iran and on a small scale in Morocco, India, Greece, Italy, Spain and France. In addition to being a widely used food additive, saffron is used in the traditional medicine for the treatment of numerous diseases including depression, cognitive disorders, seizures and cancer [6, 7]. Crocin (crocetin glycoside), crocetin and safranal are the major active constituents of saffron [8]. Previous studies have demonstrated a wide variety of pharmacological effects of saffron and its active constituents such as anti-tumor, anti-genotoxic, memory and learning enhancing, neuroprotective, analgesic and anti-inflammatory, anti-convulsant, anti-anxiety, anti-depressant, anti-hypertensive and anti-hyperlipidemic effects [6, 7]. Further, saffron, crocin, crocetin and safranal have high antioxidant activities [9, 10, 11].

Crocetin has increased insulin sensitivity and ameliorated abnormalities related to insulin resistance such as impaired glucose tolerance, hyperinsulinemia, dyslipidemia and hypertension due to high-fructose diet and dexamethasone injection in rats [12, 13]. Crocetin attenuated the palmitate-induced insulin insensitivity in the rat adipocytes [14]. Also, crocetin improved the insulin resistance induced by high-fat diet in rats [15]. The antioxidant effects of crocetin may, at least in part, explain the ability of this compound to attenuate insulin insensitivity [13, 14]. In addition, crocetin inhibited adhesion of leukocytes to the bovine aortic endothelial cells (BEC) induced by advanced glycation end products (AGEs) and AGEs-induced BEC apoptosis possibly through its antioxidant activity and thus it has been suggested that crocetin may prevent diabetes-associated vascular complications [16, 17]. Oxidative stress can cause insulin resistance and the long-term complications of diabetes. Antioxidants may be very important in mitigating impaired insulin secretion and action in insulin resistance and prevent diabetes complications [18, 19].

Various studies have shown that both type 1 and type 2 diabetes are associated with increased oxidative stress and decrease in antioxidant potential due to increased formation of free radicals [18]. Antioxidants have been demonstrated to increase glucose disposal [20]. Furthermore, treatment modalities that focus on reducing oxidative stress may be good choices for diabetes therapy [21]. Also, plants used for the

[Downloaded from jmp.ir on 2026-11-03]

symptoms of diabetes or its complications have been shown to have high antioxidative potentials [20].

Alloxan-induced diabetes in the rat is an animal model of type 1 diabetes [22]. Saffron extract had anti-hyperglycemic and hypoglycemic effects in alloxan-diabetic and non-diabetic rats respectively. Further, saffron extract increased serum insulin levels in alloxan-diabetic and non-diabetic rats and caused regeneration of β-cells in alloxan-diabetic rats [23, 24]. However, the effects of saffron active constituents (crocin and safranal) on the blood levels of glucose, glycosylated hemoglobin (HbA1c) and insulin and liver/kidney function tests and the effects of saffron on the blood HbA1c levels and liver/kidney function tests in the diabetic rats have not been evaluated so far. Thus, we tested the effects of saffron, crocin and safranal on the blood fasting glucose, HbA1c, insulin, creatinine and the liver enzymes SGOT and SGPT levels in the model of alloxan-induced diabetes of the rat.

Materials and Methods

Saffron

The stigmas of *Crocus sativus* were collected from the lands of Ghaen in the Iranian province of southern Khorasan in December and dried in shade followed by grinding. The identity of *Crocus sativus* was authenticated by a botanist (Y. Ajani) and a voucher specimen of the plant (number 1023) was deposited in the Tehran University Central Herbarium.

Preparation of the saffron extract

The dried stigmas powder (260 g) was extracted with methanol/water (80/20) as the solvent in a percolator three times, the solvent was completely removed from the methanolic extract at 42 ºC by Rotavapor and 30 g dried extract was produced.

