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BIOCHEMICAL EFFECT OF *VICINE* AND *DIVICINE* EXTRACTED FROM *FAVA BEANS* (*VICIA FABA*) IN RATS

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A B S T R A C T

Vicine and its aglycone *Divicine* are two natural phenolics extracted from the tropical plant *Fava bean (Vicia faba; broad bean, horse bean)*. In our study, *Divicine* was obtained by acid hydrolysis of *Vicine*. Structural elucidation of the extracted compound *Vicine* and its aglycon *Divicine* was proven using elemental analysis, infra-red and mass spectral data. Both the phenolic compounds were tested for their ability to inhibit peroxidation induced by free radicals, named; Fe^{2+}, superoxide, hydrogen peroxide and hydroxyl radicals. In addition, the results were compared with natural and synthetic antioxidants, such as α- tocopherol, ascorbic acid, butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and trolox. *Vicine* and *Divicine* exhibited a strong reducing power, chelating activity on Fe^{2+}, free radical-, hydrogen peroxide- and hydroxyl radical scavenging activities. All the mentioned spectral data and structural conditions explain that, *O-deglycosylation* of *Vicine* to give its aglycone, Divicine and increase its ability to inhibit peroxidation and reaction with peroxyl radicals.

Key words: *Vicine*, *Divicine*, *Fava bean*, antioxidants proper, free radicals scavenging.

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1. INTRODUCTION

Oxidative stress is a widely accepted participant in the development and progression of diabetes and its complications [4, 8]. Diabetes is usually accompanied by increased production of reactive oxygen species [6, 10, 50] or impaired antioxidant defenses [17, 39, 32] Streptozotocin is often used to induce diabetes mellitus in experimental animals through its toxic effects on pancreatic β-cells. Streptozotocin-induced diabetes mellitus is associated with the generation of reactive oxygen species causing oxidative damage .Traditional medicines provide better health coverage for 80% of the world population, especially in the developing countries [29] . *Faba bean* (*Vicia faba*) (broad bean, horse bean) is an important member of the legume family with highly useful characteristics. A studies investigating the anti-diabetic and free radical scavenging effects of *Faba bean* showed a hypoglycemic and antioxidant response [50]. The presence of *Visine* or *Divicine* in faba beans seeds [7], may be is one of reasons of its antidiabetic activity. On the other, the hypoglycemic effect of *Vicine* and anti-inflammatory property of *Vicine* and its aglucone *Divicine* isolated from *Faba bean* (*Vicia faba*) was reported [12] and [21]. As an extension of our interested research program in the extraction and therapeutic evaluation of medicinal plants [21] and [23]);

we report herein, a facile route to explain the antidiabetic and antioxidant activities of *Vicine* and its aglucone *Divicine* isolated from *Fava beans* (*Vicia faba*) and their effect on lipid peroxides and enzymatic antioxidant in streptozotocin (STZ)-induced diabetic rats, in which may pave the way for possible therapeutic application.

2. Materials and methods

2.1. Chemistry

Melting points were determined on Gallen-kamp melting point apparatus and are uncorrected. The infrared (IR) spectra were recorded on shimadzu MR 470 infrared spectrophotometer using the KBr pellets. Micro analytical data (C, H, N) was determined at the Micro analytical Centre, Cairo University, Egypt. Mass spectra were run using HP Model MS-5988.

Tested compounds

1. *Vicine* pure crystalline sample was extracted from mature seeds of fava beans (*Vicia faba*) according to the procedure described by [3]
2. *Divicine* pure crystalline sample was obtained by acid hydrolysis from *Vicine* according to the method described by [30]

2.2. Animals:

Male albino rats weighing around 200 ± 10 gms were purchased from Faculty of Veterinary Medicine, Cairo University. All the rats were given a period of acclimatization for 15 days before starting the experiment. Animals were provided with standard diet and water *ad libtum* and were kept on a 12 h light/12 h dark cycle, in a room with the temperature regulated to 21–25◦C and humidity at roughly 56%. The animals were used accordingly to guidelines of the Committee on Care and use of Experimental Animal Resources of Faculty of Pharmacy October 6 University, Egypt.

