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EFFECT OF THE β-PYRMIMIDINE GLYCOSIDE VICINE EXTRACTED FROM FABA BEAN ON THE BIO-ANTIOXIDANT DEFENSE SYSTEM IN MALE REBBITS

Nasr A. Ziada

Nut. Deficiency Disease Unit. Animal Health Dept., Desert Res. Cenre, Cairo, Egypt.

ABSTRACT

This study was done to investigate the peroxidative effects of the β-pyrimidine glycoside compound (vicine) extracted from the faba been by studying the chemical alterations of malondialdehyde (MDA) as a maker of free radical generation. This necessitate the evaluation of superoxide dismutase (SOD) in whole blood as these parameters are part of the antioxidant defense system. 48 rabbits were classified as three treated subgroup: 7, 14, 21 days vicine treated groups and 7, 14, 21 days saline injected control groups for comparison. The result showed that vicine from faba bean is a toxic substance and the effect was dose -time dependent. It enhance the free radical generation and alter TG and the activities of both GPx and SOD. It was concuded that the reactive pyrimidine glycoside (vicine) of faba bean is a toxic substance and considered as free radical generator.

INTRODUCTION

Mager, et al., (1969); ***Belsey, (1973)*** stated that the vicine is a glycoside (β-pyrimiding glycoside) in Faba beans (Vicia Faba L.) which is one of the most palatable important food crop in Egypt and is an excellent source of proteins. Vicine is hydrolysed by the intestinal flora to aglycones divicine and isouramile. ***Tanaka and Valentine (1986)*** reported that, Faba bean increased red cell calcium, decreased calcium adenosine triphoshate and altered membrane proteins during faba bean hemolysis in glucose-6-phosphate dehydrogenase 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 malondialdehyde (MDA) as a marker of the rate of lipid peroxidation, and this necessitates the determination of blood glutathione peroxidase (GPx) and superoxide dismutase (SOD) enzyme activities as both enzymes are involved in the lipid peroxidation prevention process in male rabbits(***Chance, et al., 1979; Chui, et al., 1982***).

MARERIAL AND METHODS

This experiment was done at Marriott research station, Desert Research Center, at 35 k of Alexandria.

I- Experimental animals:

A total of 48 Male pure New Zealand white rabbits, aged 3 months and weighing 2.3 - 3.6 kg and housed (one per cage) in an animal room at 25°C with a 12-h light:dark cycle. They fed on the basal diet which contained (gm/kg diet): Corn starch 688, Dl-methionine 2, corn oil 50, salt mix 40, fat 1.2%, 17% proteins and 21% water-mineral-cellulose ***(Tae-Yoal, et al., 1995)***. The animals were divided into two major groups of 12 each and further sub-grouped and treated as the following:

Saline treated control groups:

The first 12 animals were subdivided into three sub-groups, 4 animals each and I.P injected with a daily dose of 2 ml saline and were classified into three control groups according to the time of saline injection as follows:

- 7-days saline injected group (4 animals).
- 14-days saline injected group (4 animals).
- 21- days saline injected group (4 animals).

Each sub-group was used as control for the corresponding vicine treated group of the same period of vicine treatment.

Vicine treated control groups:

The second 12 animals were as on case of the first 12 animals were sub-divided into thre sub-groups (of 4 animals each) and injected with a daily dose of 206 mg/kg body weight of vicine as specified by ***Harraz, (2000)*** dissolved in 2 ml saline and used as vicine treated sub-groups. According to the time of treatment, they were classified into:

- 7-days vicine treated sub-group (4 animals).
- 14-days vicine treated sub-group (4 animals).
- 21- days vicine treated sub-group (4 animals).

The result of each subgroup was compared to each control saline-injected one.

At the end of 1,2 and 3 weeks, one saline treated subgroup (control) and one subgroup of the vicine-treated animals were taken for investigation.

