EFFICACY OF HERBAL BINDER IN AFLATOXIN B1 INDUCED TOXICITY ON HAEMATOLOGICAL PROFILE IN WISTAR RATS

Prajapati A., Y. Verma*, M. Swamy and A. Dubey

Department of Veterinary Pathology

College of Veterinary Science and A.H., N.D.V.S.U., Jabalpur – 482001

Corresponding author e-mail: dryaminiverma@rediffmail.com

Received 30-7-2013 Accepted 25-8-2013

ABSTRACT

A study was conducted in forty eight male wistar rats to evaluate the efficacy of herbal binder on AFB1 induced toxicity on haematological profile. Aflatoxin adversely affected haematological profile. Administration of AFB1 was found to be immunosuppressive, leading to significant reduction in total erythrocyte count, haemoglobin concentration, packed cell volume and lymphocyte percent. There was significant increase in total leukocyte count and neutrophil in rats of group II as compared to the control group animals. However supplementation of herbal binder diminished the toxicity produced by aflatoxin administration in rats.

KEY WORDS : AflatoxinB1 (AFB1), hematological, herbal binder, rats

INTRODUCTION

Aflatoxins are secondary toxic metabolites produced by certain strains of fungi, *Aspergillus flavus and Aspergillus parasiticus spp* (Manafi *et al*., 2011) and *Penicillium spp.* Aflatoxin B1 is the most toxic among all aflatoxins. Surveillance of poultry and animal feed samples showed moderate to high percent of aflatoxin in India (Gupta, 2011). Chronic aflatoxicosis due to prolonged intake of low level of aflatoxins retards growth, reduces feed conversion ratio and increases susceptibility of chicks to infectious diseases. Aflatoxicosis leads to immunosuppression, characterized by decreased immune response (Bakshi *et al*., 2000) and breakdown of vaccine immunity (Panisup *et al*., 1982). Deleterious effects of Aflatoxin could be overcome or at least diminished by adsorbents/ herbal mycotoxin binder (HMB) in rats (Abdel-Wahhab *et al*., 2002). Rats have been used extensively for decades as a model for human/livestock mycotoxicosis. Studies on effect of combination of antioxidants and adsorbents in experimental animals are scanty. Hence the present investigation was carried out to study the protective role and efficacy of herbal toxin binder product in rats during induced aflatoxicosis.

MATERIALS AND METHODS

Forty eight adult male wistar rats weighing 140-150 g were procured from Bharat Traders, Bhopal. Prior to experiment all the rats were kept at laboratory condition for a period of 7 days. They were maintained in good hygienic environment and kept on a standard feed and water *ad libitum* during experimental period. The experimental work on rats was performed with approval of Institutional Animal Ethics Committee (IEAC) of College of Veterinary Science and Animal Husbandry, Jabalpur (M.P.) and all the protocols were followed according to the guidelines given by Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA). Rats were randomly divided into 4 groups with 12 (two replicates of 6 each) rats in each group. All the four groups were housed under identical managemental and environmental conditions. Rats of control group (group I) were fed with standard feed and water throughout the experiment and treated with olive oil @ 2 ml /Kg b. wt. single dose I/P on the 1st day of experiment. Rats of Group II were fed with standard diet throughout the experiment and treated with Aflatoxin B1 reconstituted with 2 ml olive oil @

2 mg/Kg b. wt. single dose I/P on the 1st day of experiment. Rats of Group III were fed with the standard diet mixed with herbal mycotoxin binder (HMB) @ 0.2% of the diet from the day of procurement to throughout the experiment and also treated with olive oil @ 2 ml /Kg b. wt. single dose I/P on the 1st day of experiment. Animals of Group IV were fed diet as per Group III and also treated with Aflatoxin B1 reconstituted with 2 ml olive oil @ 2 mg/Kg b.wt. single dose I/P on the 1st day of experiment.

Chemicals: Aflatoxin B1 procured from HIMEDIA was used (@ 2mg/Kg body weight). Olive oil was used as vehicle for the toxin. The herbal mycotoxin binder was prepared using autoclaved clay containing yeast (50%), esterase (25%), diatomaceous earth mineral (15%), and curcumin (10%) and used at the rate of 0.2 % in feed.

