RESEARCH ARTICLE

Protective effect of *Butea monosperma* leaves extract against Aaflatoxin induced haemolysis

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ABSTRACT

The present investigation is an attempt to evaluate the possible ameliorative effect of *Butea monosperma* leaves extracts on aflatoxin induced haemolysis. Blood sample were collected from healthy adult human being (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 *Butea monosperma* leaves extracts (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 haemolysis. However, concurrent addition of aflatoxin (2.0µg/mL) and extracts of *Butea monosperma* leaves caused concentration dependent retardation in aflatoxin induced hemolysis.

Keywords: aflatoxin, , *Butea monosperma*, retardation, haemolysis

INTRODUCTION

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. (Shank *et al*.,1972; Stoloff, 1977; Busby and Wogan, 1984). Aflatoxins have been implicated in acute hepatitis, hepatocarcinogenesis and mutagenesis. (Busby and Wogan, 1984; Groopman *et al*., 1988; Verma and Raval, 1992). The decreased RBC count during induced chronic aflatoxicosis in rabbits (Verma and Raval, 1992). Aflatoxins cause oxidative stress by increasing lipid peroxidation and decreasing enzymatic and non-enzymatic antioxidants in aflatoxin treated animals.(El-Gibaly *et al*., 2003).

Butea monosperma is a medium sized deciduous tree which belongs to family Fabaceae. It is a commonly used Ayurvedic plant with important medicinal values and is widely used by tribal and rural people in different parts of India to cure many disorders. The presence of triterpenes, flavonoids, butein, butin, stigmasterol, í-carotene, í-sitosterol, myristic, palmitic, stearic, oleic and linolenic acids. Sindhia and Bairwa (2010). The plant provides vast pharmacological potential. It has been reported to have anticonvulsive, antidiabetic, (Ahmed *et al*., 2012) anti-inflammatory, hepatoprotective, antihelmintic, antioxidant, anti-stress, antimicrobial activities (Kumar and Samant, 2012). Moreover, the leaves of *Butea. Monosperma* possess antioxidant and anticancer activity which is a prerequisite for anticlastogenic activity. The present investigation was an attempt to evaluate ameliorative effects of aqueous, alcoholic and flavonoids extracts on aflatoxin induced haemolysis.

MATERIAL AND MATERIALS

Aflatoxin was produced by growing *Aspergillus sps* on medium (sucrose, 20 g; magnesium sulfate, 0.5 g; potassium nitrate, 3 g; yeast extract, 7 g; and distilled water, 1000 mL) for 10 days at 28 ± 2^0C as described by(Diener and Davis,1966). Obtained culture filtrates were extracted with chloroform. *Butea monosperma* leaves were collected from Bhavan college campus and was used to make aqueous, alcoholic and flavonoid extracts. The flavonoid extract was preparedas described by Subramanian and Nagarajan (1969). The extract was subjected to qualitative chemical tests to determine the nature of the phytoconstituents. (Horbone, 1998).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 1000rpm 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. For examining the haemolysis due to aflatoxin on RBC and its amelioration by antioxidants.

First sets of test tubes were prepared to check toxic level of aflatoxin.

Second sets of the tubes were prepared as follows:

1. Control tubes containing 2.0 mL of RBC suspension.

2. Antioxidants control tubes containing 100µg/mL *Butea monosperma* leaves extracts added to 2.0 mLof 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/flavonoid extracts (1µg/mL to 100 µg/mL)of *Butea monosperma* leaves added to RBC suspension treated with 2 µg/mL of aflatoxin. Aflatoxin solutions and extracts of *Butea monosperma* leaves were prepared in normal saline (0.9 % NaCl). Total volume of each tube was made up to4.0 mL by adding saline. All the tubes were incubated at 37^0C for 4 h. Morphological alterations in RBC were observed after staining with Leishmanís Stain. Tubes were centrifuged at 1000 rpm for 10min and color density of supernatant was measured spectrophotometrically at 540 nm.

$$\text{Percent haemolysis} = \frac{\text{Absorbance of individual tubes}}{\text{Absorbance with 100 \% haemolysis}} X100$$

Percent retardation with different concentration of antioxidants was calculated with the following formula (Raval and Verma,1993):

$$\text{Percent Retardation} = \frac{A - B}{A} X100$$

Where,

A = aflatoxin-induced haemolysis;

B = haemolysis caused by concurrent addition of aflatoxin and antioxidant.Studentís t-test was used for statistical analysisof the data.