2.3. Induction of diabetes:

STZ-induced diabetes has been described as a useful experimental model to study the activity of hypoglycemic agents [25]. After an overnight fasting (deprived of food for 16 hours had been allowed free access to water), diabetes was induced in rats by intraperitoneal injection of STZ (Sigma, St. Louis, Mo) dissolved in 0.1 M sodium citrate buffer pH 4.5 at a dose of 55 mg/Kg b.w. The normal control rats received the same amount of 0.1 M sodium citrate buffer. The animals were allowed to drink 5% glucose solution overnight to overcome the drug-induced hypoglycemia. After a week time for the development of diabetes, the rats with moderate diabetes having glucosuria and hyperglycemia (blood glucose range of above 250 mg/dl) were considered as diabetic rats and were used for the further experiments. The change in the body weight experimental animals was observed throughout the treatment period.

2.4. Experimental set up:

The animals were classified into six groups with eight animals in each. A suspended solution of 1.0g/ 100 ml saline was prepared for intragastric intubation of rats.

Group I: Normal control (was given physiological saline solution). Group II: Normal control treated with *Vicine* (50mg/kg b.w/day) suspended in saline, orally for 30 days [21]. Group III: Normal control treated with *Divicine* (50mg/kg b.w/day) suspended in saline, orally for 30 days [21]. Group IV: Diabetic control (STZ-induced diabetic rats, was given physiological saline solution) [25]. Group V: Diabetic rats treated with *Vicine* (50mg/kg b.w./day) suspended in saline, orally for 30 days [21]. Group VI: Diabetic rats treated with *Divicine* (50mg/kg b.w./day) suspended in saline, orally for 30 days [21].

After 30 days of treatment the fasted rats were sacrificed by cervical decapitation and plasma glucose [38], insulin [45], triacylglycerols [11], total cholesterol [14] , HDL- cholesterol [2], LDL-cholesterol [13]

from the formula (LDL-cholesterol = total cholesterol – triacylglycerols/5 – HDL-cholesterol),VLDL-cholesterol concentration [15] from the formula (VLDL-cholesterol = triacylglycerols/5), the atherogenic index [log (TG/ HDL-C)] was also calculated [11], Serum iron [9], transferrin [18], ferritin [47] total iron bending capacity (TIBC) [35], blood Glutathione 6 phosphate dehydrogenase (G6PD) [19] in blood and liver reduced glutathione (GSH) [40] and respectively, superoxide dismutase (SOD) [33], catalase (CAT) [1], glutathione peroxidase (GPx) [37], glutathione-S-transferase (GST) [16], thiobarbaturic acid reactive substances (TBARs) [46], total- and glycated haemoglobin and liver [48] were determined using diagnostic kits (Sigma, St. Louis, Mo).

2.5.Statistical analysis:

All the data were statistically evaluated with SPSS/13 software. Hypothesis testing methods included one way analysis of variance (ANOVA) followed by least significant difference (LSD) test. P values of less than 0.05 were considered to indicate statistical significance. Diabetic control rats were compared with normal control rats. Experimental groups were compared with diabetic control. All the results were expressed as mean ± SD for eight animals in each group.

