Hematological and Hemostatic changes in aflatoxin, curcumin plus aflatoxin and curcumin treated rat.

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A B S T R A C T

The present study carried out by using aflatoxin (AF), curcumin plus aflatoxin (R+AF) and curcumin (R). Four groups of rats were used each one consists of 5 rats; control group (C); which received distilled water intragastric daily for 6weeks. aflatoxin-treated group (AF); which received AF at a concentration 38 ppb in diet daily for 6 weeks. Curcumin plus aflatoxin group (R+AF) which received curcumin intragastric at a dose of 200 mg /kg B. wt. with aflatoxins at a dose 38 ppb in diet daily for 6 weeks. Curcumin-treated group (R); which received curcumin intragastric at a dose of 200 mg /kg B. wt. daily for 6 weeks. Aflatoxin-treated group showed macrocytic hypochromic anemia with significant decrease in lymphocyte. Meanwhile, Curcumin plus aflatoxin-treated group showed an improvement in haematological changes (RBC, Hb, Hct, MCV, MCH, MCHC, WBCs and granulocytes) induced by aflatoxin. Concerning haemostatic markers, there were significant prolongations of PT and TT, as well as reduction in platelet count and aggregation percentage in both groups and in curcumin-treated group when compared with control. The results of this study demonstrate that AF cause hemostatic disturbance with toxic effects on hematopoietic system. Additionally, curcumin has antithrombotic and antiplatelet effects.

***Keywords*:** Hemostatic markers, Aflatoxin, Curcumin, platelet Aggregation, Hematological parameters.

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1. INTRODUCTION

Aflatoxins are mycotoxins produced by mould which occurring as a result of unsuitable storage of food and foodstuff *(ÇELIK et al., 2000).* Aflatoxins B1, B2, G1 and G2 were occurred on different foodstuffs when exposed to certain strains of *Aspergillus flavus and Aspergillus parasiticus (Casado et al., 2001).* AFB1 is first metabolized mainly by the cytochrome P-450 enzyme (CYP450) system found in the microsome. This metabolism will produce a variety of metabolites such as AFB1 epoxide and hydroxylated metabolites (AFM1, AFP1, AFQ1, AFB 2α and aflatoxicol) *(Tulayakul et al., 2005).* The carcinogenicity and mutagenicity of aflatoxins are considered to be as a result of the formation of a reactive epoxide at the 8, 9-position of the terminal furan ring and its subsequent covalent binding to nucleic acid *(Chrevatidis et al., 2003).* Aflatoxin showed decreased erythrocyte count, leukocyte count, hemoglobin, and hematocrit levels *Donmez et al., (2012),* prolonged prothrombin time (PT) and activated partial thromboplastin time (APTT). Curcumin, a yellow pigment from *Curcuma longa*, is a major component of turmeric and one member of the ginger family. It has a wide range of pharmacological and physiological actions such as antioxidant, radioprotective, antibacterial, antifungal, antiviral, antiinflammatory, antiaflatoxogenic, antiproliferative, proapoptotic and antiatherosclerotic effects (*Ghoniem et al., 2012; Kedia et al., 2014*). Curcumin showed an enhancement in the hematological parameters as *Abdel-Moneim et al., (2015)* found that rats treated with curcumin after lead intoxication showed improvement in RBCs count, Hb concentration, total leukocytic count, eosinophils and monocytes toward normal. Curcumin that have better anticoagulant action as it caused significant increases in PT, APTT and inhibition the activities of thrombin and FXa *(Kim et al., 2012)*. Therefore, the aim of the study is the investigation of the changes in hematological and hemostatic pictures in experimentally treated rats by aflatoxin(AF), curcumin and curcumin plus aflatoxin (R+AF).

2. MATERIALS AND METHODS

2.1. Animals and experimental design:

A total number of 20 apparently healthy adult male white Albino rats (180–190 gm) body weight. The animals were obtained from faculty of veterinary medicine, Cairo, Egypt and housed for one week at constant environmental and nutritional conditions similar to those under which the experiment was performed for accommodation. Rats were housed in suitable cages away from any stressful stimuli, and supplied with diet and water *ad libitum*. Rats were allocated randomly into four main groups each group consists of 5 rats: control group (C); served as a control and received distilled water intragastric daily for 6weeks. Aflatoxin-treated group (AF): received aflatoxins after its preparation according to *(Stubblefield et al., 1967)* at a concentration 38 ppb in diet daily for 6 weeks. Curcumin plus aflatoxins-treated group (R+AF): received curcumin intragastric at a dose of 200 mg /kg B. wt. *(Park et al., 2000)* with aflatoxins at a concentration 38 ppb in diet daily for 6 weeks.

