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ORAL ADMINISTRATION OF PIPERINE FOR THE CONTROL OF AFLATOXIN INTOXICATION IN RATS

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ABSTRACT

Aflatoxins are mycotoxins that have important toxic effects on human and animal health, even if consumed at low doses. The oral administration of piperine (1.12 mg/kg) during 23 days in rats seemingly interfered with the toxicity of aflatoxins, decreasing hepatic injuries and the leukocyte depletion in experimentally intoxicated animals.

Key words: aflatoxins, piperine, experimental intoxication, rats.

Aflatoxins are mycotoxins produced by several *Aspergillus* species and widely detected in agricultural products (6). Several animal species are sensitive and acute tissue toxicity, mutagenic, carcinogenic and teratogenic effects may be included among the metabolic and genetic alterations (10). The liver is the main target and hepatic carcinogenesis is the most important effect of subacute toxicity (3). Additionally, aflatoxins interfere with immunological responses, predisposing animals to infections and parasitical diseases and decreasing vaccinal performance (5, 8).

Piperine (1-piperol piperidine) is an amide found in Piper species that non-specifically increases the bioavailability of several drugs and nutritional supplements, involving different P-450 cytochrome types (2, 11) and plays a chemoprotector role against the procarcinogenic toxicity of benzo(a)pyrene, heavy metals and aflatoxins (9, 12, 13).

Considering that piperine can be a powerful chemopreventive agent against the activation of procarcinogens *in vitro*, such as aflatoxins, this research was carried out *in vivo* in order to evaluate whether piperine, orally supplied to rats, is able to decrease the toxic effect of aflatoxins on white blood cells and the liver.

Piperine was obtained according to Ikan's method (7), in a 5-7% yield and 98% purity, determined by GC-MS. The melting point (128-129 ºC) and spectrometric data (1H and 13C NMR, IR and MS) identical to values reported in the literature (1, 14). Piperine was dissolved in 1.0 ml DMSO/ethanol 10% right before use. Aflatoxins were obtained from 11-day *Aspergillus parasiticus* CMDB 0460 NRLL 2999 cultures, in Yes culture media (Micromed) at 37° C and by HPLC on a normal phase system, according to DIREITO (4). After chloroform evaporation, the aflatoxin solution (containing 62.89% of AFB1 and 37.09% of AFG1) was dissolved in maize oil and vigorously homogenized in an ultrasound bath.

Piperine and aflatoxins doses were elected based on previous assays (data not shown): the oral piperine dose (1.12 mg/kg) confirmed the results of Dogra *et al*. (5), being a safe dose for mice. The 7.2 µg/kg aflatoxins dose was chosen based on the capability to induce hepatic alterations and white blood

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cell reduction without causing other impairments.

The experiments were carried out using LOU-M rats, kept under standard environmental conditions, appropriately fed and given water *ad libitum*. The animals were randomly divided into three experimental groups (n = 10) and orally supplied once a day, according to the following treatments: [Group 1] 200 µL of piperine (1.12 mg/kg) diluted in PBS pH 7.2 and mixed v/v in maize oil for 23 days; [Group 2] 200 µL of aflatoxin (7.2 µg/kg) mixed v/v in maize oil for 21 days; [Group 3] 200 µL of piperine (1.12 mg/kg) + 200 µL of aflatoxin (7.2 µg/kg); piperine were administrated for 23 days and aflatoxin were provided for 21 days, starting at the 3rd day of inoculation with piperine.

At the end of the experiments, animal blood samples were collected by intra-cardiac puncture. Blood samples were added of EDTA and a leukogram was done. Samples not added of EDTA were submitted to aspartate aminotransferase (AST) alanine aminotransferase (ALT) determinations (Laborlab, SP/Brazil). The animals were euthanized in a CO_2 chamber and the livers were fixed in formalin 10%, pH 7.8, stained with hematoxilin-eosin (HE) and processed for microscopic analysis.

The results of weight again, liver weight, enzyme dosage and leukogram profile (Table 1). Were expressed as mean ± S.D. of ten animals in each group. The significant mean of different parameters between treatment groups was analysed using one-way analysis of variance (ANOVA) after ascertaining the homogeneity of variance between treatments. The difference was considered statistically significant when $p<0.05$.