3. Results

Table 1 shows the changes in blood glucose, insulin, total- and glycated haemoglobin levels in normal control and experimental groups of rats. There was a significant increase in blood glucose and glycated haemoglobin by 173.7% (p<0.01) and 62.2% (p<0.01), respectively, decrease insulin and total haemoglobin by 62.34% (p<0.01) and 32.56% (p<0.01), respectively, in diabetic control group compared to normal control rats. Administration of *Vicine* and *Divicine* tends to bring down the blood glucose by 24.9% (p<0.01) and 51.28% (p<0.01), and glycated haemoglobin by 19.09% (p<0.05) and 32.24% (p<0.01), respectively compared to untreated diabetic rats. Treatment of animals with *Vicine* significantly increase the level insulin and haemoglobin by 35.45% and 19.20% (p<0.05) compared to untreated diabetic rats. While, treatment of animals with *Divicine* significantly increase the level insulin and haemoglobin by 62.36% (p<0.01) and 28.86% (p<0.01) compared to untreated diabetic rats. The effect was more pronounced in case of simultaneous administration of *Divicine*- treatment compared to administration of *Vicine*-treatment. Table 2 shows the changes in plasma TG, TC, HDL-C, LDL-C, vLDL-C and atherogenic index levels in normal control and experimental groups of rats. There was a significant increase in plasma TG, TC, LDL-C, vLDL-C and atherogenic index levels by 157.7% (p<0.01), 123.83% (p<0.01), 268.2% (p<0.01), 156.99% (p<0.01) and 110.28% (p<0.01) respectively, and decrease HDL-C by 13.68% (p<0.05) in diabetic control group compared to normal control rats. Treatment of animals with *Vicine* significantly decrease the level TG, TC, LDL-C, vLDL-C and atherogenic index levels by 30.04% (p<0.01), 27.69% (p<0.01), 38.43% (p<0.01), 30.10 (p<0.01) and

Table 1. Effect of *Vicine* and *Divicine* on plasma glucose, insulin, blood total- and glycosylated hemoglobin in control and experimental groups of rats

Groups	Glucose (mg/dl)	Insulin (µU/ml)	Haemoglobin (g/dl)	HbA1c (%)
Normal Control	99.33± 6.71	54.76 ± 6.05	13.51± 1.66	7.36 ± 0.16
Control + *Vicine*	102.67 ± 11.29	43.99 ± 5.35	12.82± 2.07	6.84 ± 0.09
Control + *Divicine*	83.83 ± 6.70	50.10 ± 7.854	12.44± 2.13	7.22 ± 0.11
Diabetic Control	272 ± 21.42**	20.62 ± 2.47**	9.11± 0.77**	11.94 ± 0.25**
Diabetic + *Vicine*	204.17 ± 14.07**	27.93 ± 2.02**	10.86± 0.84*	9.66 ± 0.08**
Diabetic + *Divicine*	132.50 ± 12.84**	33.48 ± 1.49**	11.74± 2.08**	8.09 ± 0.17**

Values are given as mean ± SD for groups of eight animals each. Values are statistically significant at **P*<0.05 & ***P*<0.01. Diabetic control rats were compared with normal control rats. Experimental groups were compared with the diabetic control rats.

Table 2 Effect of *Vicine* and *Divicine* on plasma triglyceride (TG), total Cholesterol (TC), HDL-cholesterol (HDL-C), LDL- cholesterol (LDLC), vLDL- cholesterol (vLDLC) and atherogenic index in control and experimental groups of rats.

Groups	TG (mg/dl)	TC (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	vLDL-C (mg/dl)	Atherogenic index
Normal Control	117.50 ± 9.76	103.50±4.80	43.83 ± 4.53	36.17±6.1	23.6 ± 4.3	0.428 ±0.006
Control + *Vicine*	112.67 ± 6.52	110.00 ± 6.47	47.17 ± 6.92	40.50± 4.40	22.33 ±3.6	0.378±0.009
Control + *Divicine*	108.00 ± 4.62	102.33 ± 9.47	44.33 ± 4.77	36.4± 5.22	21.6± 4.7	0.386±0.006
Diabetic Control	302.83 ± 7.51**	231.67 ± 8.54**	37.83 ± 9.74*	133.19 ± 8.7**	60.56± 5.8**	0.90±0.017**
Diabetic + *Vicine*	211.67± 8.87*	167.50 ± 13.12*	43.17± 3.54*	82.00 ± 5.3**	42.33 ± 7.2**	0.69±0.019*
Diabetic + *Divicine*	102.17 ± 4.02**	149.67 ± 5.00**	44.00± 4.23*	85.24± 6.9**	20.4±3.4**	0.365±0.017**

Values represent the mean ± SE (n=8). Diabetic control rats were compared with normal control rats. Experimental groups were compared with the diabetic control rats.

