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Original Research

Efficacy of N-Acetylcysteine on Aflatoxicosis in Rabbits

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Abstract

The aim of this study was to determine the clinical, hematological, biochemical and pathological findings, and to evaluate the efficiency of n-acetylcysteine (NAC) in experimentally induced aflatoxicosis in rabbits. Our study divided 42 rabbits into 6 groups. The groups received the following treatment: Group 1: control group without treatment, Group 2: aflatoxin (AF) (0.4 mg/kg body weight (bw)), Group 3: AF (0.4 mg/kg bw) plus NAC (250 mg/kg bw), Group 4: AF (0.4 mg/kg bw) plus NAC (500 mg/kg bw), Group 5: NAC (250 mg/kg bw), Group 6: NAC (500 mg/kg bw). N-acetylcysteine was administered intramuscularly on day 1 following the administration of AF for 5 days. At the beginning of the study and on days 1, 4 and 7 following the administration of AF, blood samples were collected for haematological and biochemical analysis. Necropsy and histopathological examination were performed. Clinical signs were observed starting from day 1 following the administration of AF. The signs of toxicosis included decreased feed and water consumption, dullness, dehydration, emaciation and convulsion. Four rabbits died in Group 2 and 2 rabbits died in Groups 3 and 4 treated with NAC. During the study, white blood cell (WBC) counts, mean platelet volume (MPV), mean corpuscular volume (MCV) and red blood cell distribution width (RDW) values, serum urea levels and aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and gamma glutamyl transpeptidase (GGT) activities increased whereas red blood cell (RBC) and platelet counts, hemoglobin (Hgb) and hematocrit (Hct) values, total protein, total cholesterol, triglyceride and glucose levels decreased for Group 2 given only AF compared with controls. These parameters approximated to control levels for Groups 3 and 4 after the treatment with NAC. Histopathological examination showed that the main affected organ was liver. Lipid degeneration, destruction and fibrosis in the liver were detected for Group 2. Histopathological changes in the liver were observed to be less severe for Groups 3 and 4. The study demonstrated that administration of NAC might be useful for the treatment of aflatoxicosis in rabbits.

Keywords: N-acetylcysteine, aflatoxicosis, rabbits

Introduction

Aflatoxins are secondary toxic fungal metabolites designated as mycotoxins and produced by toxigenic strains of *Aspergillus fumigatus* and *A. parasiticus*. They are toxic to a wide variety of animals [1-3]. Rabbits are one of the most sensitive animals for AF [2, 4]. Many studies related to natural [5] or experimentally [4, 6-8] induced aflatoxicosis in rabbits have been reported. Signs of the aflatoxicosis in rabbits include dullness, de-

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creased feed and water consumption, weight loss, dehydration, lethargy, emaciation, icterus ve finally death [5, 6]. It is apparent from experiments carried out on animals and from clinical observations of people that short-term exposure to large amounts of AF leads to toxicity, which may be lethal. Ingestion of feedstuffs contaminated with aflatoxins has resulted in hepatobiliary lesions and deaths in many species of animals [9-13]. Therefore, intensive research has been continued out to find cost-effective and safe procedures and agents that reduce the deleterious effects of AFs [14-19]. Although many agents have been used for the treatment of aflatoxicosis, the desired effect has not been fully achieved [4, 6, 8, 11, 20]. N-acetylcysteine is already commercially available for use in humans in several countries and has been used safely in daily doses as high as 500 mg/kg. The drug has been administered as a mucolytic drug in a variety of respiratory illnesses. It also appears to have beneficial effects in conditions such as toxic agents, neoplasia, heart disease and atmospheric pollutants [21, 22]. In addition, Valdivia et al. [23] have reported that NAC have a preventive effect against aflatoxin B_1 intoxication in broiler chickens. Therefore, we believe that n-acetylcysteine is a beneficial and easily available drug that may be used in the treatment of aflatoxicosis cases in rabbits.

The aim of this study was to determine the clinical, hematological, biochemical and pathologic findings, and to evaluate the efficiency of NAC in experimentally induced aflatoxicosis in rabbits.