Spectrophotometric analysis of the saffron extract

To standardize the saffron extract, ultraviolet–visible spectra were recorded using a PerkinElmer model Lambda 25 spectrophotometer in the range from 200 to 700 nm. The spectra were run by using 50 µl of the working solutions that were further diluted to 1 ml with water and poured directly into the quartz cuvette. Absorbance readings at 257 nm, 330 nm and 440 nm were related back to the 1% solution and expressed as $E^{1\%}$ (257) bitterness, $E^{1\%}$ (330) fragrance and $E^{1\%}$ (440) coloring strength, according to the ISO 3632-2-1993 (ISO, 2003) [25].

Drugs

Crocin, safranal, alloxan and glibenclamide (purity above 99%) were purchased from Sigma. For dilution, crocin, safranal, alloxan and the extract were dissolved in physiological saline and glibenclamide was dissolved in physiological saline by DMSO (dimethyl sulfoxide) (Merck) (10%). All drugs were prepared immediately before use.

Animals

Male adult Wistar rats (175 ± 25 g) from our own breeding colony were used. Animals were maintained under standard environmental conditions and had free access to standard rodent feed and water.

Induction of diabetes

Animals were given alloxan in a single intraperitoneal injection at a dose of 125 mg/kg. Two weeks after the injection of alloxan, diabetic rats with fasting (after food deprivation for 8 h) blood glucose levels above

[Downloaded from jmp.ir on 2026-11-03]

200 mg/dl were used for the experiments.

Experimental protocol

The animals were randomly divided into eleven groups (N = 10 in each group):

Group I: Normal healthy control rats received physiological saline.

Group II: Normal healthy control rats received 10% DMSO in physiological saline (3 ml/kg).

Group III: Alloxan-diabetic rats received physiological saline.

Group IV: Alloxan-diabetic rats received 10% DMSO in physiological saline (3 ml/kg).

Group V: Alloxan-diabetic rats received the extract (80 mg/kg).

Group VI: Alloxan-diabetic rats received the extract (240 mg/kg).

Group VII: Alloxan-diabetic rats received crocin (50 mg/kg).

Group VIII: Alloxan-diabetic rats received crocin (150 mg/kg).

Group IX: Alloxan-diabetic rats received safranal (0.25 ml/kg).

Group X: Alloxan-diabetic rats received safranal (0.5 ml/kg).

Group XI: Alloxan-diabetic rats received glibenclamide (5 mg/kg in 3 ml/kg DMSO 10%).

The extract, crocin, safranal and glibenclamide were administered at doses that when given orally once, lowered the fasting blood glucose levels of the alloxan diabetic rats significantly compared with the control diabetic rats. Further, the data given here relate to the doses that not only did not cause any mortality in the diabetic rats after 6 weeks' daily oral administration but also the effect of each dose on the blood glucose level at the endpoint was significantly different from the other dose of the same substance. Each animal was used only once in all experiments. Animals were treated by oral gavage once a day for 6 weeks. At the end of the sixth week, while the rats had been deprived from food for 8 h, blood was drawn from their tail veins and the blood glucose, HbA1c, insulin, creatinine and the liver enzymes SGOT and SGPT levels were measured. The research ethics committee of the Medicinal Plants Institute approved the protocol.

Biochemical assays

Fasting blood serum glucose level was measured by the glucose oxidase method (Pars Azmoon kit), blood glycosylated hemoglobin (HbA1c) level was measured by ion-exchange chromatography using DSS Pink-300 test kit (Drew Scientific Limited, UK) and the serum creatinine and SGOT and SGPT levels were determined using standard enzymatic kits produced by the Pars Azmoon company (Tehran, Iran) and an auto analyzer (Hitachi 902, Japan) from the whole blood immediately after drawing the blood sample. The serum insulin levels were measured by a sensitive rat insulin radioimmunoassay kit (Linco Research, Inc., St. Charles, MO).

Statistical analysis:

All data are expressed as mean ± standard error of the mean. Groups of data were compared with one way analysis of variance followed by Tukey's post hoc test. Data were considered statistically significant when $p < 0.05$.

Results

Spectrophotometric analysis of the saffron extract

$E^{1\%}$ (257) bitterness, $E^{1\%}$ (330) fragrance and $E^{1\%}$ (440) coloring strength of the extract were 95.8, 42.3 and 254 respectively.