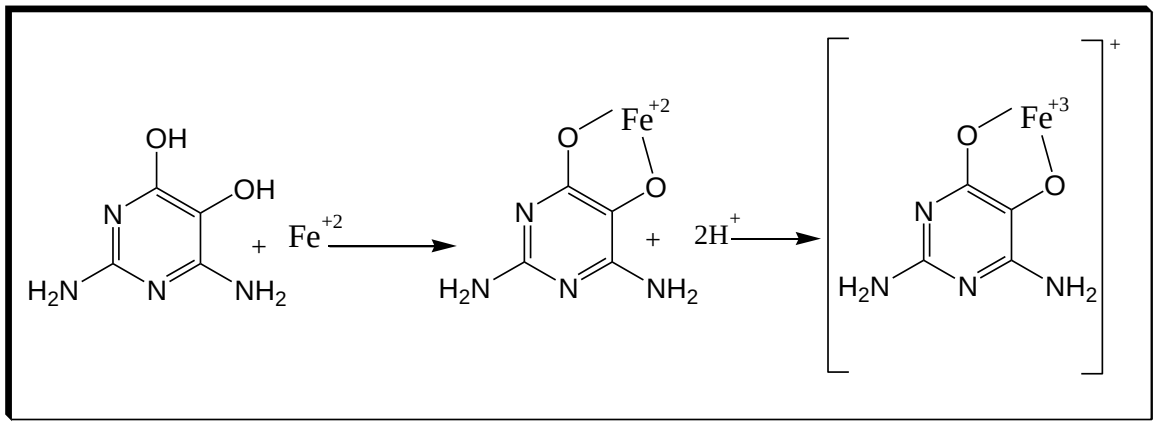
LDL-C (mg/dl) = TC-HDL-[TG / 5], vLDL-C(mg/dl) = [Triglycerides/5], Atherogenic index = log (TG/HDL-C)

- *Significantly different from control group at $p < 0.05$.
- **Significantly different from control group at $p < 0.01$.

Table 3 Effect of *Vicine* and *Divicine* on serum iron, transferrin, ferritin and total iron bending capacity (TIBC) in control and experimental groups of rats

Groups	Iron (mg/dl)	Ferritin (mg/dl)	Transferrin (mg/dl)	(TIBC) (mg/dl)
Normal Control	161.35 ± 9.12	29.45 ± 2.44	215.30 ± 8.89	457.33 ± 19.10
Normal Control + Vicine	126.79 ± 8.70	27.70 ± 3.26	223.42 ± 10.28	469.72 ± 18.80
Normal Control + Divicine	107.89 ± 7.92	27.64 ± 3.20	211.92 ± 9.37	474.11 ± 17.00
Diabetic Control	70.15 ± 5.09**	12.03 ± 4.09**	244.68 ± 7.48*	508.25 ± 25.85*
Diabetic + Vicine	100.72 ± 9.70**	16.09 ± 2.67**	172.40 ± 13.25*	364.78 ± 16.52**
Diabetic + *Divicine*	106.67 ± 6.49**	18.75 ± 2.48**	148.61 ± 10.44**	417.99 ± 15.46*

Values are given as mean ± SD for groups of eight animals each. Values are statistically significant at *P<0.05 & **P<0.01. Diabetic control rats were compared with normal control rats. Experimental groups were compared with the diabetic control rats.



Scheme 1: Chelating effect of Divicine

23.33% (p<0.01) and increase HDL-C by 14.12% (p<0.05) compared to untreated diabetic rats. While, treatment of animals with *Divicine* significantly decrease the level TG, TC, LDL-C, vLDL-C and atherogenic index levels by 66.26% (p<0.01), 35.39% (p<0.01), 36.00% (p<0.01), 66.31(p<0.01) and 60.0% (p<0.01) and increase HDL-C by 16.30% (p<0.05) compared to untreated diabetic rats. The effect was more pronounced in case of simultaneous administration of *Divicine*- treatment compared to administration of *Vicine*-treatment. Table 3 shows the changes in plasma iron, ferritin, transferrin and total iron bending capacity (TIBC) levels in normal control and experimental groups of rats. There was a significant decrease in plasma iron and ferritin by 56.52% (p<0.01) and 59.15% (p<0.01), respectively, and increase transferrin and total iron bending capacity (TIBC) by 13.64% (p<0.05) and 11.13% (p<0.05), respectively, in diabetic control group compared to normal control rats. Treatment of animals with *Vicine* significantly increase the level iron and ferritin by 37.7% (p<0.01) and 33.47% (p<0.01), respectively, and decrease transferrin and total iron bending capacity