2.2. Chemicals

Aflatoxins (AF) were produced through fermentation of corn by Aspragillus parasaticus EMCC 274 as described by Stubblefield et al., (1967) and curcumin a golden yellow powder obtained from El-Gomhoria Company.

2.3. Reagents and kits

Commercial diagnostic kits for determination of Prothrombin time (PT), Activated Partial thromboplastin time (APTT) were obtained from Biosystems diagnostic (Germany). Thrombin time (TT) was obtained from Biomed diagnostic (Spain). Kits for Adenosine diphosphate (ADP) was obtained from Hart Biologicals (Germany).

2.4. Sampling:

Blood samples were obtained from retro-orbital venous plexus of the animals after 6 week of treatment for whole blood, plasma and platelet rich and poor plasma. Whole blood was used for hemogram evaluation (RBCs count, hemoglobin determination, hematocrit, total WBCs count and differential leukocytic count). Plasma samples were used for coagulation study (PT, APTT and TT). The collected PRP and PPP were used for platelet aggregation study by aggregometer using ADP agonists.

2.5. Clinicopathological analysis:

The hematological studies including erythrogram and leukogram were determined according to Thrall et al., (2012). While, platelets count was done by manual method using improved Neubauer hemocytometer according to Feldman et al., (2000). PT, APTT, TT and platelet aggregation were determined according to method described by Biggs and Macfarlane (1962); Hoffman and Neulendijk (1978); Key et al., (2009) and Days and Holmsen (1972) respectively.

2.6. Statistical Analysis

Statistical analysis was performed using the statistical software package for social science (SPSS) for Windows (Version 16.0; SPSS Inc., Chicago, IL). The significance of differences between the experimental groups was evaluated by one-way analysis of variance (ANOVA). If one-way ANOVA indicated a significant difference, then differences between individual groups were estimated using Duncan as a post hoc. Results are expressed as the mean ± standard error of mean. A *P*-value of less than 0.05 was considered significant (Kinnear and Gray 2006).

3. RESULTS

Data demonstrating the effects of aflatoxin, curcumin and curcumin plus aflatoxin on hemogram and hemostatic markers were presented in table (1, 2, 3 and 4). Concerning RBCs count, Hb and Hct results, there were significant reductions in rats administrated aflatoxin when compared with control group. On the other hand, there were significant increases in RBCs count, Hb and Hct in curcumin plus aflatoxin when compared with aflatoxin-treated groups. MCV showed significant increase in aflatoxin-treated group when compared with control group but, curcumin plus aflatoxin-treated group revealed a significant decrease in MCV when compared with aflatoxin-treated group. On the other hand, MCH and MCHC showed significant decreases in aflatoxin-treated group when compared with control indicating that aflatoxin induce macrocytic hypochromic anemia curcumin plus aflatoxin-treated group revealed a significant increase in MCHC and non-significant modifications in MCH when compared with aflatoxin-treated group. Meanwhile, curcumin-treated group revealed non-significant changes in RBCs, Hb, Hct, MCV, MCH and MCHC when compared with control group after 6 weeks.

Rats treated with aflatoxin showed significant leukopenia and lymphopenia when compared with control group, but curcumin plus aflatoxin-treated groups revealed non-significant changes in TLC and lymphocyte count when compared with aflatoxin-treated group. Non-significant alterations were observed in granulocyte and monocyte count in aflatoxin when

compared with control group and in curcumin plus aflatoxin-treated groups when compared with aflatoxin-treated one. Curcumin-treated group showed a significant monocytosis and non-significant changes in TLC, lymphocyte and granulocyte when compared with control group

PT and INR showed significant increases in aflatoxin-treated groups when compared with control group. Among treated groups, aflatoxin-treated groups showed significant reductions in percentage activity of PT when compared with control. Meanwhile, curcumin plus aflatoxin-treated group revealed non-significant alterations in PT, percentage activity of PT and INR when compared with aflatoxin-treated group. Non-significant changes were observed in APTT and R in Aflatoxin-treated group when compared with control and in curcumin plus aflatoxin-treated group when compared with aflatoxin group. Concerning to TT and R results, aflatoxin-treated group showed significant increases when compared with control group. On the other hand, curcumin plus aflatoxin-treated group revealed significant decreases in TT and R when compared with aflatoxin treated group. Curcumin-treated group showed significant increases in PT, INR, APTT, R, TT and R of TT, and significant decrease in percent activity of PT after 6 weeks.