[Downloaded from jmp.ir on 2026-11-03]

Effects on the blood glucose levels

There was no significant difference between the blood glucose levels of the normal control rats receiving physiological saline (group I) and the normal control rats receiving 10% DMSO in physiological saline (group II) after 6 weeks ($p > 0.05$). The blood glucose level of the diabetic control group receiving 10% DMSO in physiological saline (group IV) was not significantly different from the blood glucose level of the diabetic control group receiving physiological saline (group III) at the end of 6 weeks ($p > 0.05$). The blood glucose levels increased significantly in the diabetic control groups III and IV after 6 weeks of alloxan administration compared with the groups I and II ($p < 0.01$). The extract, crocin, safranal and glibenclamide reduced the blood glucose levels of the diabetic rats significantly compared with the diabetic control groups after 6 weeks of administration (Table 1).

Effects on the blood HbA1c levels

The blood HbA1c level of the normal control group receiving physiological saline (group I) was not significantly different from the blood HbA1c level of the normal control group receiving 10% DMSO in physiological saline (group II) after 6 weeks ($p > 0.05$). There was no significant difference between the blood HbA1c level of the diabetic control group receiving 10% DMSO in physiological saline (group IV) and the blood HbA1c level of diabetic control group receiving physiological saline (group III) at the end of 6 weeks ($p > 0.05$). However the blood HbA1c levels in the diabetic control groups (groups III and IV) were significantly higher than the

Table 1- Effects of the saffron methanolic extract and its active constituents, crocin and safranal, on the fasting blood glucose, glycosylated hemoglobin (HbA1c) and insulin levels after daily oral gavage for six weeks in rats.

Treatment groups (N = 10 in each group)	Blood glucose (mg/dl)	Blood HbA1c (%)	Blood insulin (µU/ml)
Normal control (physiological saline)	68.72 ± 2.61	7.36 ± 0.08	14.53 ± 0.28
Normal control (10% DMSO in physiological saline)	71.25 ± 2.36	7.26 ± 0.07	14.36 ± 0.32
Diabetic control (physiological saline)	361.51 ± 2.81*	12.2 ± 0.17*	6.22 ± 0.16*
Diabetic control (10 % DMSO in physiological saline)	364.92 ± 2.17*	12.26 ± 0.06*	6.12 ± 0.33*
Saffron extract (80 mg/kg)	230.36 ± 2.33**	9.25 ± 0.13**	11.19 ± 0.22**
Saffron extract (240 mg/kg)	145.07 ± 2.62**	8.3 ± 0.16**	12.15 ± 0.13**
Crocin (50 mg/kg)	252.12 ± 3.08**	9.72 ± 0.11**	10.92 ± 0.28**
Crocin (150 mg/kg)	125.63 ± 2.17**	6.85 ± 0.12**	12.23 ± 0.31**
Safranal (0.25 ml/kg)	137.64 ± 2.21**	7.12 ± 0.07**	12.81 ± 0.26**
safranal (0.5 ml/kg)	73.51 ± 2.48***	7.9 ± 0.09**	13.81 ± 0.12**
glibenclamide (5 mg/kg in 3 ml/kg DMSO 10%)	115.83 ± 3.25**	6.16 ± 0.05**	12.97 ± 0.27**

The data are given as mean ± standard error of the mean
*p<0.01, compared with normal healthy rats
**p<0.01, compared with diabetic control rats
***p<0.05, compared with diabetic control rats

[Downloaded from jmp.ir on 2026-11-03]

blood HgA1c levels of the normal control groups (groups I and II) ($p< 0.01$). The extract, crocin, safranal and glibenclamide reduced significantly the blood HbA1c levels of the diabetic rats compared with the control diabetic groups after 6 weeks ($p< 0.01$) (Table 1).