Haematological studies: At the 14th and 28th day rats were sacrificed using mild chloroform anesthesia. Blood from individual rat was collected at the time of sacrifice in dry sterilized vials containing anticoagulant for haematological analysis. The haematological estimations, such as haemoglobin concentration (Hb), packed cell volume (PCV), total erythrocyte count (TEC), total leukocyte count (TLC), differential leukocyte count (DLC) was studied as per method described by Benjamin (2001). The values of mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were derived from the standard formula accordingly.

Statistical analysis: Analysis was done by one way analysis of variance (ANOVA) and Duncan's multiple range tests (DMRT) to study the significant effect of different groups (Snedecor and Cochran, 1994). The p-value of <0.05 was considered as statistically significant.

RESULTS AND DISCUSSION

Average haematological values of experimental rats observed at 14th and 28th day of experiment are presented in Table 1. The haematological parameters, Hb,PCV,TEC and MCHC reduced significantly (<0.05) wheres MCV was increased significantly (<0.05) in AFB1 fed rates as compared to control group during both periods of experiment. A significant alteration in haematological values indicates development of anaemia due to direct effect of the toxin on haematopoeitic system. This type of macrocytic hypochromic anaemia (increase in MCV and decrease in MCHC) were in close conformity with earlier reports (Salem *et al*., 2007 and Gupta, 2011) .The reduction in Hb concentration observed during aflatoxicosis may be due to reduced protein synthesis, reduced size of RBC and impaired biosysnthesis of haem in bone marrow (Sawarkar *et al*., 2011 and Verma *et al*., 2001). The low percentage PCV were recorded in aflatoxin induced hemolysis arising from lipid peroxidation of the plasma membrane (Verma *et al*., 2001). There was a significant increase in total leucocyte count and differential cell count revealed that mean percent of neutrophil are increased where as lymphocytes count is decreased in the AFB1 treated rats as compared to control rats. An insignificant variation was observed in eosinophils, monocytes and basophils (data not shown). Increase in WBC count may attribute to the toxic effect of AFB1 on haemopoietic tissue. Oral administration of herbal binder along with aflatoxin caused significant amelioration in aflatoxin-induced effects in the blood parameters as compared to the aflatoxin alone treated groups, as herbal mycotoxin binder (HMB) is a unique combination of minerals (extra purified clay containing diatomaceous earth minerals), antioxidants (curcuminoids extracted from turmeric) and enzymes (Epoxidases and Esterases). Diatomaceous earth mineral is a powerful natural adsorbent and which might effectively adsorb the toxins through its polar ends (Gowda *et al*., 2008). Moreover, presence of curcuminoids and enzymes in the binder used might have acted as antioxidants and detoxified epoxides, respectively. Curcumin induces drug metabolizing enzymes like glutathione-s-transferase which results in efficient detoxification of toxin effects (Srinivas *et al*., 1992; Soni *et al*., 1992).

Table 1. Mean values of haematological parameters in different groups of rats at both intervals

Groups	Interval	Hb (g/dl)	PCV (%)	TEC (millions/µl)	MCV(fl)	MCH (pg)	MCHC (g/dl)	TLC (thousands/µl)
Group I Control	**14th Day**	14.75[a] ± 0.09	42.73[a] ± 0.11	8.60[a] ± 0.08	49.68[a] ± 0.11	17.15[a] ±0.07	34.51[a] ± 0.14	09.42[d] ± 0.17
	28th Day	14.58[a] ± 0.07	43.70[a] ± 0.12	8.95[a] ± 0.16	48.82[a] ± 0.12	16.29[a] ± 0.11	33.36[a] ± 0.09	09.21[d] ± 0.15
Group II AFB1	**14th Day**	11.73[d] ± 0.19	39.70[c] ± 0.17	7.62[c] ± 0.19	52.09[b] ± 0.10	15.09[d] ± 0.09	29.55[d] ± 0.11	14.87[a] ± 0.19
	28th Day	12.07[d] ± 0.12	40.12[c] ± 0.27	8.03[c] ± 0.12	49.96[b] ± 0.11	15.03[d] ± 0.10	30.08[d] ± 0.14	12.60[a] ± 0.10
Group III HMB	**14th Day**	15.12[a] ± 0.10	43.03[a] ± 0.15	8.87[a] ± 0.07	49.30[a] ± 0.12	17.04[a] ±0.09	35.13[a] ± 0.11	11.13[c] ± 0.15
	28th Day	15.07[a] ± 0.09	44.13[a] ± 0.27	9.00[a] ± 0.15	49.03[a] ± 5.98	16.74[a] ± 0.09	34.14[a] ± 0.17	11.50[c] ± 0.08
Group IV AFB1+HMB	**14th Day**	12.63[c] ± 0.06	41.03[b] ± 0.16	8.33[b] ± 0.12	49.25[b] ± 0.09	15.16[c] ± 0.08	30.78[c] ± 0.13	13.12[b] ± 0.14
	28th Day	13.17[c] ± 0.13	42.12[b] ± 0.09	8.57[b] ± 0.12	49.15[ab] ±0.07	15.36[c] ± 0.06	31.26[c] ± 0.12	12.18[b] ± 0.12

