EFFECT OF INTERNAL PARASITIC INFESTATION ON ANTIOXIDANT ENZYMES IN BUFFALO-CALVES

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Abstract

This study aimed to investigate the effect of the internal parasites on the erythrocyte oxidative status through determining the activities of the antioxidant enzymes, catalaze (CAT), glutathione peroxidase (GSH-Px) and superoxide dismutase (DOS), in buffalo-calves. A total of 102 faecal samples were collected randomly from both sex buffalo-calves of one week – 12 months old, belonging to some private farms in Sharkia Province. The microscopic examination of faecal samples revealed that the prevalence rate of gastro-intestinal nematodes was 24.5% (*Toxocara vitulorum* 10.8%, *Oesophagostomum radiatum* 7.8% and *Trichostrongylus* spp. 5.9%). The infestation rate of trematodes (*Paramphistomum*) and Cestodes (*Moniezia* spp.) was 3.9% for both. Lastly, the percentage of *Eimeria* spp. and *Cryptosporidium* sp. was 4.9% and 2.9%, respectively in examined samples. The blood samples with anticoagulant were collected from only infested calves and used for determination of haemoglobin and evaluation of the level of malondialdehyde (MDA) and the activities of the antioxidant enzymes (CAT, GSH-Px and DOS) in the hemolysate. The results of this study revealed a significant increase in both the activities of the antioxidant enzymes and the level of MDA in the parasite infested calves than the healthy group, where the parasitic infestation as a stress factor led to release voluminous amounts of oxyradicals (free radicals and oxygen reactive species). So, large amounts of antioxidant enzymes are produced to neutralize these free radicals.

INTRODUCTION

The antioxidant is defined as any substance which when present at low concentration compared to those of an oxidizable substrate, significantly delays or inhibit oxidation of that substrate. The antioxidant enzymes include superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px), while, the non-enzymatic antioxidant compounds include vitamins A, E, & C, albumin and glutathione (GsH) (Frei, 2001).

Antioxidants represent the first line of body defence against oxidative stress produced by the generation of free radicals and reactive oxygen species (ROS). They convert ROS to safe compounds before causing severe damage to cells and tissues, and also, protect lipids from the peroxidation by free radicals.

Glutathione peroxidase (GSH-Px) found in erythrocytes protects hemoglobin against oxidative damage by hydrogen peroxide, using glutathione and selenium-glutathione peroxidase to reduce H_2O_2 to H_2O (Reilly, 1996).

Superoxide dismutase (SOD) is present in the cell by a group of metalo-enzymes (Copper/Zinc SOD. Manganese containing SOD and iron containing SOD) SOD catalyze the dismutation of superoxide ions into oxygen and hydrogen peroxide (H_2O_2) (Goldstein and Czapski, 1996).

Catalase is an iron-containing enzyme found in the small membrane-enclosed cell components called peroxisomes. It serves to detoxicate hydrogen peroxide by catalyzing a reaction between two hydrogen peroxide molecules, resulting in the formation of water and O_2 (Frei, 2001).

Malondialdehyde (MDA) is one of the main lipid peroxidation products. Its presence in serum and tissue indicates a high level of lipid peroxidation with impaired antioxidant status. Lipid peroxidation severely damages the cell membrane and compromises cell growth rate (Julian 1998).

Effects of helminths on the production are well documented allover the world. The anorexia and reduction in feed intake, loss of blood and plasma proteins in gastro-intestinal tract, alteration in protein metabolism, decrease in levels of minerals, enzymes and diarrhoea, all contribute to loss in weight gain. Internal parasites adversely affect the health and productivity of animals, and also, decrease the resistance of animals to various diseases which may ultimately lead to higher mortality (Soulsby, 1983).

Buffaloes are the prime source of good quality meat and milk in Egypt and some other developing countries. These animals are mainly reared in small holder farms and suffer from a lot of stress conditions such as mal-nutrition and parasitism. Therefore, the present study was designed to throw light on the effect of gastrointestinal parasites on the activity of the antioxidant enzymes (glutathione peroxidase, superoxide dismutase and catalase) in buffalo-calves.

MATERIALS AND METHODS

I. Animals

A total of 102 buffalo-calves 1 week to 12 months age belonging to some private farms in Sharkia Province, were used in this study. The calves showed emaciation, poor coat, constipation, mild diarrhoea and paleness of mucous membranes.