RESULTS AND DISCUSSION

The phytochemical screening of extracts revealed the presence of bioactive constituents like alkaloid, carbohydrate, phytosterols, flavonoids, proteins and diterpenes. 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 haemolysis 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 (Verma and Nair, 1999). The concurrent addition of aqueous and alcoholic extracts of *Butea monosperma* leaves and flavonoid extracts (1 µg/mL to 100 µg/mL) to the RBC suspension significantly reduced aflatoxin induced haemolysis. An almost concentration-dependent effect was observed. flavonoid extracts was found to be most effective, followed by alcoholic extract; aqueous extract was comparatively less effective. The mechanism of action of *Butea monosperma* leaves aqueous, alcoholic and flavonoid extracts on aflatoxin induced hemolysisis could be due to antioxidative property of *Butea monosperma* leaves and other compounds in case of aqueous extracts ansd alcoholic extracts.

Table1: Aflatoxin induced haemolysis

Aflatoxin µg/mL	Haemolysis
0	0.08
0.5	3.02
1	10.01
1.5	16.01
2	23.12

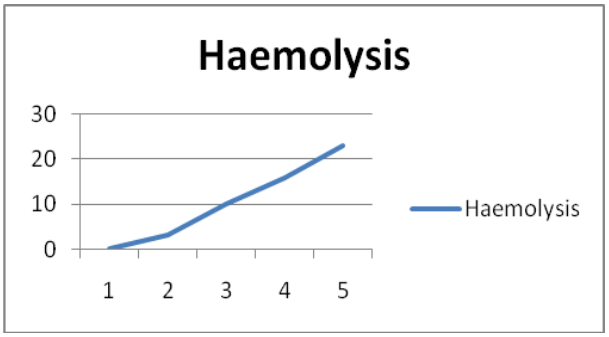

Fig.1: Aflatoxin induced haemolysis

Table 2: Retardation of aflatoxin-induced hemolysis by aqueous and alcoholic extracts and flavonoid extract of *Butea monosperma*(µg/ml)

Aflatoxin (µg/mL)	Aqueous extract/ Alcoholic extract/ flavonoid extract of *Butea monosperma* (µg/mL)	Hemolysis (%)		
		Aqueous extract) (µg/mL)	Alcoholic extract/ (µg/mL)	Flavonoid extract (µg/mL)
0	0	0.07±0.005	0.07±0.005	0.07±0.005
0	100	2.31±0.01	1.22±0.01	0.62±0.05
0	0	23.12±0.009	23.12±0.009	23.12±0.009
2	1	21.03±0.005	20.03±0.005	18.01±0.005
2	2)	20.06±0.005	18.03±0.005	16.01±0.005
2	3	18.02±0.009	16.01±0.009	14.01±0.009
2	4	16.01±0.009	14.02±0.005	10.02±0.005
2)	5)	15.03±0.009	12.01±0.005	8.01±0.005
2	10	13.01±0.005	10.02±0.005	6.03±0.005
2)	25)	11.05±0.01	9.01±0.01	4.0±0.009
2	50)	7.06±0.009	6.08±.0.01	3.23±0.01
2)	75	5.23±0.01	4.04±0.01	2.45±0.005
2	100	4.59±0.01	2.05±0.01	0.55±0..1

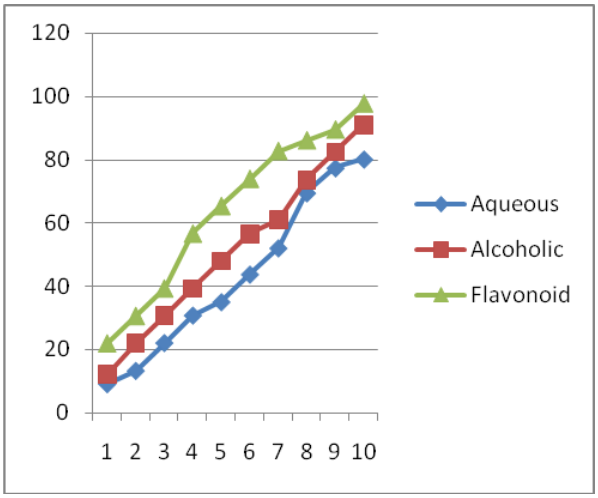

Fig.2: Retardation of aflatoxin

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 (Souza *et al*., 1999). So it can be said that these micronutrients can the restriction aflatoxin production, they can also reduce cytotoxicity in the body.

CONCLUSION

It can be concluded that as compared to other extracts of *Butea monosperma* leaves the flavonoid extract is the most active compound and plays an important role in ameliorating the aflatoxin-induced haemolysis.

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