Materials and Methods

Animals

In this study, 42 New Zealand rabbits, healthy, aged between 6 and 8 weeks, weighing between 1 and 1.5 kg were used. The rabbits were housed individually in wire cages and fed with commercial pellet food, and the water was supplied *ad libitum*. The animals were divided into 6 groups, each including 7 rabbits. Group 1 was designed as the control group. Rabbits in the Group 2, 3 and 4 were given AF at a dose of 0.4 mg/kg bw in dimethylsulphoxide (DMSO), whereas rabbits in the Group 1, 5 and 6 were given the same dose of DMSO free from AF by a catheter directly into their stomachs. At the same time on day 1 following the administration of AF, NAC was administered intramuscularly (IM) for 5 days at a dose of 250 mg/kg bw for Groups 3 and 5 and at a dose of 500 mg/kg bw for Groups 4 and 6. The purified AF was dissolved in DMSO. At the beginning of the study (period I) and on days 1 (period II), 4 (period III) and 7 (period IV) following the administration of AF, blood samples were collected from each rabbit into tubes with ethylenediaminetetraacetic acid for hematological examination and into tubes with coagulant free for serum biochemical analysis.

This study was approved by the Ethics Committee of University of Erciyes, Faculty of Veterinary Medicine, Approval number 2002-10.

Aflatoxin Production

Aflatoxin used in this study was produced in accordance with the method reported by Demet et al. (24) based on the technique reported by Shotwell et al (25). Their rates were determined according to the method reported by Nabney and Nesbit (26) as 78.80%, 10.80%, 6.80% and 3.70% for aflatoxin B_1, B_2, G_1 and G_2, respectively. The analysis of total AF (B1+B2+G1+G2) purified from rice starch was performed using a Ridascreen® label kit and ELISA reader.

Haematological and Biochemical Measurements

The determination of WBC, RBC and platelet counts and measurement of Hgb, Hct, MCV, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), RDW and MPV values were performed using an automated haematology cell counter (Vet Medonic CA 530, Stockholm, Sweden). Sera was separated by centrifugation at 3000 rpm for 10 min after 1 hour incubation at room temperature and stored at –20°C until analysis. Serum AST, ALT, ALP and GGT activities, urea, glucose (Biolabo, France), total protein, creatinine (Chema, Italy), total cholesterol and triglyceride (Biosystems, Spain) levels were determined with commercial kits using a Shimadzu-UV 1208 model spectrophotometer.

Necropsy Examination

Necropsies and histopathological examination were performed on the animals that died throughout the study and survived until the end of the study. Rabbits were humanely euthanized after anaesthesia with xylazine (10 mg/kg) (Rompun, Bayer) and ketamine (35 mg/kg) (Ketalar, Parke-Davis). Tissue samples were taken from the liver, kidneys, lungs and brain and fixed in 10% buffered formalin, and embedded in paraffin, sectioned (5-6μm), mounted on glass slides. The sections were stained with hematoxylene and eosine (HE), and examined using a light microscope.

Statistical Analysis

Statistical analyses of data were carried out using SPSS 10.0 version for Windows. One-way analysis of variance (ANOVA) was used to determine the differences between the groups. When the F value was significant, Duncan's Multiple Range Test performed. All data were expressed as means ± SEM.

Results

Clinical Findings

Clinical signs were observed starting from day 1 following the administration of AF. Clinical signs included decreased feed and water consumption, dullness, dehydration, emaciation and convulsion. In Group 2, 1, 2 and 1 rabbits died on days 2, 3 and 5 following the administration of AF, respectively. One rabbit died on day 3 and 5 following the administration of AF from Group 3 and on day 2 and 6 following the administration of AF from Group 4. During the treatment, feed, water consumption and general condition of the rabbits in Groups 3 and 4 improved.

Haematological and Biochemical Parameters

On day 1 following AF administration, an increase in WBC counts, MPV values and serum ALT, ALP, and GGT activities ($p<0.05$) and a decrease in RBC and platelet counts, hct and hgb values, serum AST activity, total protein, glucose and triglyceride levels ($p<0.05$) were determined for Groups 2, 3 and 4 compared to Group 1. The increase in the serum urea level was significant ($p<0.05$) for Groups 3 and 4. For the rabbits in Group 2, apart from an insignificant increase in the RDW value, a significant increase in serum AST activity ($p<0.05$) and a decrease in the total cholesterol level ($p<0.05$) on days 4 and 7 following the administration of AF and a significant increase in the MCV value ($p<0.05$) on day 7 following the administration of AF, alterations mentioned in the above parameters were also determined on days 4 and 7 of the study (Tables 1-3).