II. Samples and adopted methods

(A) Faecal samples

Faecal samples collected from rectum of 102 buffalo-calves were examined microscopically using direct smear, flotation and sedimentation techniques (Coles, 1986). Identification of gastrointestinal parasite eggs was carried out depending on the microscopical morphology of the eggs. A modified McMaster technique was used for faecal egg count**,** individuals harbouring > 500egg per gram (EPG) were considered clinically ill (Soulsby, 1983).

(B) Blood samples

These wre collected from only infested calves (n = 41), as well as the clinically healthy and parasitologically free calves (n = 10) used as a control group. The samples were collected (10ml) in tubes containing anticoagulant (heparin). These samples were used firstly for hemoglobin determination according to Coles (1986). Then, the fresh heparinized blood was centrifuged for 10 minutes at 3500 rpm, and the plasma was separated. The red cells were washed twice with cold saline solution, the cells were lysed with 20% parts of cold redistilled water. The lysate was divided into 4 parts in sterile ependorff tubes for determining the activity of glutathione peroxidase, catalase, superoxide dismutase and the concentration of malondialdehyde according to the method described by Pagalla and Valentine (1967), Sinha (1972), Packer and Glazer (1990) and Esterbauer *et al*. (1982) using shimudzu type spectrophotometer.

III. Statistical analysis

Data were performed by means of Software Computer Program, and correlation coefficients were carried out according to Snedecore and Cochran (1967).

RESULTS AND DISCUSSION

The parasitological examination of buffalo-calves in this study (n = 102) revealed that 41 calves were infested with gastrointestinal parasites, showing variable degrees of non-specific clinical manifestation from loss of body weight, paleness of mucous membranes, softness of faecal matter and ill-thriftiness. The different species of parasites in examined calves included Nematodes (*Toxocara vitulorum*, *Oesophagostomum radiatum* and *Trichostrongylus* spp.), the Trematode (*Paramphistomum*), the cestode (*Moniezia* spp.) and Protozoa (*Eimeria* spp. and *Cryptosporidium* spp.) (Tables 1 and 2 and Figure 1), with percentages 24.5%, 3.9%, 3.9% and 7.8%, respectively.

Table 3 illustrated the percentage prevalence of GI parasites in different age groups of buffalo-calves where, the age of animals is considered to be the major factor

in the prevalence of parasitic infections. These results agree with those of Maichomo *et al*. (2004).

The activities of antioxidant enzyme in erythrocytes (CAT, GSH-Px, SOD) of buffalo-calves infested with GI parasites (Table 4) revealed significant increase in their level than normal ones ($P < 0.01$, $P < 0.05$ and $P < 0.01$).

The internal parasites causes severe gastro-intestinal problems, causing damage in the cellular lining of gastro-intestinal tissue. This damage is one of the stress conditions that generates free radical production, causing oxidative stress. The antioxidant enzymes are a primary line of defence against these radicals, where they are scavenging them at their site of generation. So, the increase of activity of these enzymes indicates a higher exposure of the erythrocytes to the risk of oxidative stress, where this increase is an indirect compensatory response of cells to increase oxidant challenge during the stress conditions (Frei, 2001).

The elevation in the activity of anti-oxidant enzymes in this study could represent an adaptable change to scavenge excess reactive oxygen species. This compensatory increase in anti-oxidant enzymes has been previously reported in different situations that course with oxidative stress as cancer, heart diseases, rheumatoid arthritis and several gastro-intestinal disorders, where the imbalance between the production of free radicals and their safe disposal induce the oxidative stress (Bernabucci *et al*. 2002). The obtained results are in agreement with those of Kolodzie *et al*. (2005).

Also, Deger *et al*. (2008) found an increase in activity of antioxidant enzymes in sheep infested with liver trematodes (*F. hepatica*, *F. gigantica* and *Dicrocoelium dentriticum*), where these parasites cause the release of reactive oxygen species. Lastly, Adam and Yucel (2008) found a significant increase in the erythrocyte SOD, CAT and GSH-Px activities in calves with coccidiosis than that in the healthy ones.

In contrast of the results of this study, many authors (Abd Ellah *et al*. 2008) reported a decrease of activities of antioxidant enzymes in animals with parasitic infestation. This may be attributed to the severity of parasitic infestation and its stage, and presence of other stress factors as heat stress, humidity, or nutritional deficiency.

