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INFLUENCE OF *CURCUMA LONGA* AND *CURCUMIN* ON BLOOD PROFILE IN MICE SUBJECTED TO AFLATOXIN B_1

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ABSTRACT

In the present study, toxicity was developed by oral administration of AFB_1 at a dose of (2µg/kg body weight) for 45 days in male mice. *Curcuma longa* (100 and 200 mg/kg body weight) and *Curcumin* (50 and 100 mg/kg body weight) were given individually and in combination with AFB_1 for 45 days. At the end of 45 days, the blood samples were collected from various groups by heart puncture in eppendroff tubes with anticoagulant for hematological assays. Noncoagulated blood was tested for total erythrocyte count, total leukocyte count, hemoglobin, differential leukocyte count and for other blood indices. The results of present study suggest that Turmeric rhizome powder (*Curcuma longa*) and *Curcumin* showed protective effects against AFB_1 induced toxicity by modulating red blood cell count, white blood cell count and Hb percentage to some extent.

INTRODUCTION: Aflatoxins are secondary fungal metabolite produced by *Aspergillus flavus* and *A. parasiticus* groups. AFB_1 has been reported to be the parent compound of the common toxins [1]. This toxin is known to be hepatotoxic, nephrotoxic and carcinogenic to a wide variety of animals [2, 3]. The current effects of AFB_1 in livestock have been well documented and include hepatotoxicosis, immunosuppression, reduced growth and performance, carcinogecity or death [4, 5, 6].

AFB_1 induced immunosuppression has been demonstrated in domestic animals, chicken and turkey as well as in laboratory animals [7, 8]. Pier and Mcloughlin [9] summarized the effect of AFB_1 on the animals immune system as: Aflatoxins (AFs) impairs immunogenesis without suppressing antibody formation; they suppress formation of nonspecific humoral substance related to resistance and immunity and suppress phagocytosis by macrophage; they causes thymic aplasia and suppress cell mediated immunity and leukocyte migration.

Curcuma longa or turmeric is a medicinal plant widely used and cultivated in tropical regions. Plant extracts were found to have antifungal [10], imunomodulatory [11], antioxidative [12] and antimutagenic [13] activities. Moreover, Soni *et al*. [13] proved the protective effect of Curcuma *longa* food additives on aflatoxin-induced mutagenicity and hepatocarcinogenicity. Antony *et al*., [11] indicated the immunostimulatory activity of *Curcumin* which is the active ingredient extracted from *Curcuma longa*.

Keeping into consideration the possible beneficial role of *Curcuma longa* and *Curcumin,* the present paper was designed to investigate the effect of *Curcuma longa* and *Curcumin* on the blood profile of aflatoxicated male mice.

MATERIALS AND METHODS:

Chemicals: Aflatoxin B_1 was purchased from HIMEDIA (India). All other chemicals used were of analytical grade and obtained from Sisco Research Laboratories (India), Qualigens (India/Germany), SD Fine Chemicals (India), HIMEDIA (India), and Central Drug House (India).

Animals: Male Swiss albino mice weighing 15-30g (2-2.5 months) were obtained from Haryana Agricultural University, Hissar, India for experimental purpose. The Animal Ethics Committee of Banasthali University, Banasthali, India has approved the experimental protocols. All experiments were conducted on adult male albino mice (*Mus musculus* L) weighing 25-35 g (3-4 months old). They were housed in polypropylene cages in an air-conditioned room with temperature maintained at 25°C±3°C, relative humidity of 50%±5% and 12-h alternating light and dark cycles. The mice were provided with a nutritionally adequate chow diet (Hindustan Lever Limited, India) and drinking water *ad libitum* throughout the study.

Plant Material: Experimental plant *Curcuma longa* was collected from local market, Newai in powdered form and was identified by a plant taxonomist/botanist of our department as a local variety and *Curcumin* was purchased from CDH pvt. Lmt.

Preparation of Ethanolic Extract of *Curcuma longa*: The coarse powder of *Curcuma longa* (50 g) was extracted with ethanol (300ml) using a Soxhlet's apparatus for 48 h at 60°C. The ethanolic extract thus obtained was dried under reduced pressure at a room temperature not exceeding 40^{o}C to get the solid extract. The yield was 2.5g/kg and it was stored at 4°C.

Animal treatment and Sample collection: In the present study 60 adult Male Swiss albino mice (*Mus musculus* L) weighing 25-35 g (3-4 months old) were used for blood profile studies.