Value (Mean±SEM, n=6) bearing different superscript in the same column differ significant (pd"0.05)

REFERENCES

Abdel-Wahhab, M.A., Nada, S.A. and Khalil, F.A. (2002). Physiological and toxicological responses in rats fed aflatoxin-contaminated diet with or without sorbent materials. *Animal Feed Science and Technology,* **97(3)**:209-219.

Bakshi, C. S., Sikdar, A., Johri, T. S., Malik Meenakshi and Singh, R. K. (2000). Effect of grade dietary levels of aflatoxoin onhumoral immune response in commercial broilers. Ind. J. Comp. Microbiol. Immuniol. *Infect. Dis*. **21**:163-164.

Benjamin, M.M. (2001). Outline of Veterinary Clinical Pathology 3rd Edn. Kalyani Publication, New Delhi, pp 25-37.

Gowda, N. K. S., Ledoux, D. R., Rottinghaus, G. E., Bermudez, A. J. and Chenty, Y. C. (2008). Efficacy of Turmeric (*Curcuma longa*), Containing a Known Level of Curcumin, and Hydrated Sodium Calcium Aluminosilicates to Ameliorate the Adverse Effects of Aflatoxin in Broiler Chicks. *Poult. Sci.,* **87:** 1125-1130.

Gupta, R.C. (2011). Aflatoxins, ochratoxins and citrinin in reproductive and Manafi, M., Mohan, K. and Noor Ali, M. (2011). Effect of Ochratoxin A on Coccidiosis-Challenged Broiler Chicks. *World Myco. J.,* **4(2):** 177-181.

Panisup, A.S., R.I.Shah, K.C. Verma and G.C. Mohanty (1982). Atypical Ranikhet disease in broiler chicks vaccinated at frequent intervals. *Ind. J. Poult. Sci.***17**:224-226.

Salem, R.M., Mogda, K., Mansour, M.A., Rashid and Koratum, K.M. (2007). Comparative study on the efficacy of salicylic acid and silicate compounds in adsorption of aflatoxins. Egypt. *Journal of Comparetive Pathology and Clinical Pathology*, **20 (1)**: 149- 164.

Sawarkar, A.R., P.M., Sonkusale, N.V.Kurkure, C.R.Jangade, S.Maini and K.Ravikanth (2011) . Experimental Alfa and Orchratoxin induced mixed mycotoxicosis in Broilers and its amelioration with herbomineral toxin binder 'Toxiroak Gold. *Int.J. poultry Sci*. **10(7)**, 560-566

Snedecor, G. W. and W. G. Cochran (1994). Statistical method Publ., Oxford and I.B.S. publishing Co. New Delhi, 455 p.

Soni, K. B., Lahiri, M., Chackradeo, P., Bhide, S. V. and Kuttan, R. (1997). Protective Effect of Food Additives on Aflatoxininduced Mutagenicity and Hepatocarcinogenicity. *Cancer Letters,* **115(2):** 129-33.

Srinivas, L., Shalini, V. K. and Shylaja, M. (1992). Turmerin: A Water Soluble Antioxidant Peptide from Turmeric (*Curcuma longa*). Arch. Biochem. Biophys., **292(2):** 619-623.

Verma, A., Singh, A., Sudha, Sahay, N., Sharma, J., Roy, A., Kumari, M., Rana, D.,Thakran, S., Deka, D., Bharati, K., Franken, P., Hurd, T., Blechert, 0., Rexer K-H. Kost, G., Hahn, A., Hock, B., Maier, W., Walter, M., Strack, D. and Kranner, I. (2001). A cultivable mycorrhiza-like endosymbiotic fungus. In: Mycota IX Series, Chapter 8, edited by B Hock. SpringerVerlag, Berlin. pp 123-150.

❑