Effects on the blood insulin levels

No significant difference was observed between the blood insulin levels of the normal control rats receiving physiological saline (group I) and the normal control rats receiving 10% DMSO in physiological saline (group II) after 6 weeks ($p> 0.05$). There was no significant difference between the blood insulin levels of the diabetic control group receiving 10% DMSO in physiological saline (group IV) and the diabetic control group receiving physiological saline (group III) at the end of 6 weeks ($p > 0.05$). The blood insulin levels of the diabetic control groups III and IV decreased significantly after 6 weeks of alloxan administration compared with the groups I and II ($p < 0.01$). The extract, crocin, safranal and glibenclamide increased the blood insulin levels of the diabetic rats significantly compared with the diabetic control groups after 6 weeks of administration (Table 1).

Effects on the blood SGOT, SGPT and creatinine levels:

The extract, crocin, safranal and glibenclamide did not have any significant effects on the blood SGOT, SGPT and creatinine levels in the diabetic rats compared with the diabetic control groups after 6 weeks of administration.

Discussion

The saffron extract, crocin, safranal, and glibenclamide reduced the blood glucose and HbA1c levels but increased the blood insulin levels significantly without any significant effects on the liver and kidney functions in the alloxan-induced diabetic rats compared with the diabetic control rats. Oral crocin is not absorbed and is hydrolyzed to crocetin in the intestines which is absorbed in the rat (26). Thus, it follows that crocetin as a constituent of saffron and an active metabolite of crocin in the body may be involved in the anti-hyperglycemic effect of saffron and crocin in rats.

Regarding that HbA1c has usually served as an indicator of glycemic control over the preceding 2- to 3- month period in the diabetic patients (26), the present study demonstrates that saffron, crocin and safranal, may effectively control glycemia in the alloxan-induced diabetes model of the rat without hepatic and renal toxicities. Thus, it seems that crocin, crocetin and safranal may be involved in the effects of saffron in the alloxan-diabetic rats.

The mechanisms by which saffron, crocetin and safranal reduce the glucose level and increase the insulin level cannot be explained from the present study and so need further investigations. However, an earlier study demonstrated that saffron caused β cell regeneration in the alloxan-diabetic rats (23). Moreover, it seems that further research into the effects of saffron in the other animal models of diabetes and conduction of clinical trials on the anti-hyperglycemic effect of saffron in type 1 and type 2 diabetic patients are warranted.

Acknowledgements

We are grateful to the ACECR (Iranian Academic Center for Education, Culture and Research) for sponsoring this study.

[Downloaded from jmp.ir on 2026-11-03]

References

1. Shane-McWhorter L. Botanical dietary supplements and the treatment of diabetes: What is the evidence? *Curr. Diab. Rep.* 2005; 5: 391 - 8.

2. Zareba G, Serradell R, Castaner R, Davies SL, Prous J and Mealy N. Phytotherapies for diabetes. *Drugs Future.* 2005; 30: 1253-1282.

3. Dey L, Attele AS and Yuan CS. Alternative therapies for type 2 diabetes. *Altern. Med. Rev.* 2002; 7: 45 - 58.

4. Gilbert MP and Pratley RE. Efficacy and safety of incretin-based therapies in patients with type 2 diabetes mellitus. *Eur. J. Intern. Med.* 2009; 20: S309 - S318.

5. Marles R and Farnsworth NR. Antidiabetic plants and their active constituents. *Phytomedicine* 1995; 2: 137 - 46.

6. Schmidt M, Betti G and Hensel A. Saffron in phytotherapy: pharmacology and clinical uses. *Wien. Med. Wochenschr.* 2007; 157: 315 - 9.

7. Bathaie SZ and Mousavi Z. New applications and mechanisms of action of saffron and its important ingredients. *Crit. Rev. Food Sci. Nutr.* 2010; 50: 761 - 86.

8. Liakopoulou-Kyriakides M. and Kyriakidis DA. *Crocus sativus* biological active constituents. *Studies Nat. Prod. Chem.* 2002; 26: 293 - 312.

9. Assimopoulou AN, Sinakos Z and Papageorgiou VP. Radical scavenging activity of *Crocus sativus* L. extract and its bioactive constituents. *Phytother. Res.* 2005; 19: 997 - 1000.

