

AFLATOXIN INDUCED HEMOLYSIS AND ITS AMELIORATION BY TURMERIC EXTRACTS AND CURCUMIN *IN VITRO*

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Abstract: The present investigation is an attempt to evaluate the possible ameliorative effect of turmeric extracts/curcumin on aflatoxin induced hemolysis *in vitro*. Blood samples were collected from healthy adult human beings (25-30 years old) in EDTA vials and were used for preparation of RBC suspension in saline. Saline suspension of RBC was treated with aflatoxin (0.5-2.0 µg/mL) with and without turmeric extracts/curcumin (1-100 µg/mL). The results revealed that addition of aflatoxin (0.5-2.0 µg/mL) to RBC suspension caused significant dose-dependent increase in the rate of hemolysis. However, concurrent addition of aflatoxin (2.0 µg/mL) and extracts of turmeric and curcumin caused concentration-dependent retardation in aflatoxin-induced hemolysis.

Keywords: aflatoxin, turmeric, curcumin, retardation, haemolysis

Aflatoxins are secondary toxic metabolites produced by molds of *Aspergillus flavus* and *Aspergillus parasiticus* which are found to be growing on grains, groundnuts and other food stuffs. Occurrence of aflatoxin in various food commodities have been widely reported from various countries, being most prevalent in tropical and subtropical countries where environmental conditions are more favorable for moldy growth and toxin production (1-3).

Aflatoxins have been implicated in acute hepatitis, hepatocarcinogenesis and mutagenesis (3-5). Verma and Raval (5) have reported decreased RBC count during induced chronic aflatoxicosis in rabbits. Aflatoxins cause oxidative stress by increasing lipid peroxidation and decreasing enzymatic and non-enzymatic antioxidants in aflatoxin-treated animals (6).

Curcumin, one of the most active compound of turmeric administered to experimental animals, showed no adverse effects on both growth and the level of erythrocytes, leucocytes, blood constituents such as hemoglobin, total serum protein, alkaline phosphatase etc. (7). Human clinical trials also indicate that curcumin has no toxicity when administered at doses of 1-8 g/day (8) and 10 g/day (9). The ability of curcumin to scavenge superoxide may be responsible for protecting haemoglobin from nitrite by preventing autocatalytic activity (10). Curcumin exerts its protective effect against nicotine-induced

lung toxicity by modulation of the biochemical marker enzymes, lipid peroxidation and augmenting antioxidant defence mechanism (11).

The present investigation was an attempt to evaluate ameliorative effects of turmeric extracts/curcumin on aflatoxin induced hemolysis *in vitro*.

EXPERIMENTAL

Aflatoxin was produced by growing *Aspergillus parasiticus* (NRRL 3240) on SMKY liquid medium for 10 days at $28 \pm 20^\circ\text{C}$ as described by Diener and Davis (12). Obtained culture filtrates were extracted with chloroform and concentration of aflatoxin was quantified spectrophotometrically (13).

Dry yellow turmeric (*Curcuma longa*, Linn, Family: *Scitamineae*) was purchased from local market and was used to make alcoholic and aqueous extracts. Curcumin was purchased from Hi-Media Laboratories Pvt. Ltd. Mumbai, India. Random blood samples were collected into EDTA vials intravenously from healthy humans (25-30 years age group) having normal RBC counts. After dilution with saline, the samples were centrifuged at 1000 rpm for 10 min. Supernatant was discarded and the RBC pellet was further washed twice with saline by centrifugation. Final RBC suspension was prepared in saline to have 2×10^4 cells/mL. For examining the hemolysis due to aflatoxin on RBC and its amelio-

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ration by antioxidants such as aqueous and alcoholic extracts of turmeric and curcumin, four sets of the tubes were prepared as follows:

1. Control tubes containing 2.0 mL of RBC suspension.

2. Antioxidants control tubes containing 100 µg/mL turmeric extracts/curcumin added to 2.0 mL of RBC suspension.

3. Treated tubes containing different concentrations (0.5 µg/mL to 2 µg/mL) of aflatoxin added to 2.0 mL of RBC suspension.

