Sci., J.Fac., Sci., Minufiya Univ., 2005 Vol. XIX, 81 - 87

BIOCHEMICAL EFFECTS OF VICINE INJECTED INTO MALE RABBITS

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ABSTRACT

The effects of the β-pyrimidine glycoside extracted from *Vicia faba* L., on the malondialdehyde (MDA), glutathione (GS) and glutathione peroxidase (GP_X) and superoxide dismutase (SOD) activities in blood of male rabbits were studied. The major groups of 48, eight animals each, the 1st group is I.P. injected with saline and severed as controls daily for three weeks; untreated groups, the 2nd animals were injected I.P. with 206 mg/kg b.w. daily for three weeks and considered as vicine-treated rabbits, at the end of each week one of control animals and one of the treated animals were taken for blood investigation. Treatment of vicine increased markedly MDA and decrease blood glutathione level as well as decreased the activities of both GPx and SOD antioxidant enzymes.

Conclusion: overall, evidence has been produced that there is a dose reponse to the increased pereoxides of the pyrimidine glycoside. Vicine extracted from *Vicia faba* both in the oxiditative stress produced and in the decreasing of GPx and SOD activities and decreased GS level.

INTRODUCTION

Vicine is a glycoside (β-pyrimidine glycoside) in faba beans (*Vicia faba* L.) which is one of the most palatable important food in many country and is an excellent source of proteins (Harraz, 2000). Vicine is hydrolyzed by the intestinal flora to aglycones divicine and isouramile (Mager *et al.*, 1969; Escobar *et al.*, 1964; and Belsey, 1973). Tanaka and

Valentine (1968) and Turrini, *et al.* (1985) reported that faba bean increased red cell calcium, decreased calcium adenosine tirphosphate and altered

memberane proteins during faba bean hemolysis in glucose-6-phosphate dehydrogenease deficient individuals. Harraz (2000) demonstrated that vicine affects the levels of blood glutathione plasma glucose and serum enzyme markers (ALT, AST and alkaline phosphatase) and concluded that vicine is a major risk factor causing cell toxicity.

The toxicity of vicine has not been completely conducted. Hence, the present study aimed to evaluate the level of MDA malondialdehyde as a marker of the rate of lipid peroxidation, and this necessitate the determination of blood GPx and SOD enzyme activities as both enzymes are involved in the lipid peroidation prevention process in male rabbits (Chance *et al.*, 1979 and Chui *et al.*, 1982).

MATERIALS AND METHODS

A total of 64 adult male Newzeland rabbits 200±10 g/b.w. each rabbit under study were accommodated at the animal house and fed on the basal diet which contained (g/kg diet): corn starch 668, DL-methionine2, corn oil 50, salt mix 40, vitamins mix 10, and cellulose 50 (approximate 59% carbohydrate, 3% fat, 17% proteins, and 21% water-mineral cellulose (Tae-Youl *et al.*, 1995). The experiment was lasted after 3 weeks and two groups of 8 animals each were used as follows:

Group I : 24 rabbits I.P. injected with 2 ml saline daily for 1,2,3 weeks and used as control groups. One subgroup (8 animals) was taken at the ends of 1,2,3 weeks to be used as control subgroups for treated animals after 1,2,3 or 4 weeks of the beginning of the experiment.

Group II : 32 rabbits received 206 mg/kg b.w. as specified by Harraz (2000) inlapritonially vicine dissolved in 2 ml saline daily and one subgroup (8 animals) was taken after 1,2,3 or 4 weeks after vicine administration.

At the end of each week, one subgroup (control) and one subgroup of the vicine-treated animals. Two blood samples were immediately collected from heart into two clean, sterile labeled tubes. The first tube was heparinized to obtain whole blood sample in which glutathione peroxidase (GPx) activity was assayed using the enzymatic method of Paglia and Valentine (1967), superoxide dismutase (SOD) activity as assayed according to the enzymatic

method of Wooliams *et al.* (1983) and total GS according to Beutler *et al.* (1963).

The second tube without any anticoagulant. The blood was allowed to clot at room temperature and the serum sample was obtained by centrifugation at 3000 r.p.m. for 10 min The clear supernatant sample was transferred into dry sterile and labeled stoppered vial and kept at 4 °C till analyzed for MDA.

Determination of serum malondialdehyde (MDA) :

According to the method of Yoshioko *et al.* (1979); this method is based on the measurement MDA as one of the main end product of lipid peroxidation by reacting with thiobarbituric acid to yield a pink-colored complex exhibiting on absorption maximum at 530-532 nm and the color density is presumed to be proportional to MDA concentration. Statistical evaluation of the analytical data was done using student's *t*-test and *p*-value of < 0.05 was considered to be significant.

RESULTS

As may be seen from Table (1), peroxidation as indicated by malondialdehyde (MDA) level was seen to increase linearly with the increased duration (dose) of vicine. This linear increase in malondialdehyde (MDA) was paralleled by a linear decease of GPx activity. A decreases of blood glutathion (GS) level and superoxide mutase (SOD) activities also observed. The results obtained were summarized statistically calculated in Table (1).