On day 4 following the administration of AF, an increase in WBC counts, serum AST, ALT, ALP and GGT activities ($p<0.05$) for Groups 3 and 4 and increase in RDW values ($p<0.05$) in Group 4 were determined compared to Group 1. While there was a significant decrease in RBC and platelet counts, hgb and hct values and serum total protein, glucose and triglyceride levels for both groups ($p<0.05$), the decrease in the serum total cholesterol level was determined to be significant for Group 3 ($p<0.05$). There was a slight decrease in WBC counts, MPV values and serum GGT activity whereas a slight increase in RBC and platelet counts, hct and hgb values and serum total protein, glucose, triglyceride and total cholesterol levels for Groups 3 and 4 compared to Group 2. While the decrease in serum ALT activity was significant for Groups 3 and 4 ($p<0.05$), the serum AST activity for Group 3 and ALP activity for Group 4 decreased significantly ($p<0.05$) (Table 1-3).

At the end of the study, WBC, RBC and platelet counts, Hct, Hgb, RDW and MPV values recorded for Groups 3 and 4 were determined to approximate those of Group 1. Serum AST, GGT and ALP activities in both groups, urea level for Group 3 and ALT activity for Group 4 were found to be higher compared to Group 1 ($p<0.05$). On the other hand, a decrease in serum total protein, glucose, triglyceride and total cholesterol levels ($p<0.05$) were determined for both groups. However, when these groups were compared to Group 2, a decrease in serum AST, ALP and GGT activities ($p<0.05$), an increase in total protein, glucose and triglyceride levels ($p<0.05$) and an insignificant increase in total cholesterol level were found (Tables 1-3).

Necropsy Findings

In the rabbits that died throughout the study and survived in Group 2 at the end of this study, the livers were observed to be congested and slightly yellowish in colour. Microscopical examination revealed dissociation in the remark cords of the liver. Healthy hepatocytes were present in only a few restricted areas. Sharp edged, round shaped lipid vacuoles at different sizes were found to be widespread in hepatocytes of the liver. In many areas, these lipid vacuoles were observed to unite and the liver tissue to gain an appearance similar to a honeycomb. Furthermore, paranchymal degeneration leading to necrosis was detected in hepatocytes. An increase in fibrous tissue was determined between lobes and more evident in portal interspace. Lymphoid cell infiltration was observed in these regions. Hyperplasia was detected in the bile duct epitelium (Fig. 1. A, B). Sharp edged and round shaped lipid vacuoles in different sizes in some of the hepatocytes located in the acini were observed in survived rabbits in the Group 3 at the end of the study. Slight fibrosis and lymphoid cell infiltration was detected in portal interspace and interlobar areas (Fig. 2. A, B). Focally paranchymal degeneration was observed in some hepatocytes. Similar findings also were observed in Group 4 (Fig. 3. A, B). No macroscopical or microscopical finding was determined in rabbits in Groups 1, 5 and 6.

Discussion

N-acetylcysteine has been used safely in humans and other mammals [21, 22]. According to the results of the present study, administration of NAC at doses of 250 and 500 mg/kg bw for 5 days did not cause any haematological, biochemical or histopathological side effect in rabbits, which in turn supported the opinion that this compound might be used safely. Similar to the reports of various researchers [5, 6, 10, 12, 27], our study also revealed the decrease in feed and water consumption, dullness, dehydration, emaciation, convulsions and death in AF-dosed rabbits. Improvement in feed and water consumption rates and the general health condition of the animals during the treatment period with NAC might be considered indicators of the beneficial effects of NAC.

Valdivia et al. [23] have reported that NAC provided protection against negative effects on performance, liver and renal damage, and biochemical alterations induced by

Table 1. Haematological parameters in control and experimental groups.

