


Original Article

Antidiabetic effect of hydroethanolic extract of *Crocus sativus* stigmas, tepals and leaves in streptozotocin-induced diabetic rats

Sabir Ouahhoud^{1*} , Iliass Lahmass¹, Mohamed Bouhrim², Amine Khoulati¹, Assia Sabouni¹, Redouanae Benabbes¹, Abdeslam Asehrou¹, Mohammed Choukri³, Mohamed Bnouham², Ennouamane Saalaoui¹

1. Laboratory of Biochemistry and Biotechnology, Department of Biology, Faculty of Sciences, University Mohamed Premier, Oujda, Morocco

2. Laboratory of Physiology, Genetic and Ethnopharmacology, Department of Biology, Faculty of Sciences, University Mohamed Premier, Oujda, Morocco

3. Laboratory of Biochemistry, Central Laboratory Service - CHU, Mohammed VI, Faculty of Medicine and Pharmacy, University Mohamed Premier, Oujda, Morocco

Abstract

Introduction: The present study investigated for the first time, the antihyperglycemic effect of tepals and leaves of *Crocus sativus* in streptozotocin-induced diabetic rats. The effect of these by-products were compared with that of saffron stigma and glibenclamide.

Methods: Hydroethanolic extracts (stigmas, tepals and leaves) and glibenclamide were administered orally in aqueous solution daily for 21 days. In the present study, 36 male rats were used. Six rats were used in each group. Group 1: normal control rats (N), received distilled water; group 2: diabetic control rats (D), received distilled water; group 3: treated diabetic rats (TPL), received tepal (TPL) extract; group 4: treated diabetic rats (STG), received stigma (STG) extract; group 5: treated diabetic rats (LF), received leaf (LF) extract and group 6: treated diabetic rats (GLB), received glibenclamide (GLB). Blood glucose, body weight, water intake, urine elimination, plasma triglycerides, cholesterol, urea, creatinine, aspartate amino transferase (AST) and alanine amino transferase (ALT) levels were evaluated.

Results: The present data indicated that the tepals and stigmas extracts significantly prevented decreasing body weight and protected against elevation of water intake, urine elimination, blood glucose, plasma triglycerides, cholesterol, urea, creatinine, AST and ALT levels in treated diabetic rats as compared to untreated diabetic rats. Leaves extract significantly prevented decreasing body weight. It decreased water intake, blood glucose, plasma triglycerides, cholesterol, creatinine, AST and ALT.

Conclusion: Based on our data, the oral administration of tepals, stigmas and leaves extract of *Crocus sativus* reduces blood glucose levels and improves control of diabetes complications.

Keywords:

Crocus sativus;
Antihyperglycemic effect;
Streptozotocine;
Saffron;
By-products

Received: 11 Oct 2018

Accepted: 15 Feb 2019

*Correspondence to:

S. Ouahhoud

Tel: +212-677141340

Fax: +212-536500603

Email:

s.ouahhoud@ump.ac.ma

Introduction

Diabetes mellitus is one of the principal causes of

mortality and morbidity in the world (Zimmet et al., 2016). It's a major chronic disease caused when the pancreases doesn't secrete sufficing insulin or when the body can't efficaciously use this insulin (Tousoulis

et al., 2012). The increase of oxidative stress and the alteration of the antioxidant defense system, are the main disorders associated with diabetes mellitus, which contributes to the initiation and progression of diabetes complications (Maritim et al., 2003).

Medications used to treat diabetes are expensive and associated with undesirable side effects. For example, glibenclamide tended to increase plasma insulin and risk of hypoglycemia (Segal et al., 1997). Recently, research has focused on natural agents, such as medicinal plants, that provide effective, reliable and inexpensive treatment (Samarghandian et al., 2017). Morocco is the only country in Africa and the MENA region to produce the stigma of saffron. Moroccan surfaces grown from saffron are about 1000 ha, mainly in the mountains of the Anti-Atlas. This production area take more and more importance in other regions (Mounira et al., 2015).

Crocus sativus L. (saffron) belongs to the Iridaceae family. Saffron stigmas have long been used in traditional medicine, cosmetics and as a food additive for coloring and flavoring (Caballero-Ortega et al., 2007). Further, the stigma of *Crocus sativus* is traditionally used as stimulant, aphrodisiac, antidepressant, antispasmodic and eupaptic to treat menstruation disorders, liver disease, physical suffering, cough, bronchospasm, asthma, heart disease, smallpox, fever and colds (Schmidt et al., 2007).

Numerous scientific studies have shown that saffron stigma has antioxidant properties (Farahmand et al., 2013), antitumor (Samarghandian and Borji, 2014), anti-inflammatory, antinociceptive (Hosseinzadeh and Younesi, 2002), antidepressant (Lopresti and Drummond, 2014), antitussive (Hosseinzadeh and Ghenaati, 2006), hypolipidemic (Sheng et al., 2006) and could improve memory and learning abilities in rats (Ghadami and Pourmotabbed, 2009; Papandreou et al., 2011). However, large amounts of saffron by-products are generated during processing of the stigmas. About 350kg of tepals, 1500kg of leaves needed to obtain only 1kg of dry stigmas (Smolskaite et al., 2011).

Tepals extracts have free radical scavenging (Hossein Goli et al., 2012; Zeka et al., 2015; Tuberoso et al., 2016), antidepressant (Moshiri et al., 2006), antinociceptive, anti-inflammatory (Hosseinzadeh and Younesi, 2002) and antityrosinase activities (Kubo and Kinst-Hori, 1999;

Li et al., 2004). These extracts can lower blood pressure and contractile response (Fatehi et al., 2003). They also have metal chelating properties (Sánchez-Vioque et al., 2012) cytotoxic and antifungal activity (Zheng et al., 2011; Serrano-Díaz et al., 2013). Saffron leaves are considered as source of bioactive components (Smolskaite et al., 2011). Saffron leaves extract have antioxidant and metal chelating properties (Sánchez-Vioque et al., 2012; Lahmass et al., 2018), activate proteasome in human dermal fibroblast cells from aged donors and inhibit the growth and proteasome activity of cancer cells (Sánchez-Vioque et al., 2016; Lahmass et al., 2017a).

Diabetes complications are probably linked to oxidative stress which is the result of increased blood glucose levels. Equally, the presence of bioactive molecules with a strong antioxidant properties on tepals and leaves of *Crocus sativus* thus prompted us to design the present study. For the first time, we investigated the antihyperglycemic effect of these by-products. We also examined the effect on diabetes complications. We measured plasma urea and creatinine levels to evaluate renal dysfunction. Similarly, we measured plasma alanine amino transferase (ALT) and aspartate amino transferase (AST) to study liver damage. In addition, it was reported in our laboratory and other studies that *Crocus sativus* stigma have an antihyperglycemic effect (Lahmass et al., 2017b; Samarghandian et al., 2017). For this reason we compared these by-products with *Crocus sativus* stigma and glibenclamide.

Materials and methods

Plant materials

The stigmas and tepals were harvested from a farm in Taliouine (30° 31' 54" nord, 7° 55' 25" oust, South of Morocco) and the leaves were collected from a farm in the region of Oujda (34° 41' 12" nord, 1° 54' 41" oust, East of Morocco) which the original corms were obtained from Taliouine. Saffron of both farms is cultivated without any chemical treatments. The different parts of the plant were collected manually between October and November (2016).