Table 4 level of reduced glutathione (GSH) and activities of superoxide dismutase, catalase, glutathione peroxidase, glutathione-S-transferase and glucose 6-phosphate dehydrogenase (G6PD) in blood of control and experimental groups of rats

Groups	GSH (mg/g Hb)	SOD	CAT	GPx	GST	(G6P)
Normal Control	107.33 ± 6.34	25.68 ± 2.35	33.50 ± 3.06	118.16 ± 3.78	13.89 ± 1.89	21.2 ± 2.73
Normal + *Vicine*	75.56 ± 5.38	20.27 ± 0.92	27.57 ± 2.31	110.85 ± 5.04	11.49 ± 1.35	19.89 ± 3.27
Normal + *Divicine*	97.37 ± 4.09	22.36 ± 2.60	29.61 ± 3.07	100.01 ± 4.90	10.66 ± 1.52	20.73 ± 2.86
Diabetic control	52.06 ± 4.20**	11.67 ± 4.07**	20.80 ± 2.63*	67.29 ± 3.18**	5.44 ± 0.97**	10.48 ± 1.39**
Diabetic + *Vicine*	62.20 ± 5.58*	15.68 ± 2.22**	22.12 ± 3.57	83.33 ± 5.86**	6.96 ± 0.97**	14.55 ± 3.71**
Diabetic + *Divicine*	80.64 ± 3.56**	18.40 ± 2.67**	27.56 ± 2.04**	97.54 ± 2.21**	7.27 ± 0.84**	16.43 ± 4.00**

Values are given as mean ± SD for groups of eight animals each. Values are statistically significant at **P*<0.05 & ***P*<0.01; Diabetic control rats were compared with normal control rats. Experimental groups were compared with the diabetic control rats. Activity is expressed as: 50% of inhibition of epinephrine auto oxidation per min for SOD; µmoles of hydrogen peroxide decomposed per min per gram of hemoglobin for catalase; µmoles of glutathione oxidized per min per gram of hemoglobin for GPx; units per min per gram of hemoglobin for GST.

Table 5 level of reduced glutathione (GSH) and activities of superoxide dismutase, catalase, glutathione peroxidase, glutathione-S-transferase and thiobarbaturic acid reactive substances (TBARs) in liver of control and experimental groups of rats

Groups	GSH	SOD	CAT	GPx	GST	(TBARs)
Normal Control	227.16 ± 5.22	125.27 ± 4.06	22.36 ± 1.46	37.25 ± 2.15	42.85 ± 2.75	3.12 ± 0.05
Normal + *Vicine*	251.55 ± 8.18	110.36 ± 7.11	20.52 ± 1.73	35.04 ± 3.23	38.90 ± 5.08	3.23 ± 0.08
Normal + *Divicine*	232.64 ± 7.93	115.58 ± 6.38	19.44 ± 2.16	36.68 ± 2.55	39.54 ± 4.11	3.05 ± 0.04
Diabetic control	205.44 ± 6.35**	76.59 ± 3.59**	13.25 ± 0.98*	21.40 ± 2.37**	27.61 ± 2.07**	8.37 ± 0.07**
Diabetic + *Vicine*	215.16 ± 11.48*	95.41 ± 5.37**	17.68 ± 1.33	30.94 ± 4.54**	3 3.86 ± 3.55**	5.64 ± 0.09**
Diabetic + *Divicine*	230.64 ± 9.5**	108.40 ± 4.6**	18.37 ± 1.26**	34.07 ± 3.77**	37.49 ± 3.49**	4.57 ± 0.08**

Values are given as mean ± SD for groups of eight animals each. Values are statistically significant at **P*<0.05 & ***P*<0.01; Diabetic control rats were compared with normal control rats. Experimental groups were compared with the diabetic control rats. Activity is expressed as: 50% of inhibition of epinephrine auto oxidation per min for SOD; µmoles of hydrogen peroxide decomposed per min per mg protein for catalase; µmoles of glutathione oxidized per min per mg protein for GPx; units per min per mg protein for GST.