The mean values of haemoglobin content and malondialdehyde concentration in buffalo-calves (Table 5), show significant decrease ($P < 0.001$) in haemoglobin concentration and significant increase ($P < 0.05$) in MDA level in infested calves than in apparently healthy ones. This decrease in haemoglobin level could be attributed to parasitism where bone marrow fails to produce enough erythrocytes owing to toxins produced by parasites (Karapehlivan *et al*., 2007).

Similar results were obtained by Ahmed and Hassan (2007), who reported that the increase in MDA level may reflect the work of the defence mechanisms against the lipid peroxidation during the oxidative stress in parasitic infestation. This high level of MDA in the affected calves indicated the advanced peroxidation process in the cell membrane (Karapehlivan *et al*. 2007).

The analysis of our data illustrated in table 6 revealed a non- significant positive correlation between MDA concentration and the activity of the anti-oxidant enzymes, where there is a negative correlation between the haemoglobin concentration and the activity of the anti oxidant enzymes, and this correlation is significant in catalase enzyme.

It could be concluded that the parasitic infestation acts as a stress factor on animal body and interferes with the oxidative status leading to increase the oxidative stress with subsequent adverse effect on health conditions.

Table 1. Prevalence of helminthiasis in buffalo-calves

Helminths	*No. of animals examined*	*No. of animals infected*	*% of infection*
Nematodes	102	25	24.5
Trematodes	102	4	3.9
Cestodes	102	4	3.9
Protozoa	102	8	7.8
Total	102	41	40.1

Table 2. Helminth species identified in buffalo-calves

Species	*No. of positive samples*	*% of infection*
Toxocara vitulorum	11	10.8
Oesophagostomum radiatum	8	7.8
Trichostrongylus spp.	6	5.9
Paramphistomum	4	3.9
Moniezia spp.	4	3.9
Eimeria spp.	5	4.9
Cryptosporidium sp.	3	2.9

Table 3. The percentage prevalence of gastro-intestinal helminths in different age groups of buffalo-calves.

Age groups	*Helminths species*	*No. of positive samples*	*%*
1 – 120 days old	*Cryptosporidium* sp.	3	7.3
	Toxocara vitulorum	11	26.8
121 – 240 days old	*Trichostrongylus* spp.	6	14.6
	Moniezia spp.	4	9.8
	Oesophagostomum radiatum	8	19.5
	Eimeria spp.	2	4.9
241 – 365 days old	*Eimeria* spp.	3	7.3
	Paramphistomum	4	9.8

Table 4. Activities of antioxidants enzymes in the erythrocytes of buffalo-calves, expressed in U/g Hb.

Parameters	*Apparently healthy calves (n = 10)*	*Infected calves (n = 41)*
Catalase	12.8 ± 0.432	18.326 ± 1.51**
Glutathione peroxidase	0.462 ± 0.0367	0.642 ± 0.0677*
Superoxide dismutase	79.096 ± 3.352	89.338 ± 1.045**

*Significant at (P < 0.05) **Highly significant at (P < 0.01)

Table 5. Mean values of haemoglobin and malondialdehyde concentration in buffalo-calves

Parameters	*Apparently healthy calves (n = 10)*	*Infected calves (n = 41)*
Hb (g/l)	12.06 ± 0.12	8.90 ± 0.71***
MDA (nmol/mL)	8.29 ± 0.31	9.34 ± 0.30*

*Significant at (P < 0.05) ***Very highly significant at (P < 0.001)

Table 6. The correlation between Hb and MDA concentration and the activities of antioxidant enzymes in buffalo-calves.

	CAT	*GSH-Px*	*SOD*	*Hb*	*MDA*
Hb	- 0.788**	- 0.257	- 0.563	1.000	- 0.485
MDA	0.427	0.267	0.416	- 0.485	1.000
CAT	1.000	0.305	0.530	- 0.788**	0.427
GSH-Px	0.305	1.000	0.269	- 0.257	0.267
SOD	0.530	0.269	1.000	- 0.563	0.416