The identification of the plant has been done and confirmed by a professional botanist, Professor Fennane Mohammed from Scientific Institute in

Rabat, Morocco. Three specimens of the plant have been deposited at the plant section of Herbarium University Mohammed Premier, Oujda, Morocco (HUMPOM), under the voucher number (HUMPOM210).

Chemicals

Streptozotocin (STZ) was purchased from Sigma-Aldrich (Hambourg, Allemande), glibenclamide were obtained from Sigma chemicals (USA). All other chemicals and reagents used were of analytical grade.

Animals

Adult male Wistar rats (weight 150-200g) were maintained in their own cages (In the animal house of the Department of Biology, Faculty of Sciences, Oujda, Morocco) at constant temperature ($21\pm 2^\circ\text{C}$) and in a 12h light/dark cycle with free access to food (Dry rat pellets feeds provided by SONABETAIL Society, Oujda, Morocco) and water throughout the experiments. Maintenance and handling of rats were in accordance to the international conventional standard guidelines and with the Helsinki declaration for use of laboratory animals.

Preparation of hydroethanolic extracts

Hydroethanolic extracts were prepared as described in (Liu et al., 2013). Firstly, stigmas and the bio-residues of plant (*Crocus sativus*) were separated manually. Fresh tepals or dried and ground stigmas or leaves were macerated with ethanol/water 80/20 (v/v) for 24h in the dark at room temperature. After this first extraction, the solvent was filtered ($0.45\mu\text{m}$), and the marc was recuperated for second extraction. The procedure was repeated 3 times and the total hydroethanolic phase was subsequently evaporated under reduced pressure at 37°C . Finally, the resulting extracts were kept as solid at -20°C until use.

STZ-induced diabetic rats

Diabetes was induced by STZ according to method of Su et al. (2006). Briefly, STZ (60mg/kg, freshly prepared in 0.1M citrate buffer pH 4.5) was injected intraperitoneally to animals following a 16h fast for induction of diabetes. After 72h the blood was extracted from the tail vein for the measuring of fasting glucose levels using glucose oxidase method. Only those rats with blood glucose levels situated

between 2 and 5g/l) were considered diabetic and taken for further experimentation.

Experimental design

Hydroethanolic extracts (stigmas, tepals and leaves) and glibenclamide were administered orally in aqueous solution daily using an intragastric tube for 21 days. In the present study, 36 rats (30 diabetic and 6 normal rats) were used. The rats were divided into six groups. Six rats were used in each group as follows: group 1: normal control rats (N), received distilled water (1ml); group 2: diabetic control rats (D), received distilled water (1ml); group 3: treated diabetic rats (TPL), received tepal extract (250mg/kg); group 4: treated diabetic rats (STG), received stigma extract (50mg/kg); group 5: treated diabetic rats (LF), received leaf extract (250mg/kg) and group 6: treated diabetic rats (GLB), received glibenclamide (2mg/kg)

Blood glucose level and body weights were recorded at weekly intervals. Blood glucose level was determined by the GOD-POD method after prior fasting for 12h. After 21 days of treatments, blood samples were drawn from abdominal aorta into tubes containing heparin. Blood samples were centrifuged at 1300g for separation of plasma and stored at -20°C until biochemical assays. Urines were collected during 24h at the final of treatment using metabolic cages.

Biochemical assays

Separated plasma samples were used for determination of glucose and total cholesterol by the oxidase-peroxidase method (Trinder, 1969; Allain et al., 1974), triglycerides by the GPO-PAP method (Bucolo and David, 1973), urea by urease-GLDH method (Talke and Schubert, 1965), creatinine by Jaffe reaction without deproteinization method (Henry, 1968), AST and ALT by IFCC method (Karmen et al., 1955), using common clinical diagnostic kits.

Statistical analysis

The data were expressed as mean \pm SEM, with n=6 for each group. Normality test of data indicated that all data have normal distribution. Statistical analysis of data was performed using one-way ANOVA using GraphPad Prism 5.0 statistical software. Differences between treatment groups were analyzed by Tukey's

Table 1: Effect of *Crocus sativus* tepals (TPL, 250mg/kg), stigmas (STG, 50mg/kg) and leaves (LF, 250mg/kg) on body weight, compared to glibenclamide (GLB, 2mg/kg), N: normal control rats, D: diabetic control rats.

Groups	Days			
	0	7	14	21
N	161,25 ± 3,49	174,8 ± 4,17 (12,4 ± 0,81)	187,8 ± 4,5 (25,4 ± 1,57)	198,75 ± 6,89 (38 ± 3,45)
D	171,75±7,85	169,4 ± 9,07 (-8 ± 2,47)###	160 ± 10,45 (-17,4 ± 4,03)###	151,6,8 ± 10,67 (-25,8 ± 4,98)###
GLB	179,6±6,07	187,6 ± 5,85 (8 ± 0,89)***	194 ± 6,8 (14,4 ± 1,36)***	207,6 ± 7,31 (28 ± 2)***
TPL	181,5±4,44	187,5 ± 5,79 (5,5 ± 1,66)***	191,5 ± 5,83 (9,5 ± 2,06)***	203,5 ± 7,36 (21,96 ± 4,07)***
STG	182,7±3,85	185,4 ± 3,93 (2,6 ± 0,4)***	189,4 ± 3,28 (5,4 ± 1,12)***	195,4 ± 3,1 (13 ± 5,15)***
LF	185,2±6,71	186,8 ± 6,61 (1,6 ± 0,4)***	189,8 ± 7,17 (4,6 ± 1,63)***	196 ± 7,48 (10,8 ± 1,11)***

The results were expressed by: mean±SEM (n=6); ###P<0.001 compared with (N); ***P<0.001 compared with (D).

Table 2: Effect of *Crocus sativus* tepals (TPL, 250mg/kg), stigmas (STG, 50mg/kg) and leaves (LF, 250mg/kg) on water intake and urine elimination, compared to glibenclamide (GLB, 2mg/kg) after 20 days of treatment. N: normal control rats, D: diabetic control rats.

Groups	Water intake (ml)	Urine elimination (ml)
N	35 ± 1,58	4,1 ± 1,17
D	99 ± 6,4 ###	49,4 ± 2,82 ###
GLB	45,4 ± 0,02***	17,6 ± 4,61**
TPL	47 ± 9,4***	18,3 ± 7,18**
STG	52,6 ± 4,8**	23 ± 6,63*
LF	63 ± 11,9*	35,5 ± 7,6

The results were expressed by: mean±SEM (n=6); ###P<0.001 compared with (N); *P<0.05; **P<0.01; ***P<0.001 compared with (D).

multiple comparison post-test and with significance levels of $P<0.05$, $P<0.01$ and $P<0.001$.

Results

Effect on body weight

The effects of tepals, stigmas and leaves on the body weight are shown in Table 1. The untreated diabetic rats showed a significant weight loss ($P<0.001$) compared to the normal control group. Glibenclamide (GLB, 2 mg/kg/day), tepals (250 mg/kg/day), stigmas (50 mg/kg/day) and leaves (250 mg/kg/day) significantly protect against weight loss in STZ diabetic rats at 1st, 2nd and 3rd week of treatment ($P<0.001$, Table 1) compared to the untreated diabetic rats.