(TIBC) by 19.92% (p<0.05) and 28.22% (p<0.01), respectively, compared to untreated diabetic rats. While, treatment of animals with *Divicine* significantly increase the level iron and ferritin by 52.06% (p<0.01) and 55.86% (p<0.01), respectively, and decrease transferrin and total iron bending capacity (TIBC) by 39.26% (p<0.01) and 17.75% (p<0.05), respectively, compared to untreated diabetic rats. The effect was more pronounced in case of simultaneous administration of *Divicine*- treatment compared to administration of *Vicine*-treatment. Table 4 shows the changes in blood GSH, SOD, CAT, GPx, GST and (G6P) levels in normal control and experimental groups of rats. There was a significant decrease blood GSH, SOD, CAT, GPx, GST and (G6P) levels by 51.49% (p<0.01), 35.08% (p<0.01), 37.91% (p<0.01), 43.05% (p<0.01), 60.83% (p<0.01) and 50.56% (p<0.01) respectively, in diabetic control group compared to normal control rats. Treatment of animals with *Vicine* significantly increase GSH, SOD, CAT, GPx, GST and (G6P) levels by 19.47% (p<0.05), 34.36% (p<0.01), 6.35% (p<0.05), 23.83% (p<0.01), 27.94% (p<0.01) and 38.89% (p<0.01) compared to untreated diabetic rats. While, treatment of animals with *Divicine* significantly increase GSH, SOD, CAT, GPx, GST and (G6P) levels by 54.89% (p<0.01), 57.66% (p<0.01), 32.5% (p<0.01), 44.95% (p<0.01), 33.64% (p<0.01) and 56.77% (p<0.01) compared to untreated diabetic rats. The effect was more pronounced in case of simultaneous administration of *Divicine*-treatment compared to administration of *Vicine*-treatment. Table 5 shows the changes in liver GSH, SOD, CAT, GPx, GST and TBARs levels in normal control and experimental groups of rats. There was a significant decrease liver GSH, SOD, CAT, GPx and GST levels by 9.56% (p<0.05), 38.86% (p<0.01), 40.74% (p<0.01), 42.55% (p<0.01) and 35.56% (p<0.01), respectively, and increase TBARs by 168.26% (p<0.01) in diabetic control group compared to normal control rats. Treatment of animals with *Vicine* significantly increase GSH, SOD, CAT, GPx and GST levels by 4.73% (p<0.05), 24.57% (p<0.01), 33.43% (p<0.05), 44.57% (p<0.01) and 22.64% (p<0.01), respectively, and decrease TBARs by 32.62% (p<0.01) compared to untreated diabetic rats. While, treatment of animals with *Divicine* significantly increase GSH, SOD, CAT, GPx and GST levels by 12.27% (p<0.05), 41.64% (p<0.01), 38.64% (p<0.01), 59.21% (p<0.01) and 35.78% (p<0.01), respectively, and decrease TBARs by 45.40% (p<0.01) compared to untreated diabetic rats. The effect was more pronounced in case of simultaneous administration of *Divicine*- treatment compared to administration of *Vicine*-treatment.