Parameters	Period	Groups											
		Group 1		Group 2		Group 3		Group 4		Group 5		Group 6	
		n		n		n		n		n		n	
WBC (x 10^3/µL)	I	7	6.57±2.04	7	6.21±1.41	7	6.19±1.93	7	5.31±0.53	7	6.29±1.09	7	6.16±1.30
	II	7	5.87±0.92 [a]	7	12.91±6.13 [b]	7	12.52±5.59 [b]	7	12.74±6.42 [b]	7	6.16±1.76 [a]	7	6.07±1.93 [a]
	III	7	6.16±1.22 [a]	4	12.43±4.95 [b]	6	11.95±4.43 [b]	6	10.25±2.73 [b]	7	6.15±0.85 [a]	7	5.97±0.72 [a]
	IV	7	6.20±1.95 [a]	3	10.43±4.89 [b]	5	7.78±1.44 [a]	5	7.42±1.08 [a]	7	5.75±1.55 [a]	7	5.91±1.05 [a]
RBC (x 10^6/µL)	I	7	5.54±0.63	7	5.61±0.28	7	5.70±0.64	7	5.81±0.94	7	5.27±0.55	7	5.68±0.89
	II	7	5.53±0.77[a]	7	4.33±0.55[b]	7	4.08±0.65 [b]	7	3.86±0.62 [b]	7	5.24±0.53 [a]	7	5.59±1.13 [a]
	III	7	5.60±0.46 [a]	4	3.34±0.99 [b]	6	3.91±0.60 [b]	6	3.80±0.91 [b]	7	5.23±0.56 [a]	7	5.54±0.50 [a]
	IV	7	5.65±0.87 [a]	3	3.84±0.72 [c]	5	4.87±0.37 [ab]	5	4.69±1.05 [bc]	7	5.08±0.44 [ab]	7	5.39±0.40 [a]
Htc (%)	I	7	34.67±4.47	7	36.19±2.08	7	36.41±4.51	7	35.43±4.99	7	33.33±3.21	7	34.94±6.81
	II	7	35.79±4.78 [a]	7	27.41±2.20 [b]	7	26.87±4.02 [b]	7	24.24±3.75 [b]	7	32.71±3.30 [a]	7	34.00±5.45 [a]
	III	7	34.93±3.57 [a]	4	22.05±6.29 [b]	6	26.20±2.97 [b]	6	24.43±5.00 [b]	7	34.34±3.20 [a]	7	35.63±3.25 [a]
	IV	7	34.47±3.68[a]	3	26.23±5.27[c]	5	30.76±3.47[ab]	5	29.65±2.72[bc]	7	32.10±2.11[ab]	7	34.20±2.04[ab]
Hgb (g/dl)	I	7	10.09±±1.20	7	10.24±0.48	7	10.44±1.27	7	10.26±1.49	7	9.51±0.91	7	10.11±2.11
	II	7	10.30±1.57 [a]	7	7.74±0.73 [b]	7	7.64±1.28 [b]	7	6.91±1.15 [b]	7	9.46±0.84 [a]	7	9.67±1.42 [a]
	III	7	9.96±1.07 [a]	4	6.25±1.91 [b]	6	7.45±1.02 [b]	6	6.92±1.53 [b]	7	9.90±0.83 [a]	7	10.31±1.02 [a]
	IV	7	10.03±1.04[a]	3	7.23±1.47[c]	5	9.37±0.84[ab]	5	8.42±1.20[bc]	7	9.49±0.78[ab]	7	9.71±0.64[ab]
MCH (pg)	I	7	18.14±0.39	7	18.31±0.76	7	18.60±0.60	7	17.66±1.40	7	18.11±0.85	7	17.81±1.23
	II	7	18.67±0.82	7	18.00±1.26	7	18.59±0.97	7	17.91±1.48	7	18.09±0.73	7	17.51±1.21
	III	7	17.81±0.71	4	18.73±0.50	6	18.75±0.52	6	18.48±2.29	7	18.97±0.72	7	18.64±0.60