DISCUSSION

Our results (Table 1) shows that total glutathione linearly decreased significantly in vicine treated animals after 1,2,3, and 4 weeks of treatment. This linear loss of total glutathione by passing of time maybe due to that glutathione is a part of the naturally occurring antioxidant defense system needed as preventative of the attack of the oxidative stress process and cell detoxification.The cytosolic form is used mostly in the overall tissue response to oxidative stress and it is required to maintain the functional integrity of mitochondrial respiration, which is crucial for cell metabolism. Cells need to be in a balanced reduction-oxidation (redox). The redox status of the cell and tissues can be quantitatively determined by calculating the GSH/ GSSG ratio (Sciuto, 1998). Induced by treatment of vicine, our study showed a drastic elevation of peroxides as indicated by the elevation of malondialdehyde

(MDA) level in serum. This could be therefore that vicine is ROS generator and lipid peroxidation increasing factor (Harazz, 2000; Kaplowitz, 1980; and Tan *et al.*, 1989). The decreased activity of GPx in vicine stressed animals may be due to that vicine induced generation of superoxide and hydrogen peroxide radicals in excess of the ability of the antioxidant enzyme GPx to remove these toxic species which may cause induced cell injury and cytotoxicity and consequently decreased GPx activity.

The decrease of superoxide dismutase (SOD) as shown in Table (1) which decreased by passing weeks (Table 1) may be due to that SOD is needed as antioxidant defense factor converts free super oxide anion (O_2^-) to H_2O_2 protecting the cell of the toxic effect induced by the reactive substance vicine.

In conclusion, the reactive pyrimidine divicin is a toxic substance affect the antioxidant defense system and is considered as few radical generator causes cell toxicity.

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Table 1

MDA, GS levels and GPx, SOD enzyme activities in male rabbits given the pyramidine glycosideVicine I, daily for 1,2 or 3 weeks and the results were compared with each corresponding control

Period of experim-entation weeks	Total number of doses D= 206[+] (mg/kg b.w./day)	Serum malondialdehyde (nmol/L MDA)		Whole blood: Total glutathione (G) (mg/dl G= GSH+GSSG		Whole blood: Glutathione peroxidase enzyme activity in U/L (GPx))		Whole blood: Superoxide dimutase enzyme activity in U/L (SOD)	
		Control subgroup	Treated subgroup	Control subgroup	Treated subgroup	Control subgroup	Treated subgroup	Control subgroup	Treated subgroup
I	7 days	4.11 ± 0.24	5.90 ± 0.28*	42.20 ± 1.30*	26.40 ± 1.03*	50.86 ± 3.41*	42.38 ± 3.1*	24.57 ± 2.13*	35.78 ± 2.27*
II	14 days	4.18 ± 0.24	6.86 ± 0.29*	40.10 ± 1.10*	25.81 ± 1.80*	53.3 ± 3.30*	40.2 ± 0.34*	25.42 ± 2.10*	36.41 ± 1.32*
III	21 days	4.52 ± 0.20	6.70 ± 0.24*	41.20 ± 6.83*	24.72 ± 2.20*	54.21 ± 3.0*	35.81 ± 3.10*	23.18 ± 3.65*	34.55 ± 2.78*

[+] Specified by Harraz (2000).
* Significant at $P \leq 0.005$.

الأثر السمي لبيتا – بيريميدين جليكوسيد (الفيسين) المستخلص من حبوب الفول على عوامل مضادات الأكسدة الحيوية فى الأرانب

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يهدف هذا البحث لدراسة أثر مستخلص مادة بيتا – جليكوسيد من حبوب الفول على الشقوق الحرة الناتجة من أكسدة الليبيدات ، الجلوتاثيون فى مصل الدم وكذلك نشاط إنزيمى الجلوتاثيون بيرأوكسيديز وسوبر أكسيد ديرميوتيز فى الدم الكامل.

أستخدمت ذكور الأرانب وعددها 48 أرنبا ، قسمت إلى ثلاث مجموعات معالجة بالمستخلص : 7 ، 14 ، 21 يوما ، وثلاث مجموعات ضابطة : 7 ، 14 ، 21 يوما ، كل مجموعة 8 أرانب (6 × 8 = 48) . حقنت حيوانات إحدى المجموعات المعالجة (8 أرانب) بجرعات يومية ، مقدار الجرعة 206 مجم/ كجم من وزن الحيوان ، ولمدة 7 أيام. حقنت حيوانات المجموعة الثانية (8 أرانب) بجرعات يومية مقدارها 206 مجم/ كجم ، ولمدة 14 يوما. حقنت المجموعة الثالثة (8 أرانب) بجرعات يومية مقدارها 206 مجم/ كجم ، ولمدة 21 يوما ، لكل مجموعة من المجموعات الثلاث السابقة مجموعة ضابطة خاصة بها (8 أرانب) تم حقن أفرادها بجرعات يومية مقدارها 2 مل من الماء الملحى (أستخدمت فى إذابة الفيسين) لمدد 7 أيام (8 أرانب) ، 14 يوم (8 أرانب) ، 21 يوم (8 أرنب).

الفيسين المستخلص من حبوب الفول له تأثير غير مرغوب على مكونات مصل الدم ، والدم الكلى من الجلوتاثيون والجلوتاثيون بيرأوكسيديز ، وسوبر أوكسيد ديزميوتيز ، ومولد للشقوق الحرة خاصة إذا أعطى لمدة طويلة وبصورة الاستمرار.