At the 3rd week, a significant difference ($P<0.001$) was observed on body weight between group treated with STZ induced diabetic rats (-25.8±4.98g) compared to the normal rats (+38±3.45g). However, the administration of GLB had significantly ($P<0.001$) prevented the decrease of body weight in diabetic rats (+28±2g). Also, the treatment with TPL, STG and

LF induce a significant prevent ($P<0.001$) of decreased body weight in diabetic rats (+21.96±4.07g, +13±5.15g and +10.8±1.11g, respectively).

Effect on water intake and urine elimination

Table 2 shows the water intake and urine elimination in normal and diabetic rats after 20 days of treatment. The untreated diabetic rats showed a significant increase ($P<0.001$) of water intake and urine elimination compared to the normal group. The treatment with tepals (250 mg/kg/day) and stigmas (50 mg/kg/day) extracts decreased significantly water intake ($P<0.001$ and $P<0.01$, respectively) and urine elimination ($P<0.01$ and $P<0.05$, respectively). While leaves extract decreased significantly ($P<0.05$) water intake compared to untreated diabetic rats. Administration of glibenclamide (2 mg/kg/day) showed significant reduction of water intake ($P<0.001$) and urine elimination ($P<0.01$).

Effect on hyperglycemia

Figure 1 summarizes the levels of glucose in normal

and diabetic animals. The diabetic rats exhibited significant ($P<0.001$) hyperglycemia compared to the normal rats. After 3 weeks the tepals, stigmas and leaves extracts decreased blood glucose levels in the diabetic rats compared with the untreated diabetic rats (Fig. 1). Tepals, stigmas and glibenclamide significantly decreased glucose in STZ diabetic rats at 1st, 2nd and 3rd week of treatment ($P<0.01$, $P<0.001$ and $P<0.001$, respectively). While, leaves extract reduced significantly blood glucose level at 2nd and 3rd week of the study ($P<0.01$ and $P<0.001$, respectively) compared to untreated diabetic rats (Fig. 1).

Effect on plasma triglycerides and total cholesterol

Figure 2 (A and B) shows the plasma levels of total cholesterol (A) and triglycerides (B) in normal and experimental animals in each group. The STZ-diabetic rats showed a significant increase ($P<0.001$) in plasma cholesterol and triglycerides levels compared to the normal group. The administration of the tepals, stigmas, and leaves extracts significantly decreased plasma total cholesterol ($P<0.01$, $P<0.01$ and $P<0.05$, respectively) and plasma triglycerides

($P<0.001$, $P<0.01$ and $P<0.05$, respectively). There was a significant ($P<0.001$) reduction in plasma cholesterol and triglycerides of rats diabetic treated with glibenclamide compared to untreated diabetic rats.

Effect on creatinine and plasma urea levels

On day 21, the plasma levels of creatinine and urea in normal and diabetic animals in each group was showed in Figure 3 (A and B). Creatinine and urea levels in plasma were increased in untreated diabetic rats compared with the normal group ($P<0.01$). The administration of the tepals, stigmas and leaves extracts significantly decreased plasma creatinine ($P<0.01$, $P<0.01$ and $P<0.01$, respectively). In addition, rats treated with tepals and stigma showed a significant decrease in plasma levels of urea ($P<0.05$ and $P<0.05$, respectively). While, there was no significant decrease on plasma levels of urea in group treated with leaves. Equally, there was a significant ($P<0.05$) reduction in plasma creatinine and urea of rats diabetic treated with glibenclamide (Figs. 3A and B).

Effect on plasma ALT and AST levels

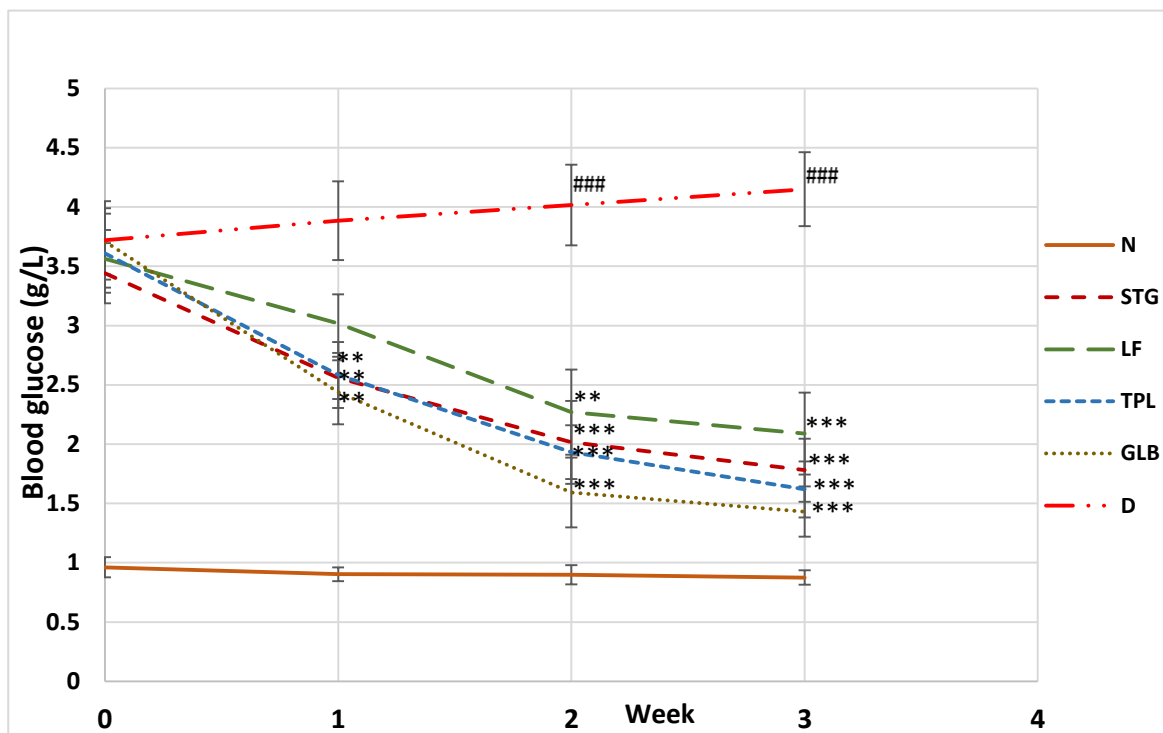


Fig.1. Effect of *Crocus sativus* tepals (TPL, 250mg/kg), stigmas (STG, 50mg/kg) and leaves (LF, 250mg/kg) on blood glucose levels, compared to glibenclamide (GLB, 2mg/kg) in STZ induced-diabetic rats during 21 days of treatment. The results were expressed by: mean \pm SEM (n=6); ### $P<0.001$ compared with normal control rats (N); ** $P<0.01$; *** $P<0.001$ compared with diabetic control rats (D).