	IV	7	17.94±1.37	3	18.87±0.35	5	18.56±0.33	5	18.56±2.00	7	18.79±0.96	7	18.09±0.84
MCHC (g/dl)	I	7	29.17±0.58	7	28.41±0.90	7	28.91±0.43	7	28.89±0.74	7	28.60±0.78	7	28.96±1.01
	II	7	28.84±1.23	7	28.29±0.61	7	28.56±0.72	7	28.49±1.05	7	28.97±0.60	7	28.86±0.67
	III	7	28.59±1.17	4	28.30±0.88	6	28.67±0.82	6	28.32±0.89	7	28.90±1.18	7	29.04±1.46
	IV	7	29.23±1.07	3	27.70±0.17	5	28.18±0.94	5	28.22±1.84	7	29.24±1.55	7	28.96±0.39
MCV (fL)	I	7	62.61±1.43	7	64.67±3.94	7	64.73±0.98	7	61.44±1.39	7	63.41±2.90	7	61.87±2.71
	II	7	64.63±1.19	7	64.17±4.84	7	65.36±1.14	7	62.90±3.09	7	62.44±2.41	7	61.24±1.20
	III	7	62.39±2.63	4	66.15±1.73	6	66.70±0.77	6	64.57±6.11	7	65.63±2.40	7	64.13±1.52
	IV	7	60.99±2.11 [a]	3	68.17±0.64 [b]	5	63.10±0.94 [a]	5	62.18±1.76 [a]	7	63.30±2.45 [a]	7	63.61±2.96 [a]
RDW (%)	I	7	15.54±1.67	7	15.26±2.73	7	16.13±2.68	7	16.43±3.37	7	15.69±1.68	7	15.30±1.73
	II	7	16.24±2.10	7	17.08±2.78	7	16.83±2.25	7	17.07±3.81	7	15.16±3.11	7	15.64±2.73
	III	7	15.70±1.98 [ab]	4	19.38±1.80 [bc]	6	18.65±3.77 [bc]	6	19.88±5.12 [c]	7	14.63±2.93 [a]	7	16.23±1.07 [abc]
	IV	7	15.37±1.83	3	20.03±0.83	5	16.58±1.19	5	17.41±1.04	7	15.50±3.91	7	15.91±2.53
Plt (x 10^5/µL)	I	7	370±155.49	7	418.57±63.90	7	403.86±74.58	7	433.29±167.33	7	407.86±71.55	7	364.14±105.33
	II	7	459.43±62.58 [a]	7	183.29±118.74 [b]	7	160.29±102.14 [b]	7	170.43±138.80 [b]	7	387.86±105.18 [a]	7	402.71±129.17 [a]
	III	7	375.57±121.79 [a]	4	182.00±53.82 [b]	6	255.00±79.49 [b]	6	251.17±78.52 [b]	7	384.14±64.89 [a]	7	378.86±111.56 [a]
	IV	7	322.57±70.26 [bc]	3	173.33±57.95 [a]	5	310.40±75.63 [bc]	5	272.00±35.74 [ab]	7	380.00±69.74 [bc]	7	413.29±140.60 [c]
MPV (fL)	I	7	6.86±0.87	7	6.84±0.97	7	6.44±0.95	7	6.73±0.49	7	6.83±0.81	7	6.13±0.49
	II	7	6.24±0.32 [a]	7	7.93±1.47 [b]	7	7.81±1.26 [b]	7	8.19±0.84 [b]	7	6.57±0.78 [a]	7	6.64±0.53 [a]
	III	7	6.64±0.89	4	7.95±1.51	6	7.00±0.75	6	7.47±0.92	7	6.47±0.84	7	6.46±0.74
	IV	7	6.47±0.94[a]	3	7.80±1.25[b]	5	6.54±0.54[a]	5	6.86±0.91[ab]	7	6.37±0.33[a]	7	6.87±0.40[ab]