The Groups for each parameter were treated orally, once, daily as follows;

- Group 1, received 2ml of DMSO served as Control.
- Group 2, received Aflatoxin B_1 (2µg/Kg body weight) dissolved in DMSO.
- Group 3 and 4, were administered with ethanolic *Curcuma longa* extract at a dose of 100 and 200mg/kg body weight, respectively.
- Group 5 and 6, were administered with *Curcumin* at a dose of 50 and 100mg/kg body weight, respectively.
- Group 7 and 8, were administered with aflatoxin B_1 (2µg/Kg body weight) and *Curcuma longa* (100 and 200mg/kg body weight) respectively.
- Group 9 and 10, were administered with aflatoxin B_1 (2µg/Kg body weight) and *Curcumin* (50 and 100mg/kg body weight) respectively.

Curcuma longa extract and *Curcumin* were given at an interval of 30 minutes of AFB_1 administration. The dose of aflatoxin B_1 was decided on the basis of previously published report [14] and mentioned doses of *Curcuma longa* and *Curcumin* were selected on the basis of earlier published report [15] and on the basis of practicals performed in the laboratory. The duration of treatment for each group was of 45 days. After administration of last dose, the animals were given rest overnight and then on the next day they were sacrificed under light ether anesthesia. Blood was collected by heart puncture in eppendroff tubes with anticoagulant for various hematological assays.

Total erythrocyte count and total leukocyte count were done by method given by Berkson *et al.*[16]. Hemoglobin estimation was performed using Haden's method [17]. Hematocrit (PCV) was calculated by Wintrob [18] method. Platelet count was performed by method of Dorland [19], and differential leukocyte count was done by Leishman's staining method.

Statistical Analysis: Data are expressed as the mean ±S.E. Statistical analysis was done using one way analysis of variance (ANOVA) followed by Tukey's multiple comparison tests using the Statistical Package for the Social Sciences (S.P.S.S. 11). The level of significance was set at $p<0.05$, $p<0.02$, $p<0.01$, $p<0.001$.

RESULTS: Table 1 illustrates total erythrocytes count (TEC), total leukocyte count (TLC), hemoglobin concentration (Hb), packed cell volume (PCV), and total platelet count (PLTC) in control and experimental group of animals.

In the animals group given with AFB_1, the average values ($p>0.05$) of RBC count was lesser after 45 days, when compared to control group. Administration of low dose and high dose of *Curcuma longa* and *Curcumin*, to animals in groups III-VI showed improved RBC count as compared to control group. Mice treated with low and high dose of *Curcuma longa* and *Curcumin*, along with AFB_1 showed insignificant increase in RBC values in groups VII-X when compared to AFB_1 treated group respectively. Oral administration of AFB_1 caused insignificant decrease in WBC count compared to control group. Treatment of mice with low and high dose of *Curcuma longa* and *Curcumin*, (groups IV-VI, except group III) showed increased WBC values compared to control group. In animals given with low dose and high dose of *Curcuma longa* and *Curcumin*, along with AFB_1, WBC count showed increased values as compared to AFB_1 treated group.

The effect of AFB_1 on Hb content was similar to its action on TEC and TLC. After administration of AFB_1, Hb content was lowered ($p<0.001$), as compared to control group. Oral administration of low and high dose of *Curcuma longa* and *Curcumin*, to mice raised Hb content as compared to control group. Mice when administered with low and high dose of *Curcuma longa* and *Curcumin*, along with AFB_1 showed protective effects, in comparison to AFB_1 treated group. In case of PCV, AFB_1 exposure produced an insignificant decline in PCV of mice as compared to control group. Administration of low and high dose of *Curcuma longa* and *Curcumin* showed significant increase in PCV content ($p<0.001$) as compared to control group.

TABLE 1: PREVENTIVE EFFECT OF *CURCUMA LONGA* AND *CURCUMIN* ON SOME HEMATOLOGICAL PARAMETERS IN AFLATOXIN B_1 EXPOSED MICE

Group	Parameters	Total erythrocyte count (10*6/uL)	Total leukocyte count (10*3/uL)	Hemoglobin (gm/dL)	PCV (%)	Platelet count (10*3/uL)
I	Control	5.60±0.17	5.15±0.20	15.11±0.24	36.79±0.16	847.33±33.58
II	Aflatoxin B_1 (2µg/kg body weight)	5.31±0.16	5.03±0.24	15.01±0.23^k	36.67±0.17	782.83±41.80
III	C.L. (low dose) 100mg/kg body weight	5.71±0.18	5.14±0.19	15.20±0.18	39.64±0.24^k	971.50±44.13
IV	C.L. (high dose) 200mg/kg body weight	5.78±0.16	5.21±0.19	15.28±0.25	42.79±0.18^k	1100.66±54.20^l
V	*Curcumin* (low dose) 50mg/kg body weight	5.72±0.15	5.21±0.19	15.10±0.16	41.07±0.29^k	977.83±55.73
VI	*Curcumin* (high dose) 100m g/kg body weight	5.35±0.62	5.27±0.20	15.20±0.30	42.96±0.26^k	1282.66±30.56^k
VII	C.L. (low dose) + AFB_1	5.46±0.16	5.09±0.19	15.06±0.29	39.55±0.24^a	943.83±33.92
VIII	C.L. (high dose) + AFB_1	5.61±0.16	5.17±0.19	15.41±0.33	42.68±0.19^a	1076.33±48.19^a
IX	*Curcumin* (low dose)+AFB_1	5.67±0.17	5.16±0.19	15.53±0.30	40.98±0.31^a	1016.50±53.29^c
X	*Curcumin* (high dose)+AFB_1	5.70±0.17	5.26±0.20	15.65±0.18	42.85±0.26^a	1254.16±27.76^a