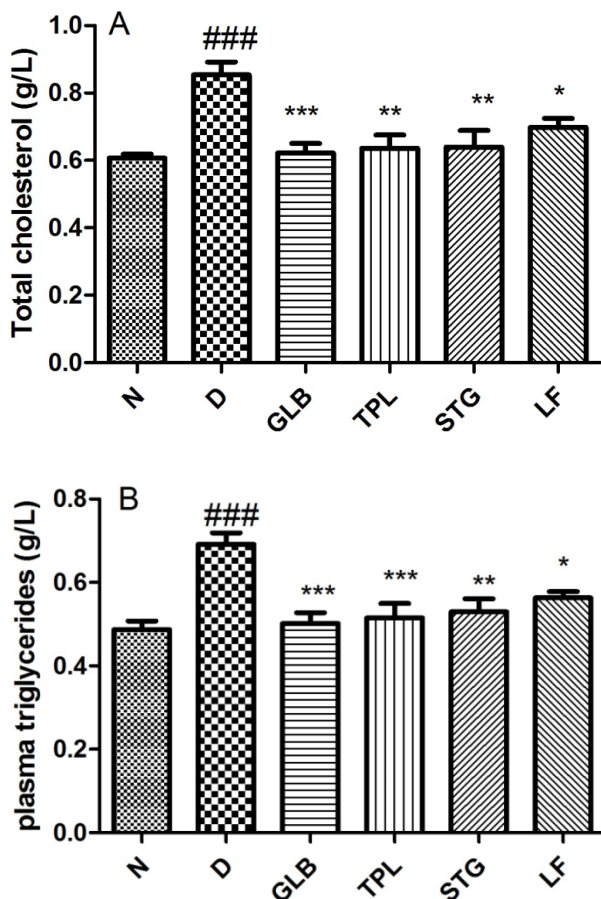


Fig.2. Effect of *Crocus sativus* tepals (TPL, 250mg/kg), stigmas (STG, 50mg/kg) and leaves (LF, 250mg/kg) on plasma levels of total cholesterol (A) and triglycerides (B), compared to glibenclamide (GLB, 2mg/kg) in STZ induced-diabetic rats after 21 days of treatment. The results were expressed by: mean±SEM (n=6); ^{###} $P<0.001$ compared with normal control rats (N); ^{*} $P<0.05$; ^{**} $P<0.01$; ^{***} $P<0.001$ compared with diabetic control rats (D).

Figure 4 (A and B) shows the plasma levels of ALT and AST in normal and experimental animals in each group. The STZ-diabetic rats showed a significant increase ($P<0.001$) in the level of plasma ALT and AST compared with the normal control group. The administration of tepals, stigmas and leaves extracts significantly decreased plasma ALT ($P<0.001$, $P<0.01$ and $P<0.05$, respectively) and plasma AST ($P<0.001$, $P<0.01$ and $P<0.05$, respectively). There was a significant ($P<0.01$) reduction in plasma ALT and AST of diabetic rats treated with glibenclamide (Figs 4A and B).

Discussion

The present data indicated that the tepals and

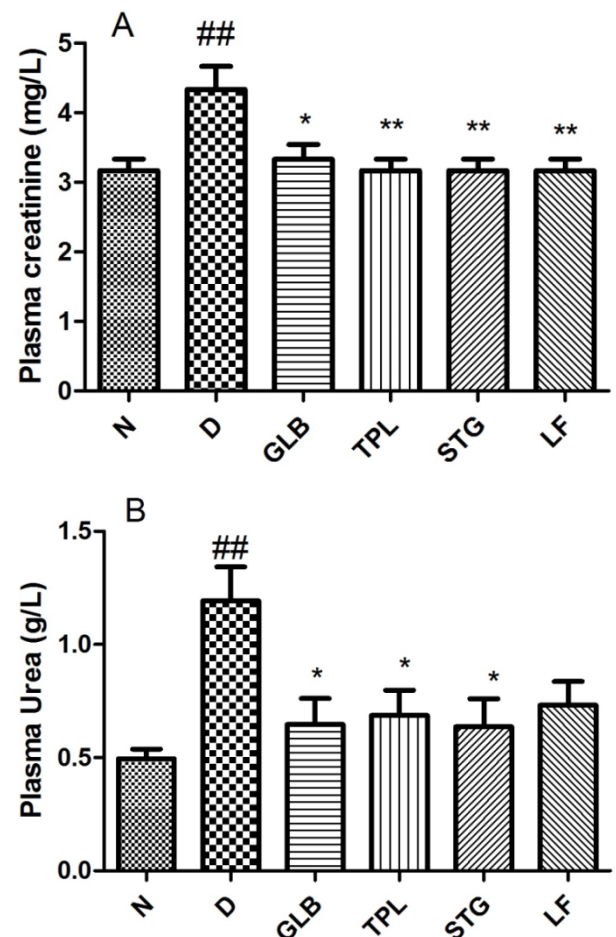


Fig.3. Effect of *Crocus sativus* tepals (TPL, 250mg/kg), stigmas (STG, 50mg/kg) and leaves (LF, 250mg/kg) on plasma levels of creatinine (A) and urea (B), compared to glibenclamide (GLB, 2mg/kg) in STZ induced-diabetic rats after 21 days of treatment. The results were expressed by: mean±SEM (n=6); ^{##} $P<0.01$ compared with normal control rats (N); ^{*} $P<0.05$; ^{**} $P<0.01$ compared with diabetic control rats (D).

stigmas extracts significantly prevented weight body loss and protected against elevation of water intake, urine elimination, blood glucose, plasma triglycerides, cholesterol, urea, creatinine, AST and ALT levels in treated diabetic rats compared with untreated diabetic rats. Moreover, leaves extract significantly prevented weight body loss. It decreased all other parameters. While, the latter shows that there was no significant decrease on urine elimination volume and plasma levels of urea.

The results observed after the injection of streptozotocin which indicate a polyphagic state and weight loss that could be due to excessive degradation of the protein tissue (Chatterjea and Shinde, 2011; Altinoz et al., 2014; Samarghandian et al., 2014) and may be due to dehydration and