4. Tubes containing different concentrations of alcoholic/aqueous/curcumin (1µg/mL to 100 µg/mL) added to RBC suspension treated with 2 µg/mL of aflatoxin.

Aflatoxin solutions and extracts of turmeric and curcumin were prepared in normal saline (0.9 % NaCl). Total volume of each tube was made up to

4.0 mL by adding saline. All the tubes were incubated at 37°C for 4 h. Morphological alterations in RBC were observed after staining with Leishman's Stain. Tubes were centrifuged at 1000 rpm for 10 min and color density of supernatant was measured spectrophotometrically at 540 nm. Percent hemolysis was calculated by the formula as:

$$\text{Percent hemolysis} = \frac{\text{Absorbance of individual tubes}}{\text{Absorbance with 100 \% hemolysis}} \times 100$$

Percent retardation with different concentration of antioxidants was calculated with the following formula (14):

$$\text{Percent Retardation} = \frac{A-B}{A} \times 100$$

where, A = aflatoxin-induced hemolysis; B =

Table – 1 Aflatoxin induced haemolysis *in vitro*

Aflatoxin (µg/mL)	Hemolysis (%)
0.0 (Control)	0.11 ± 0.02
0.5	3.65 ± 0.35*
1.0	24.53 ± 3.11**
1.5	57.07 ± 0.93**
2.0	70.08 ± 2.87**

Values are mean ± S.E.M.; N = 10

Significant at the level : *p < 0.01 ; **p < 0.001

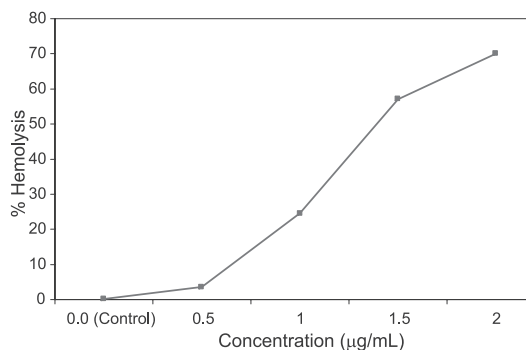


Figure 1. Aflatoxin-induced hemolysis

Table 2. Retardation of aflatoxin-induced hemolysis by aqueous and alcoholic extracts of turmeric and curcumin *in vitro*

Aflatoxin (µg/mL)	Aqueous extract/ Alcoholic extract of turmeric/curcumin (µg/mL)	Hemolysis (%)		
		Aqueous extract	Alcoholic extract	Curcumin
0.0	0	0.11 ± 0.02	0.11 ± 0.02	0.11 ± 0.02
0.0	100	3.21 ± 0.07	1.44 ± 0.05	0.74 ± 0.11
2	0	70.08 ± 2.87**	70.08 ± 2.87**	70.08 ± 2.87**
2	1	57.19 ± 0.36**	55.52 ± 0.28**	45.64 ± 0.14**
2	2	42.90 ± 0.25**	42.38 ± 0.34**	32.20 ± 0.32**
2	3	33.82 ± 0.31**	30.69 ± 0.16**	24.70 ± 0.17**
2	4	23.82 ± 0.11**	20.32 ± 0.12**	14.78 ± 0.19**
2	5	16.35 ± 0.10**	12.18 ± 0.15**	7.78 ± 0.07**
2	10	13.01 ± 0.16**	10.06 ± 0.10**	6.52 ± 0.06**
2	25	10.23 ± 0.07**	7.71 ± 0.12**	4.06 ± 0.06**
2	50	8.76 ± 0.05**	5.73 ± 0.13**	3.17 ± 0.05**
2	75	7.54 ± 0.06**	4.59 ± 0.09**	2.37 ± 0.03**
2	100	6.16 ± 0.09**	3.46 ± 0.14**	0.55 ± 0.03**