4. Discussion:

Diabetics and experimental animal models exhibit high oxidative stress due to persistent and chronic hyperglycaemia, which thereby depletes the activity of antioxidative defense system and thus promotes *de novo* free radicals generation [5] reported that aglycones and non-glycosylated polyphenols inhibited glucose uptake whereas glycosides and phenolic acids were ineffective. These data suggest that aglycones inhibit facilitated glucose uptake whereas glycosides inhibit the active transport of glucose [24]. The present study was conducted to evaluate the beneficial effects of *Vicine* and its aglycone *Divicine* isolated from *Fava bean* on antioxidant status in STZ-induced diabetic rats. This study has investigated a possible method to isolate the active phenolic compound; *Vicine* from Fava beans (*Vicia faba*) [3] and by hydrolysing it, its aglucone

Divicine was obtained [30]. Structure elucidation of *Vicine* and *Divicine* were established based on both elemental analysis and spectral data and confirmed with the results presented by [21]. The preliminary studies conducted by this work revealed the non-toxic nature of *Vicine* and /or *Divicine* on normal rats. Although no literature data have given any insight into the possible toxicological potentials of *Vicine* and its aglycone *Divicine,* the dose of 50mg/kg b.w. chosen for treating animals did not cause obvious signals of toxicity. Acute treatment of the normal control group rats with saline alone did not produce any significant change ($p > 0.05$) in the blood glucose, hemoglobin and plasma insulin concentrations of either the fasted normal or the fasted STZ-treated diabetic rats. However, *Vicine* and *Divicine*, produced significant reductions ($p < 0.01$) in the blood glucose levels of fasted STZ-treated diabetic rats (table 1). In our study, elevated blood glucose level and decrease insulin level were observed in STZ-induced diabetic rats and it may be due to vitiate glucose oxidation and reduction of insulin biosynthesis and secretion. Oral administration of *Vicine* and *Divicine* at 50 mg/kg to the diabetic rats significantly reduced blood glucose level compared to diabetic control rats. Hence, the hypoglycemic activity of *Vicine* and *Divicine* may be due to its protective action against STZ-mediated damage to the pancreas beta cells and possibly because of regeneration of damage beta cell or increased insulin release or secretion. HbA1c is the product of non-enzymatic reaction between glucose and free amino group of Hb (glycosylation) [34]. It is marker of evaluation of long-term glycemic control in diabetic patients and predicts risks for the development and/or proression of diabetic complication [44]. Our study results showed that increased level of HbA1c and decreased Hb level were observed in diabetic rats compared to normal control rats, which indicate the occurrence of glycosylation in diabetic rats due to hyperglycemia.

The vascular diseases occurred in diabetes due to disturbance in lipoprotein metabolism, which causes acceleration of atherosclerosis [31]. In diabetic condition, increased level of TC, TG and reduced level of HDL-c along with altered composition of LDL-c particles were commonly reported [20]. In the present study, administration of STZ showed alteration of normal lipid profiles such as increase of TC, TG, LDL-c and VLDL-c levels as well as decreased HDL level compared to normal control rats (table 2). These altered lipid profiles were reversed to near normal level after treatment of *Vicine* and *Divicine* in STZ-induced diabetic rats.

In the present study, a decreased total iron and serum ferritin with a significant increase in serum transferrin and total iron binding capacity (TIBC) in diabetic rats control group ($P<0.01$) (table 3). In type 2 diabetes with insulin deficiency, the rate of iron deposition in body organs was increased [35] leading to decrease the serum iron level. Oral administration of *Vicine* and *Divicine* to the diabetic group of rats significantly reverted back iron, ferritin, transferrin and total iron binding capacity (TIBC) levels to near normal values, which show the anti-lipid peroxidative property of *Vicine and Divicine* in Type 1 experimental diabetes. The mentioned structural conditions may be found in a *Divicine* molecule which, in the in vitro systems efficiently scavenges hydroxyl radical ($OH^{\bullet}$), superoxide radical ($LOO^{\bullet}$), superoxide anion radical ($O_2^{\bullet-}$), singlet oxygen ($^1O_2$), and nitrogen oxide ($NO^{\bullet}$). Nevertheless, the mentioned structural conditions not allowed to *Vicine* molecule, so it have lower chelating potency less than its aglycone *Divicine* (Scheme 1).