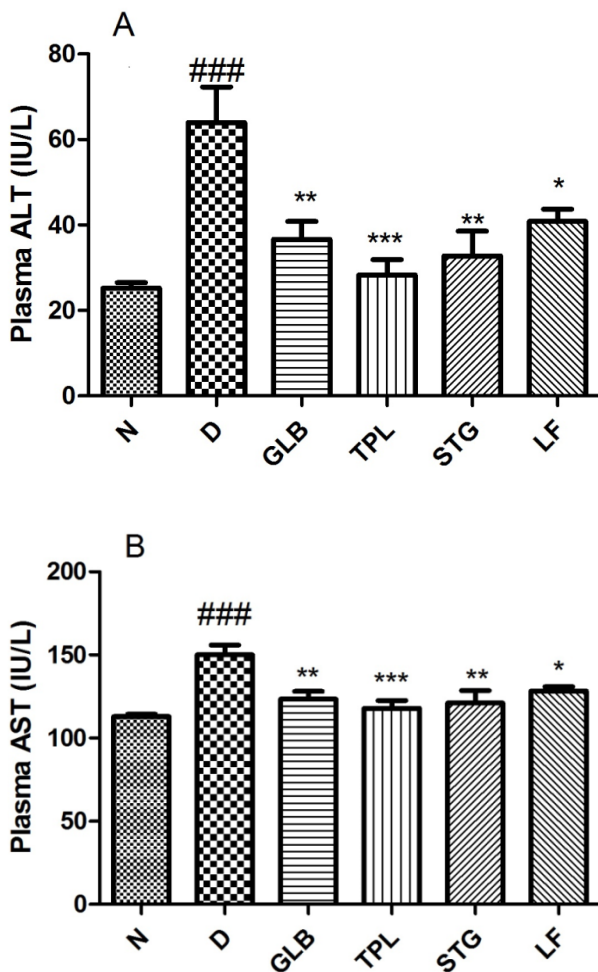


Fig.4. Effect of *Crocus sativus* tepals (TPL, 250mg/kg), stigmas (STG, 50mg/kg) and leaves (LF, 250mg/kg) on plasma levels of ALT (A) and AST (B), compared to glibenclamide (GLB, 2mg/kg) in STZ induced-diabetic rats after 21 days of treatment. The results were expressed by: mean±SEM (n=6); ### $P<0.001$ compared with normal control rats (N); * $P<0.05$; ** $P<0.01$; *** $P<0.001$ compared with diabetic control rats (D).

catabolism of fats and proteins (Hakim et al., 1997). However, administration of tepals, stigmas and leaves extracts prevented decreasing body weight in STZ-diabetic rats. This might be due to a significant control of the state of hyperglycemia in the diabetic rats. A reduction in blood glucose levels may improve body weight in rats with streptozotocin diabetes (Kamalakkannan et al., 2003; Babu and Prince, 2004). Our results agreed with Shirali et al. who reported that the aqueous extract of saffron had a protective effect against body weight loss compared to untreated diabetic rats (Shirali et al., 2012). Moreover, Administration of plants polyphenols affect a significant increase in the body weight (Aybar et al., 2001; Patel et al., 2011).

The untreated diabetic rats drank a large amount of water and produced a correspondingly high urine volume. The treatment with tepals, stigmas and leaves extracts decreased water intake and urine elimination. Additionally, Lahmass et al. showed that stigma aqueous extract regulate water intake in tartrazine induced diabetic rats treated with saffron stigma (Lahmass et al., 2017c). Furthermore, It was indicated that polyphenols of plants reduced water intake compared to diabetic rats (Rajagopal and Sasikala, 2008; Ong et al., 2011).

Streptozotocin has been widely used for inducing diabetes mellitus in a variety of animals by affecting degeneration and necrosis of pancreatic β -cells (Merzouk et al., 2000).

Several studies indicated that the level of plasma glucose increases and the level of plasma insulin decreases in STZ-induced diabetic rats (Akbarzadeh et al., 2007).

The present results agreed with Mohajeri et al. (2008) who revealed that administration of the ethanolic saffron extract (20, 40 and 80 mg/kg) has significantly decreased blood glucose and increased plasma insulin in diabetic rats by increasing the number of β -cells in the pancreas (Mohajeri et al., 2008).

Another study also showed that saffron extract has hypoglycemic effects on healthy male rats (Arasteh et al., 2010).

Oral administration of saffron extract has significantly increased plasma insulin and reduced blood glucose levels in alloxan induced diabetic rats. In addition, Histological study showed that pancreas sections of diabetic rats treated with saffron had hypertrophy and hyperplasia of β -cells of islets of Langerhans associated with pyknosis of their nuclei (Elgazar et al., 2013).

A recent study in our laboratory suggests that saffron has curative (antidiabetic) and protective (antidiabetogenic) effect against diabetes induced by tartrazine via reducing blood glucose level and creatinine (Lahmass et al., 2017b).

Several studies indicated that crocin has an antihyperglycemic effect (Rajaei et al., 2013; Shirali et al., 2013; Asri-Rezaei et al., 2015). Besides, administration of safranin affects a significant decrease in glucose, and improve the antioxidant system (Samarghandian et al., 2013).

The anti-hyperglycemic effect of petals and leaves may be due to flavonoid compounds. Consumption of

flavonoids rich foods may reduce the risk of diabetes (Bahadoran et al., 2013). This class of compounds protect from diseases like obesity, diabetes and their complications (Vinayagam and Xu, 2015), regulate the enzymes and hormones and help to maintain blood glucose levels. Improve insulin secretion, glucose uptake, lipid profile and immune system (Hanhineva et al., 2010; Hajiaghaalipour et al., 2015). Kaempferol derivatives were the most abundant flavonoids on tepals and leaves (Goupy et al., 2013; Sánchez-Vioque et al., 2016; Tuberoso et al., 2016). Oral administration of kaempferol to diabetic rats has resulted a significant reduction in plasma glucose and an increase in plasma insulin, back to near normal levels in plasma lipid peroxidation products, enzymatic, and non-enzymatic antioxidants (Al-Numair et al., 2015).

Additionally, kaempferol improved insulin secretion and synthesis in β -cells, protected pancreatic beta-cells from hyperglycemia-induced apoptosis and dysfunction (Zhang and Liu, 2011).

Diabetic patients suffer with hyperlipidemia. Furthermore, it was demonstrated that insulin deficiency in DM caused a lot of perturbation in metabolic processes. Consequently, cholesterol storage problems result an increased lipids such as TC and TG levels (Goldberg, 1981). Our results confirm this notion as the treatment with streptozotocin (60 mg/kg, i.p.) caused a significant increase of plasma TC and TG levels. In fact, treatment with stigmas, tepals and leaves significantly decreased the cholesterol and triglycerides levels compared to untreated STZ-diabetic rats.

Shirali et al. (2013) showed that crocin in stigma extract has significantly decreased the levels of triglyceride and total cholesterol in the diabetic rats by improving insulin resistance in the diabetic rats (Shirali et al., 2013).

In addition, Samarghandian et al. (2013), indicated that safranal inhibits elevation of the plasma lipid level by controlling oxidative and nitrosative stress (Samarghandian et al., 2013).

The aqueous extract of *Crocus sativus* tepals has been shown to improve dyslipidemia and insulin resistance in obese rats fed a high-fat diet (Hoshyar et al., 2016).

The beneficial effect of the tepals and leaves extract may be due to their richness on polyphenols and flavonoid compounds.

The elevation of production of ROS caused an increase of oxidative stress on the kidney. That is the result of increased uptake of glucose (Forbes et al., 2008). It was demonstrated that the increase of plasma urea and creatinine levels in diabetics are linked to renal dysfunction (Almdal et al., 1986).

Our data showed that stigma, tepals and leaves extracts decreased the plasma creatinine level in diabetic rats. Also, rats treated with extracts of tepals and stigma showed a significant decrease in urea plasma levels. Well, there was no significant decrease on plasma levels of urea in the group treated with the leaves.

Also, Kianbakht and Hajiaghaee (2011) suggested that saffron may have anti-hyperglycemic effect without renal toxicities in the alloxan - diabetic rats. It has also been reported that crocin administration could be considered as a valuable adjunct therapy for prevention against renal complication due to diabetes by its potential role in defense against free radicals (Rajaei et al., 2013). A protective effect of tepals and leaves of *Crocus sativus* against the increase of plasma creatinine and urea levels, may be due to bioactive compounds. Various studies suggest that polyphenols compounds of plants may protect against renal dysfunction in diabetes models by decreasing oxidative stress or decreasing blood glucose (Sabu and Kuttan, 2002; Kumar et al., 2011; Shivanna et al., 2013).