Regarding platelet count, aflatoxin and curcumin-treated groups showed significant reduction in platelet number when compared with control. Whereas, there were non-significant modifications in platelet count in curcumin plus aflatoxin-treated group when compared with aflatoxin-treated group. Concerning to results of aggregometer, aggregation % revealed significant decreases (lower aggregation percent) in aflatoxin, curcumin plus aflatoxin and curcumin-treated groups when compared with control. Meanwhile, there were non-significant modifications in aggregation percentage in curcumin plus aflatoxin-treated group when compared with aflatoxin-treated groups.

Table (1): Erythrogram after 6 weeks in different experimental animal groups.

Parameters & Groups	RBCs ($x10^6/\mu l$)	Hb (gm/dl)	Hct (%)	MCV (fl)	MCH (pg)	MCHC (%)
C	7.11 ± 0.08^{c}	15.14 ± 0.69^{b}	$40.45\pm0.88^{b,c}$	$57.8\pm1.01^{a,b}$	21.50 ± 0.73^{b}	38.08 ± 0.76^{b}
AF	5.81 ± 0.18^{a}	12.8 ± 0.34^{a}	36.13 ± 1.01^{a}	60.6 ± 0.50^{c}	19.72 ± 0.52^{a}	35.2 ± 0.96^{a}
R+AF	6.64 ± 0.16^{b}	14.24 ± 0.20^{b}	39 ± 0.57^{b}	$57.8\pm0.37^{a,b}$	$21.07\pm0.27^{a,b}$	37.32 ± 0.66^{b}

Results are expressed as mean ±S.E.M. Different superscripts (a, b, c and d) within the same column indicate significant differences at $P\leq0.05$

(2): Leukogram after 6 weeks in different experimental animal groups.

Parameter& Groups	WBCs ($x10^3/\mu l$)	Granulocytes ($x10^3/\mu l$)	Lymphocyte ($x10^3/\mu l$)	Monocyte ($x10^3/\mu l$)
C	$14.10\pm0.69^{b,c}$	3.09 ± 0.25^{a}	$10.18\pm0.58^{b,c}$	0.83 ± 0.02^{a}
AF	9.64 ± 0.70^{a}	3.33 ± 0.40^{a}	5.47 ± 0.61^{a}	0.83 ± 0.07^{a}
R+AF	$12.1\pm0.64^{a,b}$	3.56 ± 0.34^{a}	$7.5\pm0.76^{a,b}$	$1.04\pm0.09^{a,b}$

Results are expressed as mean ±S.E.M. Different superscripts (a, b, c and d) within the same column indicate significant differences at $P\leq0.05$

Table (3): Coagulation markers after 6 weeks in different experimental animal groups.

Parameters & Groups	PT (second)	Percentage Concentration %	INR	APTT (second)	R
C	18.16±0.50[a]	40.80±1.98[b]	1.27±0.08[a]	32.30±3.16[a]	1.14±0.04[a]
AF	23.26±0.36[b]	35±0.42 [a]	1.50±0.12[b,c]	33.87±4.52[a,b]	1.34±0.15[a,b]
R+AF	21.52±0.42[b]	37.20±1.98[a,b]	1.44±0.02[a,b,c]	32.15±3.63[a]	1.44±0.13[a,b,c]

Results are expressed as mean ±S.E.M. Different superscripts (a, b, c and d) within the same column indicate significant differences at $P \leq 0.05$

Table (4): Coagulation markers after 6 weeks in different experimental animal groups.