Values are the mean ±S.E.M; n =6; as compared to group 1 (Control), k = $p<0.001$, l = $p<0.01$, m = $p<0.02$; as compared to group 2; (Aflatoxin), a = $p<0.001$, b = $p<0.01$, c = $p<0.02$; Abbreviation: CL: *Curcuma longa*

Treatment of mice with low dose and high dose of *Curcuma longa* and *Curcumin*, along with AFB_1 also showed significant ($p<0.001$) increase in PCV % as compared to AFB_1 treated group. In mice group treated with AFB_1, the average value of PLTC count were lesser after 45 days, when compared to control group. Administration of low dose and high dose of *Curcuma longa* and *Curcumin*, to animals showed improved PLTC count, as compared to control group and when mice were treated with low dose and high dose of *Curcuma longa* and *Curcumin*, along with AFB_1 showed increased values of PLTC ($p>0.05$), ($p<0.001$), ($p<0.02$) and ($p<0.001$) in group VII- group X respectively when compared to AFB_1 treated group.

Table 2 illustrates differential leukocyte count (Lymphocyte, Neutrophil and Monocytes count) in control and experimental group of animals. In the animal group treated with AFB_1, the average value of lymphocyte was decreased ($p<0.05$) as compared to control value after 45 days, whereas mice treated with low and high dose of *Curcuma longa* and *Curcumin* (group III - VI), showed insignificant increase in lymphocyte count, as compared to control group. Similarly mice treated with low dose and high dose of *Curcuma longa* and *Curcumin*, along with AFB_1 showed insignificant increase in lymphocyte as compared to AFB_1 exposed group.

TABLE 2: PREVENTIVE EFFECT OF *CURCUMA LONGA* AND *CURCUMIN* ON DIFFERENTIAL LEUKOCYTE COUNT IN AFLATOXIN B_1 EXPOSED MICE

Groups	Parameters	Differential leukocyte count		
		Lymphocytes (%)	Neutrophil (%)	Monocytes (%)
I	Control	8.72±0.15	4.83±0.12	0.40±0.00
II	Aflatoxin B_1 (2µg/kg body weight)	8.66±0.15	4.81±0.14	0.40±0.01
III	C.L. (low dose) 100mg/kg body weight	8.82±0.17	4.76±0.14	0.40±0.01
IV	C.L. (high dose) 200mg/kg body weight	8.93±0.19	4.67±0.13	0.41±0.01
V	*Curcumin* (low dose) 50mg/kg body weight	8.86±0.18	4.66±0.14	0.41±0.01
VI	*Curcumin* (high dose) 100mg/kg body weight	8.99±0.20	4.57±0.12	0.42±0.01
VII	C.L. (low dose)+AFB_1	8.77±0.17	4.74±0.13	0.40±0.01
VIII	C.L. (high dose)+AFB_1	8.89±0.19	4.64±0.13	0.41±0.01
IX	*Curcumin* (low dose)+AFB_1	8.71±0.18	4.63±0.13	0.40±0.01
X	*Curcumin* (high dose)+AFB_1	8.91±0.21	4.54±0.12	0.42±0.01

Values are the mean ±S.E.M; n =6 Abbreviation: CL: *Curcuma longa*

Oral administration of AFB_1 caused insignificant change in neutrophil count, as compared to control value. Mice treated with *Curcuma longa* and *Curcumin* (at both dose, groups III - VI), showed insignificant decrease in neutrophil count as compared to control value. Similarly treatment of mice with low dose and high dose of *Curcuma longa* and *Curcumin* (group VII - X), along with AFB_1 showed insignificant decrease in neutrophil count as compared to AFB_1 treated group. Oral administration of mice with AFB_1 caused no change in monocyte count, but when mice treated with low dose and high dose of *Curcuma longa* and *Curcumin*, showed very slight but insignificant increase in monocyte count in groups III-VI as compared to control animals. Similarly treatment with low and high dose of *Curcuma longa* and *Curcumin*, along with AFB_1 showed no change or slight increase in values of monocytes in group VII-X as compared to AFB_1 treated group.