A specific indicator of liver damage is the elevation of ALT and AST activities (El-Demerdash et al., 2001). This elevation caused an increase on ketogenesis and gluconeogenesis observed in diabetes. These enzymes (ALT and AST) degrade the amino acids into β -keto acid, following metabolism pathways via the Krebs cycle (Maiti et al., 2004). Our results showed that administration of stigma, tepals and leaves decreased significantly those enzymes levels in plasma compared with untreated STZ-diabetic rats. The present data agreed with Altinos et al. who revealed that crocin (active constituent of saffron stigma) decreased serum ALT and AST levels compared to untreated STZ-induced diabetic rats. It may protected the liver tissue by decreasing the oxidative stress (Mohammad et al., 2011; Altinoz et al., 2014).

The effect of the tepals and leaves extract may be due to polyphenols and flavonoids compounds. Flavonoids compounds effectively protected the liver

against STZ-induced damage in rats. Therefore, application of flavonoids compounds decreased serum ALT and AST levels compared to untreated STZ-induced diabetic rats. There are reports indicating that several medicinal plants such as agyanom mixture, bolex bitters and remedia mixture can reduce these liver enzymes markers (Girish et al., 2009; Akande et al., 2010). Polyphenolic compounds and flavonoids can improve metabolism and protect cells against destructive effect of free radicals by increasing antioxidant enzymes capacity such as catalase, superoxide dismutase and glutathione peroxidase (Baer-Dubowska et al., 1998; Prakash et al., 2007).

Conclusion

Diabetes complications are probably linked to oxidative stress which is the result of increased blood glucose levels. Based on our data, the oral administration of tepals, stigmas and leaves extract of *Crocus sativus* reduces blood glucose levels and improves control of diabetes complications that are hyperglycemic results. In addition, the antidiabetic effect of these by-products may be due to the presence of bioactive molecules with antioxidant properties which may exert various health benefits.

Acknowledgments

We are very thankful to the unit of biochemistry in the University Hospital Center (Mohammed VI), Oujda, Morocco. We thank YOUSSEFI Siham and AMIROU Asmae for helping on biochemical assays. We thank also EL BEDRAOUI Mostapha for helping in animals care.

Conflict of interest

The authors declare no conflict of interest.

References

- Akande IS, Ebuehi OA, Samuel TA, Onubogu IC, Esin H. Effects of herbal remedies (Agyanom mixture, Bolex bitters and Remedia mixture) on hepatic and renal functions in male rats. *Nig Q J Hosp Med* 2010; 20:70-6.
- Akbarzadeh A, Norouzian D, Mehrabi MR, Jamshidi Sh, Farhangi A, Verdi AA, et al. Induction of diabetes by Streptozotocin in rats. *Indian J Clin Biochem* 2007; 22: 60-4.
- Al-Numair KS, Chandramohan G, Veeramani C, Alsaif MA. Ameliorative effect of kaempferol, a flavonoid, on oxidative stress in streptozotocin-induced diabetic rats. *Redox Rep* 2015; 20: 198-209.
- Allain CC, Poon LS, Chan CS, Richmond W, Fu PC. Enzymatic determination of total serum cholesterol. *Clin Chem* 1974; 20: 470-5.
- Almdal TP, Petersen KF, Hansen BA, Vilstrup H. Increased capacity of urea synthesis in streptozotocin diabetes in rats. *Diabetologia* 1986; 29: 812-6.
- Altinoz E, Oner Z, Elbe H, Turkoz Y, Cigremis Y. Protective effect of saffron (its active constituent, crocin) on oxidative stress and hepatic injury in streptozotocin induced diabetic rats. *Gene Ther Mol Biol* 2014; 16: 160-71.
- Arasteh A, Aliyev A, Khamnei S, Delazar A, Mesgari Abbasi M, Mehmannaavaz 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.
- Asri-Rezaei S, Tamaddonfard E, Ghasemsoltani-Momtaz B, Erfanparast A, Gholamalipour S. Effects of crocin and zinc chloride on blood levels of zinc and metabolic and oxidative parameters in streptozotocin-induced diabetic rats. *Avicenna J Phytomed* 2015; 5: 403-12.
- Aybar MJ, Sánchez Riera AN, Grau A, Sánchez SS. Hypoglycemic effect of the water extract of *Smallantus sonchifolius* (yacon) leaves in normal and diabetic rats. *J Ethnopharmacol* 2001; 74: 125-32.
- Babu PS, Stanely Mainzen Prince P. Antihyperglycaemic and antioxidant effect of hyponidd, an ayurvedic herbomineral formulation in streptozotocin-induced diabetic rats. *J Pharm Pharmacol* 2004; 56: 1435-42.
- Baer-Dubowska W, Szaefer H, Krajka-Kuzniak V. Inhibition of murine hepatic cytochrome P450 activities by natural and synthetic phenolic compounds. *Xenobiotica* 1998; 28: 735-43.
- Bahadoran Z, Mirmiran P, Azizi F. Dietary polyphenols as potential nutraceuticals in management of diabetes: a review. *J Diabetes Metab Disord* 2013; 12: 43.
- Bucolo G, David H. Quantitative determination of serum triglycerides by the use of enzymes. *Clin Chem* 1973; 19: 476-82.
- Caballero-Ortega H, Pereda-Miranda R, Abdullaev FI. HPLC quantification of major active components from 11 different saffron (*Crocus sativus* L.) sources. *Food Chem* 2007; 100: 1126-31.
- Chatterjea MN, Shinde R. Textbook of medical biochemistry: Wife Goes On, 2011.
- El-Demerdash FM, Yousef MI, Elagamy EI. Influence of paraquat, glyphosate, and cadmium on the activity of some serum enzymes and protein electrophoretic behavior (in vitro). *J Environ Sci Health B* 2001; 36: 29-42.
- Elgazar AF, Rezaq AA, Bukhari HM. Anti-hyperglycemic effect of saffron extract in alloxan-induced diabetic rats. *Eur J Biol Sci* 2013; 5: 14-22.
- Farahmand SK, Samini F, Samini M, Samarghandian S. Safran ameliorates antioxidant enzymes and