** Correlation is significant at the 0.01 level

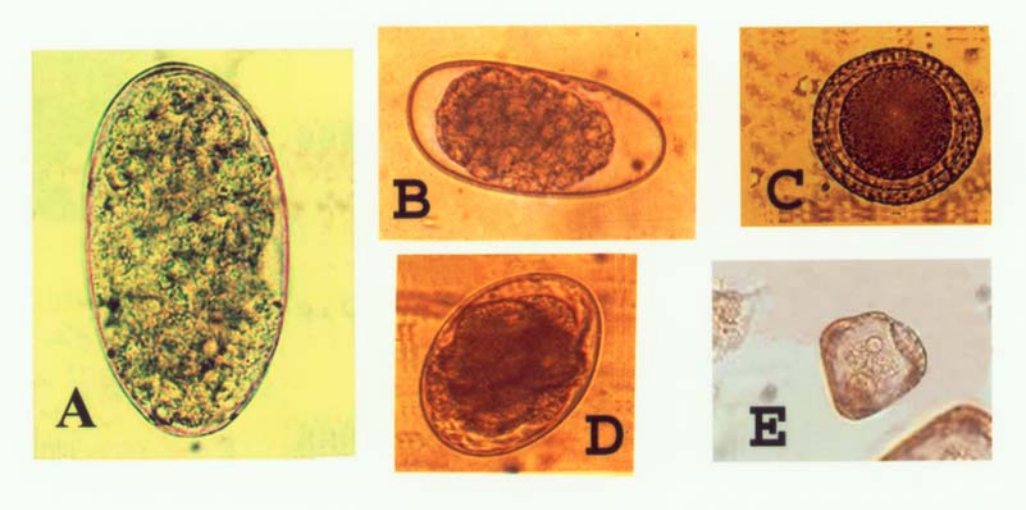

Figure 1. (A) *Paramphistomum spp. egg, (B) Trichostrongylus spp. egg, (C) Toxocara vitulorum egg, (D) Oesophagostomum radiatum egg and (E) Moniezia spp. egg*

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تأثير الإصابة بالطفيليات الداخلية علي الإنزيمات المضادة للأكسدة في عجول الجاموس

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أجريت هذه الدراسة بهدف معرفة تأثير تواجد الطفيليات الداخلية علي نشاط الأنزيمات المضادة للأكسدة المتواجدة داخل خلايا الدم الحمراء – الكتاليز والجلوتاسيون بيراكسيديز، وسوبر أوكسيد ديسميوتاز – وذلك في العجول الجاموس بإحدي المزارع الخاصة بمحافظة الشرقية.

تم إجراء هذا البحث علي عدد ١٠٢ عجل جاموس من كلا الجنسين ويتراوح أعمارها من أسبوع إلي سنة ، وقد تم تجميع عينات براز من هذه العجول حيث أظهرت نتائج الفحص الطفيلي إصابة عدد ٤١ عجلاً منها بالطفيليات الداخلية (٤٠,١%) كالتالي: إصابة عدد ٢٥ عجلاً بالديدان الأسطوانية بمعدل انتشار ٢٤,٥% (ديدان الأسكارس "تكسوكارا فيتللورم" بنسبة ١٠,٨%، طفيل الأوسفجوستومم راديـاتم بنسبة ٧,٨% وطفيل ترايكوسترونجيليس بنسبة ٥,٩%) – وكانت الإصابة بالديدان المفلطحة (البارامفستومم) بمعدل عدد ٤ عجول بنسبة ٣,٩% وكذلك الديدان الشريطية (المونيزيا) بمعدل عدد ٤ عجول بنسبة ٣,٩% وأخيراً كانت نسبة الإصابة بالطفيليات الأولية (الكوكسيديا والكربتوسبوريديم) ٧,٨% بمعدل ٥ و ٣ عجول علي التوالي لكل منها.

تم قياس نشاط الأنزيمات المضادة للأكسدة وكذلك قياس مستوي الهيموجلوبين ومستوي المالوينك داي الديهايد (***MDA***) المعبر عن الأكسدة الفوقية للدهون الخلوية – وذلك في العجول المصابة بالطفيليات الداخلية (عدد ٤١) وكذا (عدد ١٠) لمجموعة أخري سليمة إكلينيكيا وخالية من الطفيليات (مجموعة ضابطة) .

أظهرت نتائج البحث وجود زيادة في نشاط الأنزيمات المضادة للأكسدة وكذلك في مستوي المالونيك داي الديهايد في العجول المصابة بالطفيليات الداخلية عن أفراد المجموعة الضابطة وذلك كرد فعل لانطلاق كميات هائلة من العوامل المؤكسدة (***ROS***) وفي سبيل إحداث التوازن بين معدل إنتاج الشوارد الحرة ومعدل التخلص الآمن لها.

لذلك يتضح من هذه الدراسة وجود زيادة في ضغوط الأكسدة في الحيوانات المصابة بالطفيليات الداخلية مما يؤثر علي الحالة الصحية للحيوان وإنتاجيته.