Table 3 illustrates MCV, MCH and MCHC values in control and experimental group of animals. MCV content showed increased values in AFB_1 treated group compared to control group. Oral administration of low and high dose of *Curcuma longa* and *Curcumin* to mice raised MCV content as compared to control group. Oral administration of low dose and high dose of *Curcuma longa* and *Curcumin*, along with AFB_1 showed increase MCV values as compared to AFB_1 treated group.

In the animals group given with AFB_1, the average values of MCH content was higher after 45 days, when compared to control group. Administration of low and high dose of *Curcuma longa and Curcumin*, to animals showed decrease MCH content as compared to control group. Mice treated with low dose and high dose of *Curcuma longa* and *Curcumin*, along with AFB_1 also showed insignificant decrease value of MCH as compared to AFB_1 treated group respectively.

MCHC content showed decrease value in AFB_1 treated group, as compared to control group. Administration of low and high dose of *Curcuma longa* and *Curcumin*, to animals showed significant decrease of MCHC content as compared to control animals. Animals on administration with low and high dose of *Curcuma longa* and *Curcumin*, along with AFB_1 also showed decrease MCHC values as compared to AFB_1 treated group.

TABLE 3: PREVENTIVE EFFECT OF *CURCUMA LONGA* AND *CURCUMIN* ON BLOOD INDICES IN AFLATOXIN B_1 EXPOSED MICE

Groups	Parameters	MCV (fL)	MCH (pg)	MCHC (gm/dL)
I	Control	65.91±1.98	27.04±0.67	41.31±0.75
II	Aflatoxin B_1 (2µg/kg body weight)	69.58±2.04	28.34±0.75	40.93±0.59
III	C.L. (low dose) 100mg/kg body weight	69.77±2.19	26.71±0.69	38.34±0.55[l]
IV	C.L. (high dose) 200mg/kg body weight	74.25±2.33	26.51±0.94	35.70±0.46[k]
V	*Curcumin* (low dose) 50mg/kg body weight	71.98±1.91	26.44±0.53	36.76±0.34[k]
VI	*Curcumin* (high dose) 100mg/kg body weight	73.88±2.76	26.04±0.44	35.39±0.80[k]
VII	C.L. (low dose)+AFB_1	72.68±2.11	27.62±0.44	38.08±0.70
VIII	C.L. (high dose)+AFB_1	76.44±2.50	27.51±0.38	36.13±0.91[a]
IX	*Curcumin* (low dose)+AFB_1	72.27±1.99	27.44±0.35	38.05±0.64[b]
X	*Curcumin* (high dose)+AFB_1	75.45±2.59	27.52±0.82	36.52±0.51[a]

Values are the mean±S.E.M; n =6; Abbreviation: CL: *Curcuma longa;* MCV: Mean Corpuscular volume MCH: Mean Corpuscular Hemoglobin; MCHC: Mean Corpuscular Hemoglobin content as compared to group 1 (control), k = p<0.001, l = p<0.01; as compared to group 2 (aflatoxin), a = p<0.001, b = p<0.01

DISCUSSION: Aflatoxin has a harmful and stressful influence in the blood, serum, hepatic and renal tissue. In the present study, the reduced level of total erythrocyte count (TEC) is due to AFB_1 toxicity. The mechanism by which AFB_1 aggravated pathogenesis of anemia could involve down-regulation of erythropoietin activity [20] by aflatoxin B_1. Decrease in TEC has been contributed to reduction in erythropiosis in bone marrow and faster rate of destruction of peripheral RBC in spleen. Decrease in Hb can be related with reduction in size of RBC, impaired biosynthesis of heam in bone marrow or due to reduction in rate of formation of TEC.

Animals treated with AFB_1 showed lower leukocyte levels; evidently this may be due to a considerable decrease in lymphocyte count. This may be indicative of the deterioration of immune status in the mice of this group in response to the toxic effect of AFB_1. The data suggest that different doses of AFB_1 can either stimulate or suppress the immune system of the organism [21-23]. Lowered PCV in AFB_1 treated mice might be due anemia developed in aflatoxicosis.

Administration of *Curcuma longa* and *Curcumin*, were effective in reducing of the adverse effect of AFB_1 on hemopoietic system supporting the hypotheses that plant products exhibits effective antioxidative agents. From the present study, it is evident that *Curcuma longa* and *Curcumin* may stabilize the cell membrane and restore various blood variables. The lower and higher levels of *Curcumin* and *Curcuma longa* increased both erythrocyte and total leukocyte count. Antony *et al*. [11] proved that *Curcuma longa* extract administration increased the total WBC count in Balb/c mice due to its immune-stimulating activity of *Curcumin*.

CONCLUSION: The results of the present study suggest that *Curcuma longa* and *Curcumin* can be used as a dietary supplement in order to prevent aflatoxicosis to some extent.

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