- suppresses lipid peroxidation and nitric oxide formation in aged male rat liver. *Biogerontology* 2013; 14: 63-71.
- Fatehi M, Rashidabady T, Fatehi-Hassanabad Z. Effects of *Crocus sativus* petals' extract on rat blood pressure and on responses induced by electrical field stimulation in the rat isolated vas deferens and guinea-pig ileum. *J Ethnopharmacol* 2003; 84: 199-203.
- Forbes JM, Coughlan MT, Cooper ME. Oxidative stress as a major culprit in kidney disease in diabetes. *Diabetes* 2008; 57: 1446-54.
- Ghadami MR, Pourmotabbed A. The effect of Crocin on scopolamine induced spatial learning and memory deficits in rats. *Physiol Pharmacol* 2009; 12: 287-95.
- Girish C, Koner BC, Jayanthi S, Rao KR, Rajesh B, Pradhan SC. Hepatoprotective activity of six polyherbal formulations in CCl₄-induced liver toxicity in mice. *Indian J Exp Biol* 2009; 47: 257-263.
- Goldberg RB. Lipid disorders in diabetes. *Diabetes Care* 1981; 4: 561-72.
- Goupy P, Vian MA, Chemat F, Caris-Veyrat C. Identification and quantification of flavonols, anthocyanins and lutein diesters in tepals of *Crocus sativus* by ultra performance liquid chromatography coupled to diode array and ion trap mass spectrometry detections. *Ind Crops Prod* 2013; 44: 496-510.
- Hajiaghaalipour F, Khalilpourfarshbafi M, Arya A. Modulation of glucose transporter protein by dietary flavonoids in type 2 diabetes mellitus. *Int J Biol Sci* 2015; 11: 508-24.
- Hakim ZS, Patel BK, Goyal RK. Effects of chronic ramipril treatment in streptozotocin-induced diabetic rats. *Indian J Physiol Pharmacol* 1997; 41: 353-60.
- Hanhineva K, Törrönen R, Bondia-Pons I, Pekkinen J, Kolehmainen M, Mykkänen H, et al. Impact of dietary polyphenols on carbohydrate metabolism. *Int J Mol Sci* 2010; 11: 1365-402.
- Henry RJ. *Clinical chemistry; principles and technics*. New York: Hoeber Medical Division, 1968.
- Hoshyar R, Hosseinian M, Naghandar MR, Hemmati M, Zarban A, Amini Z, et al. Anti-dyslipidemic properties of saffron: reduction in the associated risks of atherosclerosis and insulin resistance. *Iran Red Crescent Med J* 2016; 18: e36226.
- Hossein Goli SA, Mokhtari F, Rahimmalek M. Phenolic compounds and antioxidant activity from saffron (*Crocus sativus* L.) petal. *J Agr Sci* 2012; 4: 175-181.
- Hosseinzadeh H, Ghenaati J. Evaluation of the antitussive effect of stigma and petals of saffron (*Crocus sativus*) and its components, safranal and crocin in guinea pigs. *Fitoterapia* 2006; 77: 446-8.
- Hosseinzadeh H, Younesi HM. Antinociceptive and anti-inflammatory effects of *Crocus sativus* L. stigma and petal extracts in mice. *BMC Pharmacol* 2002; 2: 7.
- Kamalakkanan N, Rajadurai M, Prince PS. Effect of Aegle marmelos fruits on normal and streptozotocin-diabetic Wistar rats. *J Med Food* 2003; 6: 93-8.
- Karmen A, Wroblewski F, Ladue JS. Transaminase activity in human blood. *J Clin Invest* 1955; 34: 126-31.
- Kianbakht S, Hajiaghaee R. Anti-hyperglycemic effects of saffron and its active constituents, crocin and safranal, in alloxan-induced diabetic rats. *J Med Plant* 2011; 3: 82-9.
- Kubo I, Kinst-Hori I. Flavonols from saffron flower: tyrosinase inhibitory activity and inhibition mechanism. *J Agric Food Chem* 1999; 47: 4121-5.
- Kumar S, Kumar V, Prakash O. Antidiabetic, hypolipidemic and histopathological analysis of *Dillenia indica* (L.) leaves extract on alloxan induced diabetic rats. *Asian Pac J Trop Med* 2011; 4: 347-52.
- Lahmass I, Lamkani T, Delporte C, Sikdar S, Van Antwerpen P, Saalaoui E, et al. The waste of saffron crop, a cheap source of bioactive compounds. *J Funct Foods* 2017a; 35: 341-51.
- Lahmass I, Ouahhoud S, Elmansuri M, Sabouni A, Elyoubi M, Benabbas R, et al. Determination of antioxidant properties of six by-products of *Crocus sativus* L.(saffron) plant products. *Waste Biomass Valorization* 2018; 9: 1349-57.
- Lahmass I, Ouahhoud S, Sabouni A, Elyoubi M, Benabbas R, Elmoussaoui R, et al. Antihyperlipidemic effect of crude extract of saffron (*Crocus sativus*) stigma in healthy male rats. *J Med Allied Sci* 2017b; 7: 20-25.
- Lahmass I, Sabouni A, Mohammed E, Benabbas R, Mokhtari S, Saalaoui E. Anti-diabetic effect of aqueous extract *Crocus sativus* L. in tartrazine induced diabetic male rats. *Physiol Pharmacol* 2017c; 21: 312-21.
- Li CY, Lee EJ, Wu TS. Antityrosinase principles and constituents of the petals of *Crocus sativus*. *J Nat Prod* 2004; 67: 437-40.
- Liu J, Jia L, Kan J, Jin CH. In vitro and in vivo antioxidant activity of ethanolic extract of white button mushroom (*Agaricus bisporus*). *Food Chem Toxicol* 2013; 51: 310-6.
- Lopresti A, Drummond PD. Saffron (*Crocus sativus*) for depression: a systematic review of clinical studies and examination of underlying antidepressant mechanisms of action. *Hum Psychopharmacol* 2014; 29: 517-27.
- Sabu MC, Kuttan R. Anti-diabetic activity of green tea polyphenols and their role in reducing oxidative stress in experimental diabetes. *J Ethnopharmacol* 2002; 83: 109-16.
- Maiti R, Jana D, Das UK, Ghosh D. Antidiabetic effect of aqueous extract of seed of *Tamarindus indica* in streptozotocin-induced diabetic rats. *J Ethnopharmacol* 2004; 92: 85-91.
- Maritim AC, Sanders RA, Watkins JB 3rd. Effects of alpha-lipoic acid on biomarkers of oxidative stress in streptozotocin-induced diabetic rats. *J Nutr Biochem* 2003; 14: 288-94.
- Merzouk H, Madani S, Chabane Sari D, Prost J, Bouchenak M, Belleville J. Time course of changes in serum glucose, insulin, lipids and tissue lipase activities in macrosomic offspring of rats with streptozotocin-induced diabetes. *Clin Sci (Lond)* 2000; 98: 21-30.
- Mohajeri D, Tabrizi B, Mousavi G, Mesgari Abbasi M. Anti-diabetic activity of *Crocus sativus* L.(Saffron) stigma ethanolic extract in alloxan-induced diabetic rats. *Res J Biol Sci* 2008; 3: 1102-1108.