[a, b, c]. The mean values within the same row with different letters are statistically significant ($p<0.05$).

*. Group 1, control; Group 2, AF; Group 3, AF+ NAC (250 mg/kg bw); Group 4, AF+NAC (500 mg/kg bw); Group 5, NAC (250 mg/kg bw); Group 6, NAC (500 mg/kg bw)

**. Period I, beginning of the study; period II, at the day 1, period III, at the day 4; period IV, at the day 7.

Table 2. Some serum enzymes activies in the control and experimental groups.

Parameters	Period	Groups											
		Group 1		Group 2		Group 3		Group 4		Group 5		Group 6	
		n		n		n		n		n		n	
AST (IU/L)	I	7	21.12±4.12	7	22.18±6.51	7	22.33±5.86	7	24.31±4.69	7	22.34±4.48	7	23.60±6.97
	II	7	21.46±3.97^{a}	7	5.08±1.49^{b}	7	6.51±2.97^{b}	7	4.34±1.75^{b}	7	23.95±6.76^{a}	7	25.30±5.50^{a}
	III	7	22.70±2.93^{a}	4	146.17±112.25^{c}	6	87.63±29.87^{b}	6	99.85±37.09^{bc}	7	23.45±4.11^{a}	7	24.75±5.71^{a}
	IV	7	23.77±3.01^{a}	3	174.71±82.56^{c}	5	65.33±12.57^{b}	5	62.46±8.82^{b}	7	24.48±5.32^{a}	7	26.55±4.81^{a}
ALT (IU/L)	I	7	30.76±6.49	7	37.02±13.84	7	27.68±5.66	7	36.60±10.87	7	29.69±8.52	7	36.98±9.52
	II	7	33.25±10.56^{a}	7	213.02±37.78^{b}	7	224.06±48.48^{b}	7	194.63±61.59^{b}	7	35.34±9.72^{a}	7	34.46±6.68^{a}
	III	7	29.00±4.89^{a}	4	231.58±129.64^{c}	6	154.26±57.48^{b}	6	138.32±51.21^{b}	7	32.11±5.17^{a}	7	31.98±12.25^{a}
	IV	7	36.61±10.56^{a}	3	240.56±1125.31^{c}	5	71.70±10.21^{ab}	5	84.86±9.67^{b}	7	32.92±10.90^{a}	7	35.77±4.30^{a}
GGT (IU/L)	I	7	5.94±1.97	7	6.09±3.90	7	8.72±3.82	7	8.26±3.11	7	7.48±2.33	7	7.00±2.37
	II	7	6.04±1.67^{a}	7	41.71±33.39^{b}	7	40.83±26.41^{b}	7	35.64±11.26^{b}	7	6.20±1.88^{a}	7	6.24±1.82^{a}
	III	7	5.81±1.58^{a}	4	44.70±27.42^{b}	6	37.70±20.77^{b}	6	37.00±9.85^{b}	7	7.77±2.54^{a}	7	6.35±1.61^{a}
	IV	7	6.91±2.49^{a}	3	34.24±4.59^{c}	5	16.64±4.55^{b}	5	18.51±3.79^{b}	7	6.66±2.29^{a}	7	6.34±1.73^{a}
ALP (IU/L)	I	7	306.37±67.36	7	315.02±80.53	7	327.08±61.02	7	308.78±102.73	7	303.46±82.36	7	301.86±79.09
	II	7	300.77±59.98^{a}	7	913.90±385.25^{b}	7	836.07±362.80^{b}	7	795.09±303.96^{b}	7	297.09±62.25^{a}	7	296.32±28.18^{a}
	III	7	307.93±21.11^{a}	4	1029.82±292.86^{c}	6	766.29±417.05^{bc}	6	688.45±342.62^{b}	7	289.17±88.90^{a}	7	303.72±89.98^{a}
	IV	7	311.73±65.90^{a}	3	1036.20±336.62^{c}	5	509.19±199.24^{b}	5	526.12±178.56^{b}	7	299.73±75.17^{a}	7	308.56±72.23^{a}

$^{a, b, c}$ The mean values within the same row with different letters are statistically significant ($p<0.05$).

Fig. 1. Group 2 (only given aflatoxin). (A) Increase in fibrous tissue and lymphoid cell infiltrations in the portal interspace (arrows), diffuse distribution of lipid vacuoles in liver hepatocytes. HxE, x100. (B) Hyperplasia of the bile duct epithelium (thin arrows), increase in fibrous tissue and lymphoid cell infiltration in the portal interspace (bold arrows), lipid vacuoles in hepatocytes. HxE, x200.

AFB_1 in broiler chickens. Our group reported earlier, as a part of this study [28], that AF caused oxidative damage and NAC was effective partly in the prevention of this damage.

The increase in WBC counts [29, 30], and the decrease in platelet [30] and RBC counts, hgb and hct values [11, 14, 31, 32] was also determined in this study. Furthermore, this study revealed a significant increase in MPV and MCV values on day 7 in the rabbits given only AF. The exact mechanism causing a decrease in RBC and platelet counts, hgb and hct values and increasing in MCV and MPV values are not clearly understood. These effects might result from the inhibition of haematopoiesis, defective haematopoiesis, increased rate of destruction of RBC or a combination of all three [31]. *In vitro* studies have shown that an enhanced rate of AF-induced morphological alterations and haemolysis upon the treatment of a saline suspension of RBC with AF [33]. The variabilities of parameters determined in this study might be an indicator of the depressing effect of AF on haemopoetic tissue. The increase in WBC counts was attributed to the

Table 3. Some serum metabolites in control and experimental groups.