Table 1. Influence of piperine on average weight gain, average liver weight, enzyme dosage and leukogram profile.

Parameters	Groups		
	Piperine (1.12 mg/kg)	**Aflatoxin (7.2 µg/kg)**	**Piperine + Aflatoxin**
Viability (%)	100	100	100
Weight gain[1] (mg/kg)	56.67[a] ± 6.	51.20[a] ± 10.08	55.40[a] ± 6.638
Liver weight[2] (mg/100g)	4.394[a] ± 0.246	4.303[a] ± 0.264	4.574[a] ± 0.291
ALT[3] (UI/L) (26-37)*	41.80[a] ± 12.89	65.45[b] ± 10.58	30.20[c] ± 11.20
AST[4] (U/l) (40-53)*	44.50[a] ± 21.45	58.09[a] ± 17.86	50.90[a] ± 22.92
WBC[5] ($x10^3/mm^3$) (7.30-12.66)*	10.48[a] ± 2.161	8.108[b] 1.732	10.92[a] ± 2.279
Lymphocytes ($x10^3/mm^3$) (5.07-9.07)*	8.798[a] ± 1.981	6.477[b] ± 1.409	9.012[a] ± 2.085
Neutrophils ($x10^3/mm^3$) (1.25-3,71)*	0.659[a] ± 0.304	0.531[a] ± 0.038	0.604[a] ± 0.414
Monocytes ($x10^3/mm^3$) (0.50-0.74)*	0.796[a] ± 0.229	0.793[a] ± 0.802	1.045[a] ± 0.343
Eosinophils ($x10^3/mm^3$) (0.04-0.30)*	0.297[a] ± 0.111	0.302[a] ± 0.177	0.259[a] ± 0.243

Data are expressed by the group ± standard deviation for 10 animals. Statistical analysis made by variance analysis; [1]mg/kg of body weight; [2]mg/100g of body weight; [3]alanine aminotransferase; [4]aspartate aminotransferase; [5]white blood cells; * reference values interval /Thrall *et al*. (15). [a,b]different letters mean significant differences ($p< 0.05$).

Orally administered aflatoxins caused alterations in the plasma enzyme dosage and leukogram. The increase in ALT plasmatic levels (1.8 times higher than the upper limit of the reference interval) was mild but statistically significant ($p<0.05$), in comparison to groups that received piperine (Group1) and piperine plus aflatoxin (Group 2). The same was not observed in AST levels, which remained within the reference interval (Table 1). These results suggest that 7.2 µg/kg of aflatoxin orally inoculated once a day, for 21 days, caused plasmatic membrane injuries in hepatocytes of rats. Table 2 shows the histopathological lesions observed in livers of rats, ranging from minor to intense, consistent with histopathological injuries caused by aflatoxin intoxication (10). The Aflatoxin group leukogram showed discreet leukopenia and lymphopenia within the reference interval, which were significant ($p<0.05$) when compared to the other groups (Table 1).

Piperine orally supplied with aflatoxins produced a remarkable decrease ($p<0.05$) in hepatic injuries (Table 2) and impaired the toxic effect of the aflatoxin on white blood cells (Table 1). These results suggest that piperine, orally administrated at a 1.12 mg/kg dose, can decrease the toxic effects of aflatoxins at 7.2 µg/kg doses.

These preliminary results support new *in vivo* studies, showing that piperine can prevent the suppression of leukocytes and reduce the toxicity of aflatoxins in tissues, as already observed *in vitro* (12). It is a potential *in vivo* chemical preventive agent against the toxicity of aflatoxins.

Table 2. Histopathological lesions observed in livers of rats

Histopathological Injuries	Lesion Degree[1]		
	Piperine	Aflatoxin	P + A
Megalocytosis	(+)	+++	+
Individual cell necrosis	-	+(+)	+
Hepatocytes with vacuoles	-	++[F]	-
Mononuclear cell infiltrate regions	(+)	(+)	(+)
Necrosis in hepatocytes	-	+	-
Centrilobular to paracentral necrosis	-	+++	-
Centrilobular congestion	-	++	-

[1]Lesions observed in the majority of the animals: no lesion [-]; discreet [(+)]; light [+]; light to moderate [+(+)]; moderate [++]; marked [+++].
[F] Lesions in focus.

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