Values are mean ± S.E.M.; N = 10

Significant at the level : **p < 0.001

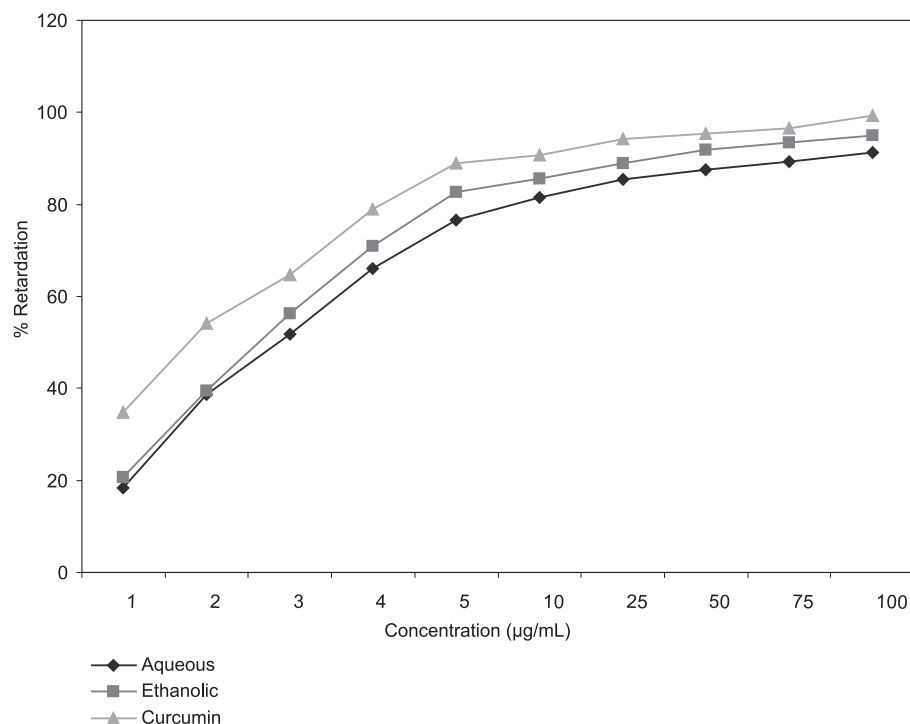


Figure 2. Comparative effect of turmeric extracts and curcumin

hemolysis caused by concurrent addition of aflatoxin and antioxidant.

Student's t-test was used for statistical analysis of the data.

RESULTS AND DISCUSSION

In case of control, it was observed that normal RBC appears as flattened indented spheres or biconcave discs. The cell pellets remained settled in the bottom of the tube and the ambient supernatant remained clear.

The addition of 2 µg/mL of aflatoxin to a RBC suspension caused a significant rise in haemolysis and swelling of the cells. The cell pellets in the bottom of the tubes reduced with reddish colored supernatant indicating hemolysis due to bursting of the cells due to excess swelling. It could be due to the direct action of aflatoxin on the plasma membrane causing lipid peroxidation, membrane permeability alterations and cell lysis (15).

The concurrent addition of aqueous and alcoholic extracts of dry yellow turmeric and curcumin (1 µg/mL to 100 µg/mL) to the RBC suspension significantly reduced aflatoxin-induced haemolysis. An almost concentration-dependent effect was observed. Curcumin was found to be most effective,

followed by alcoholic extract; aqueous extract was comparatively less effective.

The mechanism of action of curcumin alcoholic/aqueous extracts on aflatoxin induced hemolysis is not clearly understood. It could be due to antioxidative property of curcumin and other compounds in case of alcoholic and aqueous extracts.

It should be noted that other plant products such as flavonoids, lignans, citric acid, lactic acid etc., reduce aflatoxin toxicity by modifying the bioactivation process of aflatoxin, in which microsomal mixed function oxidase plays a major role (16).

The protective effect of curcumin on tissue lipid peroxidation as an antioxidant is shown in nicotine-treated Wistar rats (11). So it can be said that if these micronutrients are added to food/feedstuffs, in addition to the increase in nutritive value and the restriction of fungal growth and aflatoxin production, they can also reduce cytotoxicity in the body.

CONCLUSION

It can be concluded that as compared to other extracts of turmeric, curcumin is the most active compound and plays an important role in ameliorating the aflatoxin-induced hemolysis.

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