Parameters & Groups	TT (second)	R	Platelet ($x10^3/\mu l$)	Aggreg.%
C	45.62±0.56[a]	2.71±0.20[a]	657±10.55[c]	52.77±1.49[b]
AF	60.36±1.80[c,d]	3.65±0.18[b]	502.8±13.42[a]	15.33±2.46[a]
R+AF	50.96±2.10[b]	2.82±0.29[a]	470.6±5.81[a]	16.67±0.72[a]

Results are expressed as mean ±S.E.M. Different superscripts (a, b, c and d) within the same column indicate significant differences at $P \leq 0.05$

4. DISCUSSION

Mycotoxins are toxic metabolites produced by large number of fungi. Among those metabolites are aflatoxins that are produced by A. flavus and A. parasiticus (Casado et al., 2001) as a result of unsuitable storage of food and foodstuff (Celik et al., 2000). Consumption of aflatoxins causes serious health problems such as depression of growth and production (Magdi, 1993). Curcumin is a major component of turmeric, and is commonly used as a spice and food-coloring material. It has a wide range of pharmacological and physiological actions such as antioxidant, antifungal, antiinflammatory, antiaflatoxogenic and proapoptotic effects (Ghoniem et al., 2012; Kedia et al., 2014).

Hematopoietic system is considered the mirror of the body as it reflects any changes in animal or human body exposed to chemical, toxic agents and drugs (Yuan et al., 2014). Rats administrated aflatoxin showed macrocytic hypochromic anemia which confirmed by reduction in RBCs count, Hb concentratin, PCV%, MCH and MCHC with increase in MCV. These results agreed with that of Kececi et al., (1998); Oguz et al., (2000) and Umar et al., (2012). This anemia may be due to the hemopoietic cellular defects of AF (Abdel-Wahhab et al., 2002) as well as, aflatoxin produce hemolytic anemia by decreasing the circulating mature erythrocytes and lysis of erythrocytes (Tung et al.,1975a). On the other hand, there were significant increases in RBCs count, Hb concentration and hematocrit percentage in curcumin plus aflatoxin when compared with aflatoxin-treated group after 6 weeks. Additionally, a significant decrease in MCV and a significant increase in MCHC were observed in in curcumin plus aflatoxin when compared with aflatoxin-treated group after 6 weeks these results in accordance with Abdel-Moneim et al., (2015). This illustrated the good effect of curcumin which overcome and minimizing changes produced by aflatoxin and garlic as curcumin enhance erythropoiesis, stabilize the cell membrane and prevent cellular damage occur by free reactive oxygen species and restore blood (Banji et al., (2011) and Sharma et al., 2011).

In regard to total and differential leukocytic count, lymphocyte showed a significant decrease after 6 weeks in aflatoxin-treated group when compared with control group. This finding agreed with that of Celik et al., (2000). The lymphopenia may be attributed to aflatoxin acted as an immunosuppressant (Chang and Hamilton 1979). Curcumin-treated group showed a significant monocytosis when compared with control group which indicates that curcumin activates the animal's immune system (Yousef et al., 1999 and Çetin et al., 2010).

Concerning to hemostatic parameters in aflatoxin-treated groups, there were significant increases in PT, INR, TT and R of TT, as well as reductions in platelet number after 6 weeks. These results agreed with that observed by OğuzZ et al., (2000) and Basmacioglu et al., (2005). The coagulation defects of aflatoxicosis are primarily due to the diminished hepatic synthesis of coagulation (Bakera and Green, 1987) as well as, anticoagulant properties of aflatoxin (Bababunmi and Bassir 1969). On the other hand, curcumin-treated group showed significant increases in PT, INR, APTT, R, TT and R of TT, and significant decrease in percent activity of PT after 6 weeks. Also, there was significant reduction in platelet count when compared with control after 6 weeks. Those results in accordance with Kim et al., (2012) who explain this action as a result of methoxy group in curcumin which has a positive regulated effect on anticoagulant function of curcumin.

Concerning to results of aggregometer, aggregation % revealed significant decreases (lower aggregation percent) in aflatoxin, curcumin plus aflatoxin and curcumin-treated groups when compared with control. The result of aflatoxin on aggregation agreed with Hong and Cho (1996) who explain the antiplatelet action may be due to decrease of ATP release. The anti-aggregatory effect of curcumin is a result of inhibition of thromboxane A2 and COX, as well as preventing synthesis and signaling of Ca2 Srivastava et al., (1995). So that, it concluded that aflatoxin and curcumin causes alterations in hemostatic markers.

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