- Mohammad R, Daryoush M, Ali R, Yousef D, Mehrdad N. Attenuation of oxidative stress of hepatic tissue by ethanolic extract of saffron (dried stigmas of *Crocus sativus* L.) in streptozotocin (STZ)-induced diabetic rats. *Afr J Pharm Pharmacol* 2011; 5: 2166-73.
- Moshiri E, Basti AA, Noorbala AA, Jamshidi AH, Hesameddin Abbasi S, Akhondzadeh S. *Crocus sativus* L. (petal) in the treatment of mild-to-moderate depression: a double-blind, randomized and placebo-controlled trial. *Phytomedicine* 2006; 13: 607-11.
- Mounira L, Bernardo M, Luigi CP, Guido F, Fatima G, Khadija B, Abdelmjid Z, Luisa P. Phytochemical composition of Moroccan saffron accessions by headspace solid-phase-microextraction. *Am J Essent Oils Nat Prod* 2015; 2: 1-7.
- Ong KW, Hsu A, Song L, Huang D, Tan BK. Polyphenols-rich *Vernonia amygdalina* shows anti-diabetic effects in streptozotocin-induced diabetic rats. *J Ethnopharmacol* 2011; 133: 598-607.
- Papandreou MA, Tsachaki M, Efthimiopoulos S, Cordopatis P, Lamari FN, Margarita M. Memory enhancing effects of saffron in aged mice are correlated with antioxidant protection. *Behav Brain Res* 2011; 219: 197-204.
- Patel DK, Kumar R, Prasad SK, Sairam K, Hemalatha S. Antidiabetic and in vitro antioxidant potential of *Hybanthus enneaspermus* (Linn) F. Muell in streptozotocin-induced diabetic rats. *Asian Pac J Trop Biomed* 2011; 1: 316-22.
- Prakash D, Singh BN, Upadhyay G. Antioxidant and free radical scavenging activities of phenols from onion (*Allium cepa*). *Food Chem* 2007; 102: 1389-93.
- Rajaei Z, Hadjzadeh MA, Nemati H, Hosseini M, Ahmadi M, Shafiee S. Antihyperglycemic and antioxidant activity of crocin in streptozotocin-induced diabetic rats. *J Med Food* 2013; 16: 206-10.
- Rajagopal K, Sasikala K. Antihyperglycaemic and antihyperlipidaemic effects of *Nymphaea stellata* in alloxan-induced diabetic rats. *Singapore Med J* 2008; 49: 137-41.
- Samarghandian S, Azimi-Nezhad M, Farkhondeh T. Immunomodulatory and antioxidant effects of saffron aqueous extract (*Crocus sativus* L.) on streptozotocin-induced diabetes in rats. *Indian Heart J* 2017; 69: 151-159.
- Samarghandian S, Azimi-Nezhad M, Samini F. Ameliorative effect of saffron aqueous extract on hyperglycemia, hyperlipidemia, and oxidative stress on diabetic encephalopathy in streptozotocin induced experimental diabetes mellitus. *Biomed Res Int* 2014; 2014: 920857.
- Samarghandian S, Borji A. Anticarcinogenic effect of saffron (*Crocus sativus* L.) and its ingredients. *Pharmacognosy Res* 2014; 6: 99-107.
- Samarghandian S, Borji A, Delkhosh MB, Samini F. Safranin treatment improves hyperglycemia, hyperlipidemia and oxidative stress in streptozotocin-induced diabetic rats. *J Pharm Pharm Sci* 2013; 16: 352-62.
- Sánchez-Vioque R, Rodríguez-Conde MF, Reina-Ureña JV, Escolano-Tercero MA, Herraiz-Peñalver D, Santana-Méridas O. In vitro antioxidant and metal chelating properties of corm, tepal and leaf from saffron (*Crocus sativus* L.). *Ind Crops Prod* 2012; 39: 149-53.
- Sánchez-Vioque R, Santana-Méridas O, Polissiou M, Vioque J, Astraka K, Alaiz M, et al. Polyphenol composition and in vitro antiproliferative effect of corm, tepal and leaf from *Crocus sativus* L. on human colon adenocarcinoma cells (Caco-2). *J Funct Foods* 2016; 24: 18-25.
- Schmidt M, Betti G, Hensel A. Saffron in phytotherapy: pharmacology and clinical uses. *Wien Med Wochenschr* 2007; 157: 315-9.
- Segal P, Feig PU, Scherthaner G, Ratzmann KP, Rybka J, Petzinna D, et al. The efficacy and safety of miglitol therapy compared with glibenclamide in patients with NIDDM inadequately controlled by diet alone. *Diabetes Care* 1997; 20: 687-91.
- Serrano-Díaz J, Sánchez AM, Martínez-Tomé M, Winterhalter P, Alonso GL. A contribution to nutritional studies on *Crocus sativus* flowers and their value as food. *J Food Compos Anal* 2013; 31: 101-8.
- Sheng L, Qian Z, Zheng S, Xi L. Mechanism of hypolipidemic effect of crocin in rats: crocin inhibits pancreatic lipase. *Eur J Pharmacol* 2006; 543: 116-22.
- Shirali S, Bathaei SZ, Nakhjavani M, Ashoori MR. Effects of saffron (*Crocus sativus* L.) aqueous extract on serum biochemical factors in streptozotocin-induced diabetic rats. *Iran J Med Aromat Plants* 2012; 28: 293-3-8.
- Shirali S, Zahra Bathaie S, Nakhjavani M. Effect of crocin on the insulin resistance and lipid profile of streptozotocin-induced diabetic rats. *Phyther Res* 2013; 27: 1042-7.
- Shivanna N, Naika M, Khanum F, Kaul VK. Antioxidant, anti-diabetic and renal protective properties of *Stevia rebaudiana*. *J Diabetes Complications* 2013; 27: 103-13.
- Smolskaite L, Talou T, Fabre N, Venskutonis PR. Valorization of saffron industry by-products: bioactive compounds from leaves. In *Baltic Conference on Food Science and Technology FoodBalt-2011* 2011; 6: 67-72.
- Su HC, Hung LM, Chen JK. Resveratrol, a red wine antioxidant, possesses an insulin-like effect in streptozotocin-induced diabetic rats. *Am J Physiol Endocrinol Metab* 2006; 290: E1339-46.
- Talke H, Schubert GE. Enzymatic urea determination in the blood and serum in the warburg optical test. *Klin Wochenschr* 1965; 43: 174-5.
- Tousoulis D, Kampoli AM, Stefanadis C. Diabetes mellitus and vascular endothelial dysfunction: current perspectives. *Curr Vasc Pharmacol* 2012; 10: 19-32.
- Trinder P. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. *Ann Clin Biochem* 1969; 6: 24-7.
- Tuberoso CI, Rosa A, Montoro P, Fenu MA, Pizza C. Antioxidant activity, cytotoxic activity and metabolic profiling of juices obtained from saffron (*Crocus sativus* L.) floral by-products. *Food Chem* 2016; 199: 18-27.
- Vinayagam R, Xu B. Antidiabetic properties of dietary flavonoids: a cellular mechanism review. *Nutr Metab*

(Lond) 2015; 12: 60.

Zeka K, Ruparelia KC, Continenza MA, Stagos D, Veglio F, Arroo RRJ. Petals of *Crocus sativus* L. as a potential source of the antioxidants crocin and kaempferol. *Fitoterapia* 2015; 107: 128-134.

Zhang Y, Liu D. Flavonol kaempferol improves chronic hyperglycemia-impaired pancreatic beta-cell viability and insulin secretory function. *Eur J Pharmacol* 2011;

670: 325-32.

Zheng CJ, Li L, Ma WH, Han T, Qin LP. Chemical constituents and bioactivities of the liposoluble fraction from different medicinal parts of *Crocus sativus*. *Pharm Biol* 2011; 49: 756-63.

Zimmet P, Alberti KG, Magliano DJ, Bennett PH. Diabetes mellitus statistics on prevalence and mortality: facts and fallacies. *Nat Rev Endocrinol* 2016; 12: 616-22.