STZ utilize low affinity glucose transporter 2 in the plasma membrane and its selectivity accumulation in pancreatic beta cells and it damage other organs, which can express this

transporter, particularly kidney and liver [27]. In diabetic state, free radical generation *via* increased glycolysis, auto-oxidation of glucose and non-enzymatic protein glycation [42]. Moreover, drastic reduction of in vivo antioxidant enzymes level in various tissues was reported in diabetic condition [42]. In our study, decreased levels of blood and liver SOD, CAT, GPx, GST and GSH as well as increased liver level of TBARs were observed in STZ-induced rats (table 4&5). The reduction of above enzymes directly reflect the oxidative stress in diabetic rats and these enzyme level changes may be due to generation of free radical by auto-oxidation of glucose, glycosylation in hyperglycemic condition as well as STZ mediated generation of ROS by its NO donor property to the intracellular molecules. In the present study, increased SOD, CAT, GPx, GST and GSH as well as reduced TBARs level were noticed in diabetic rats after administration of *Vicine and Divicine*. The above action represented that antioxidant property of *Vicine and Divicine* in diabetic condition and hence *Vicine and Divicine* possesses a potential to reduce or prevent the diabetic micro- and macrovacular complication. Our data confirm that *Vicine and Divicine* possesses blood glucose lowering action in diabetic condition. Moreover, it has hypolipidemic and antioxidant activities in diabetic state; therefore, it has an ability to prevent diabetic complications. Antihyperglycaemic, hypolipidemic and antioxidant Effect of *Vicine* and its aglucone *Divicine* isolated from *Fava beans* (*Vicia faba*), in streptozotocin-induced diabetic rats has not been reported earlier to our knowledge, and this study is perhaps the first observation of its kind. In conclusion, the present study showed that oral administration of *Vicine* and *Divicine* increases insulin sensitivity and reduces metabolic complications along with oxidative stress in diabetic rats. Further studies are essential to establish the role of *Vicine* and *Divicine* in controlling type 2 diabetes and its complications.

5. References

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التأثيرات الكيمائية الحيوية لمادتي الفيسين والداى فيسين المستخلصتان من نبات الفول الأخضر في الفئران

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الملخص العربى

الفيسين والداى فيسين هما القلويدات الطبيعية يتم استخلاصها من النباتات عدة مثل الفول، الفاصوليا. في دراستنا، تم استخلاص الفيسين من بذور نبات الفول، بالتحلل الحمضي باستخدام حمض الكبريتيك المخفف للفيسين تم نزع وحدة جلوكوز والحصول على الداى فيسين. فقد تمت دراسة التأثيرات الخافضة لنسبة السكر في الدم وكذلك التأثير المثبط للشوارد الحرة لكلا من الفيسين والداى فيسين في الجرزان بعد إعطاء جرعات فموية مقدارها 50 mg/kg b.w. لمدة 30 يوم. أظهرت هذه الدراسة أن اعطاء الجرعات قد سبب انخفاض معنوي في مستوى السكر والهيموجلوبين السكرى في الدم ومستوى الحديد (Iron) والترانسفريين (Transferrin) ومعامل ارتباط الحديد (TIBC) وحمض الثيوباربيتيورك (TBARs) في المصل. كذلك مستوى الترای أثيل جليسيرول (TG) والكوليسترول الكلى (TC) والكوليسترول ذو الكثافة المنخفضة(LDL–c) والكوليسترول ذو الكثافة المنخفضة جدا (vLDL–c) ومعامل قياس مدى تصلب الشرايين (Atherogenic index) في البلازما. كذلك أظهرت الدراسة ارتفاع معنوي في معدلات محتوى البلازما من الأنسولين والكوليسترول ذو الكثافة العالية (HDL–c) والفريتين (Ferritin) في المصل والهيموجلوبين وجلوكوز 6–فوسفات ديهيدروجينيز (G6PD) بالدم. كذلك مستوى الجلوتاثيون المختزل (GSH) والسوبر أكسيد دسميوتاز (SOD) والجلوتاثيون بيروكسيداز (GPx) والكتلاليز (CAT) والجلوتاسيون أس ترانسفيريز (GST) وفي خلايا الدم ونسيج الكبد. كذلك توضح النتائج أن الداى فيسين له تأثير أقوى من الفيسين في وقاية الكبد من الشوارد الحرة.

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