Two blood samples were immediately collected from heart into two clean, sterile labeled tubes. The first tube was heparinized to obtain the whole blood sample in which (GPx) activity was assayed using the ezymatic method of ***Peglia and Valentine, (1967)*** (SOD) activity as assayed according to the ezymatic method of ***Wooliams, et al., (1983)*** and total glutathion (TG) according to ***Beutler et al., (1963).*** The second tube was without any anticoagulant. The blood was allowed to clot at room temperature and the serum sample was obtained by centrifugation at 3000 rpm for 14 min. The clear supernatant serum sample was transferred into dry sterile and labeled stoppered vial and kept at $4^{o}C$ till analysed for MDA. Determination of serum (MDA) according to ***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-coloured complex exhibiting an absorption maximum at 530-532 nm and the colour intensity is presumed to be proportional to MDA concenteration.

Statistical evaluation of the analytical data was done using Student's t-test and $P \leq 0.05$ was considered to be level of significant (***Snedecor and Cochran, 1976***).

RESULTS

As may be seen from table (1), peroxidation as indicated by MDA level in serum was seen to increase linearly with the increased duration (time) of vicine. This linear increase in malondialdehyde reported in Table (1) was paralleled by a linear decrease of GPx activity. A decrease of whole blood (TG) level and (SOD) activity werealso observed (Table 2). The results obtained were summarized and statically calculated in Table (1).

Table (1): Serum malondiadehyde levels (in nmol/L) in daily rabbits before and after administration of vicine for 1,2 and 3 weeks of treatment (data were expresses as mean ± standard error).

Period of experimentation (weeks)	Total number of doses	Serum (MDA) in nmol/L	
		Control subgroup	Treated subgroup
1	7 doses	4.11 ± 024	5.90 ± 0.28 *
2	14 doses	4.18 ± 0.24	6.86 ± 0.29 *
3	21 doses	4.52 ± 0.20	6.70 ± 0.24 *

MDA= Malondialdhyde. * The daily dose was 206 b.w for each 8 animals. **Significant at P ≤ 0. 05.

Table (2): Whole blood TG, GPx and SOD in rabbits before and after daily administration of vicine for 1,2 and 3 weeks of treatment (data were expressed as mean + standard error).

weeks	Number of doses	Whole blood					
		GSH mg/dl		(GPx) in U/L		SOD in U/L	
		Control subgroup	Treated subgroup	Control subgroup	Treated subgroup	Control subgroup	Treated subgroup
1	7 doses	42.20 ± 1.30	26.40 ± 1.03*	50.86 ± 3.41	42.38 ± 3.1*	45.7 ± 21.3	57.8 ± 22.7*
2	14 doses	40.10 ± 1.10	25.81 ± 1.80*	53.3 ± 3.3	40.2 ± 3.40*	25.42 ± 2.10	36.41 ± 1.30*
3	21 doses	41.20 ± 6.83	24.72 ± 2.29*	54.21 ± 3.0	35.81 ± 3.10*	23.18 ± 3.65	34.55 ± 2.78*

TG = Total glutathione in mg/dl. TG = GSH + GSSG. GPx = Glutathione peroxidase enzyme activity. SOD = Superoxide dismutase enzyme activity. The daily dose was 206 mg/kg b.w for 8 animals. **Signofocant at < 0. 05.

DISCUSSION

Our results (Table 1) shows that the total glutathione linearly decreased significantly in vicine treated animals 1, 2 and 3 weeks of treatment. These results agreed with that obtained with ***David, et al., (2001)*** who report that vicine rapidly stimulates HMP shunt activity and depletes TG in rat erythrocytes with concomitant formation of glutathione-protein mixed-disulfides. These biochemical alterations are associated with profound membrane skeletal protein damage and transformation of the cells to an extreme echinocytic morphology and ***Sciuto, (1998)*** who stated that the TG 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 cell and tissues can be quantitatively determined by calculating the GSH/GSSG ratio. This linear loss of total glutathione by passing of time may be due to that TG is a part of the naturally occurring antioxidant defense system needed as preventative of the attack of the oxidative stress process and cell detoxification induced by treatment of vicine (***Albano, et al.,1984***). Our study showed (Table 1) a drastic elevation of peroxides as indicated by the elevation of (MDA) level in serum. This result coincide with those obtained by ***Kaplowitz, 1981; Tan, et al., 1989; Harraz, 2000*** who illustrated these results that the vicine is reactive xygen species generator and lipid peroxidation increasing factor; ***Pedersen et al., 1988; Winterbourn, 1993*** who stated that the mixed disulfide formation could occur as a result of attack by a compound-centered free radical. In support of this concept, semiquinone radical intermediates of vicine have been detected in a cellular systems by spectroscopy; ***Chevion et al., (1982); Winterbourn., (1993)*** postulated that β-pyrimidine aglycones, liberated upon digestion of their parent