10. Kanakis CD, Tarantilis PA, Tajmir-Riahi HA and Polissiou MG. Crocetin, dimethylcrocetin and safranal bind human serum albumin: stability and antioxidative properties. *J. Agric. Food Chem.* 2007; 55: 970 - 7.

11. Chen Y, Zhang H, Tian X, Zhao C, Cai L, Liu Y, Jia L, Yin HX and Chen C. Antioxidant potential of crocins and ethanol extracts of *Gardenia jasminoides* ELLIS and *Crocus sativus* L.: A relationship investigation between antioxidant activity and crocin contents. *Food Chem.* 2008; 109: 484 - 92.

12. Xi L, Qian Z, Shen X, Wen N and Zhang Y. Crocetin prevents dexamethasone-induced insulin resistance in rats. *Planta Medica* 2005; 71: 917 - 22.

13. Xi L, Qian Z, Xu G, Zheng S, Sun S, Wen N, Sheng L, Shi Y and Zhang Y. Beneficial impact of crocetin, a carotenoid from saffron, on insulin sensitivity in fructose-fed rats. *J. Nutr. Biochem.* 2007; 18: 64 - 72.

14. Xi L, Qian Z, Xu G, Zhou C and Sun S. Crocetin attenuates palmitate-induced insulin insensitivity and disordered tumor necrosis factor-α and adiponectin expression in rat adipocytes. *Br. J. Pharmacol.* 2007; 151: 610 - 7.

15. Sheng L, Qian Z, Shi Y, Yang L, Xi L, Zhao B, Xu X and Ji H. Crocetin improves the insulin resistance induced by high-fat diet in rats. *Br. J. Pharmacol.* 2008; 154: 1016 - 24.

16. Xiang M, Qian ZY, Zhou CH, Liu J and Li WN. Crocetin inhibits leukocyte adherence to vascular endothelial cells induced by AGEs. *J. Ethnopharmacol.* 2006; 107: 25 - 31.

17. Xiang M, Yang M, Zhou C, Liu J, Li W and Qian Z. Crocetin prevents AGEs-induced vascular endothelial cell apoptosis. *Pharmacol. Res.* 2006; 54: 268 - 74.

18. Rahimi R, Nikfar S, Larijani B and Abdollahi M. A review on the role of

[Downloaded from jmp.ir on 2026-11-03]

antioxidants in the management of diabetes and its complications. *Biomed. Pharmacother* 2005; 59: 365 - 73.

19. Evans JL. Antioxidants: do they have a role in the treatment of insulin resistance? *Indian J. Med. Res.* 2007; 125: 355 - 72.

20. McCune LM and Johns T. Antioxidant activity relates to plant part, life form and growing condition in some diabetes remedies. *J. Ethnopharmcol.* 2007; 112: 461 - 9.

21. Rolo AP and Palmeira CM. Diabetes and mitochondrial function: role of hyperglycemia and oxidative stress. *Toxicol. Appl. Pharmacol.* 2006; 212: 167 - 78.

22. Parmar NS and Prakash S. Screening methods in pharmacology. Alpha Science International Ltd. India. 2006, p: 290.

23. Mohajeri D, Mousavi G and Doustar Y. Antihyperglycemic and pancrease-protective effects of *Crocus sativus* L. (saffron) stigma ethanolic extract on rats with alloxan-induced diabetes. *J. Biol. Sci.* 2009; 9: 302 - 10.

24. Arasteh A, Aliyev A, Khamnei S, Delazar A, Mesgari M and Mehmannavaz Y. Effects of hydromethanolic extract of saffron (*Crocus sativus*) on serum glucose, insulin and cholesterol levels in healthy male rats. *J. Med. Plants Res*. 2010; 4: 397 - 402.

25. ISO, 2003. Saffron (*Crocus sativus* Linnaeus) Part 1: Specifications, and Part 2: Test Methods. International Standards Organisation, Geneva, Switzerland, ISO 3632-1/2.

26. Little RR and Sacks D B. HbA_{1c}: how do we measure it and what does it mean? *Curr. Opin. Endocrinol. Diabetes Obes.* 2009; 16: 113 - 8.

[Downloaded from jmp.ir on 2026-11-03]