glucosides, vicine are absorbed into the blood and induce oxidative damage within erythrocytes as a consequence of their redox activity and ***Henrich, et al., (2004)*** also, sugested that the β-pyrimidine aglycones from vicine reduced food consumption and reduced performance, probably due to their peroxidative effect o the intestinal cell causing physiological and morphological changes in the intestinal tract. Our results in Table (1) revealed The decreased activity of GPx in vicine treated rabbits compared with control one. These results agreed with ***Floh and Eunzier, (1976); Kosower and Kosower, (1978) and Irene. (2005)*** who reported that the decreased activity of glutathione peroxidase 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.

Our results in Table (2) showed decreased activities of SOD in vicine treated rabbits compared with control group.The decrease of superoxide dismutase triggered by passing weeks and 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. This results agreed with that obtained with ***Winterbourn, (1993)***; ***Srinivasan, (2004)*** who found the Concerted action of reduced (GSH) and SOD on preventing redox cycling of dihydroxypyrimidines.

In conclusion, the reactive β-pyrimidine glycoside vicine is a toxic substance which affect the antioxidant defense system and is considered as free radical generator causes cell toxicity and consequently, suppression of immunity.

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تأثير جليكوسيد البيتا ـ بيريميدين (الفيسين) المستخلص من حبوب الفول على أنظمة مضادات الأكسدة الحيوية في الأرانب

د. نصر عبد الوكيل نصر زيادة

قسم صحة الحيوان – وحدة أمراض النقص الغذائى . مركز بحوث الصحراء . القاهرة.

يهدف هذا البحث لدراسة أثر مستخلص مادة جليكوسيد البيتا بيريميدين من حبوب الفول على الشقوق الحرة الناتجة عن أكسدة الليبيدات، على الجلوتاثيون في مصل الدم وكذلك نشاط أنزيمي الجلوتاثيون بيرأوكسيديز وسوبر أكسيد ديزميوتيز في الدم الكامل.

استخدمت ذكور الأرانب وعددها 24 أرنباً قسمت إلى ثلاث مجموعات معالجة بالمستخلص 7 ، 14 ، 21 يوماً وثلاث مجموعات ضابطة 7 ، 14 ، 21 يوماً ، كل مجموعة 8 أرانب. حقنت حيوانات إحدى المجموعات المعالجة (4 أرانب) بجرعات يومية مقدار الجرعة 206 مجم/كجم من وزن الحيوان ولمدة 7 أيام. حقنت حيوانات المجموعة الثانية (4 أرانب) بجرعات يومية مقدارها 206 مجم/كجم ولمدة

14 يوماً. حقنت حيوانات المجموعة الثالثة (4 أرانب) بجرعات يومية مقدارها 206 مجم/كجم ولمدة 21 يوماً.

لكل مجموعة من المجموعات الثلاثة السابقة مجموعة ضابطة خاصة بها (4 أرانب) تم حقن أفرادها بجرعات يومية مقدارها 2 مل من الماء المحلى (استخدم في إذابة الفيسين) لمدد 7 أيام (4 أرانب) ، 14 يوم (4 أرانب) ، 21 يوم (4 أرانب).

نتائج البحث :

الفيسين المستخلص من حبوب الفول له تأثير غير مرغوب فيه على مكونات مصل الدم والدم الكلي من الجلوتاثيون الكلى و الجلوتاثيون بيرأوكسيديز و سوبر أوكيد ديزميوتيز ومولد للشقوق الحرة خاصة إذا أعطي لمدة طويلة وبصورة الاستمرار.