Parameters	Period	Groups											
		Group 1		Group 2		Group 3		Group 4		Group 5		Group 6	
		n		n		n		n		n		n	
Urea (mg/dl)	I	7	30.29±16.15	7	29.12±4.49	7	26.22±13.45	7	28.31±10.60	7	32.52±13.73	7	34.58±14.60
	II	7	32.42±13.13 [a]	7	44.11±9.21 [abc]	7	51.29±22.55 [bc]	7	55.32±20.99 [c]	7	33.91±13.25 [a]	7	31.89±6.84 [a]
	III	7	33.80±19.68 [a]	4	86.13±69.04 [b]	6	55.74±17.03 [ab]	6	48.42±20.64 [a]	7	29.54±10.17 [a]	7	37.31±15.62 [a]
	IV	7	31.71±12.90 [a]	3	56.10±6.50 [c]	5	49.96±5.98 [bc]	5	41.08±19.03 [abc]	7	34.33±9.45 [ab]	7	36.59±14.38 [ab]
Creatinine (mg/dl)	I	7	0.57±0.12	7	0.59±0.08	7	0.73±0.06	7	0.67±0.10	7	0.67±0.10	7	0.63±0.12
	II	7	0.71±0.16	7	0.72±0.16	7	0.73±0.13	7	0.68±0.13	7	0.66±0.21	7	0.74±0.15
	III	7	0.71±0.12	4	0.64±0.15	6	0.66±0.20	6	0.73±0.10	7	0.66±0.11	7	0.66±0.17
	IV	7	0.67±0.11	3	0.71±0.11	5	0.72±0.10	5	0.73±0.09	7	0.63±0.04	7	0.68±0.21
Total Protein (g/dl)	I	7	5.50±0.80	7	5.58±0.74	7	6.07±0.77	7	5.88±0.79	7	5.53±0.84	7	5.52±0.72
	II	7	5.65±0.97 [a]	7	4.47±0.89 [bc]	7	4.44±0.63 [bc]	7	4.19±0.86 [c]	7	5.25±0.78 [a]	7	5.82±0.67 [a]
	III	7	5.36±1.18 [a]	4	3.67±0.65 [b]	6	4.25±0.36 [b]	6	4.13±0.55 [b]	7	5.34±0.59 [a]	7	5.63±0.61 [a]
	IV	7	5.54±0.90 [a]	3	3.89±0.29 [c]	5	4.74±0.39 [b]	5	4.83±0.39 [b]	7	5.47±0.44 [ab]	7	5.99±0.63 [ab]
Glucose (mg/dl)	I	7	107.15±16.48	7	106.88±20.49	7	105.24±19.58	7	112.28±29.16	7	109.66±11.42	7	111.14±17.33
	II	7	118.86±27.01 [a]	7	42.62±20.71 [b]	7	41.14±13.33 [b]	7	44.11±16.18 [b]	7	119.23±9.95 [a]	7	111.78±24.57 [a]
	III	7	105.68±15.09 [a]	4	43.33±17.36 [b]	6	67.30±40.27 [b]	6	64.34±35.16 [b]	7	107.56±11.21 [a]	7	111.12±20.78 [a]
	IV	7	115.86±10.22 [a]	3	47.22±1.75 [c]	5	84.85±13.25 [b]	5	79.57±14.18 [b]	7	113.89±9.87 [a]	7	115.91±11.64 [a]
Total Cholesterol (mg/dl)	I	7	27.53±4.71	7	27.74±5.48	7	29.26±5.21	7	27.17±3.20	7	28.30±3.42	7	26.93±7.17
	II	7	30.71±4.77	7	29.38±6.68	7	30.46±9.78	7	32.73±9.11	7	30.45±9.02	7	31.79±5.09
	III	7	26.88±3.09 [b]	4	13.47±4.71 [a]	6	15.12±3.98 [a]	6	19.83±5.30 [ab]	7	28.28±3.92 [bc]	7	27.65±11.08 [bc]
	IV	7	28.53±4.04 [a]	3	15.63±6.33 [b]	5	19.65±3.00 [b]	5	21.67±3.99 [b]	7	28.61±4.47 [a]	7	30.52±5.96 [a]
Triglyceride (mg/dl)	I	7	67.69±18.05	7	75.47±27.15	7	77.46±23.68	7	83.29±24.99	7	75.93±15.62	7	97.43±31.95
	II	7	75.12±26.48 [a]	7	40.94±20.67 [b]	7	41.55±10.04 [b]	7	35.31±15.13 [b]	7	79.50±24.05 [a]	7	92.88±35.26 [a]
	III	7	72.19±21.37 [a]	4	19.84±9.59 [b]	6	30.14±11.25 [b]	6	32.25±10.30 [b]	7	78.73±20.18 [a]	7	97.78±49.63 [a]
	IV	7	82.43±10.42 [a]	3	20.01±5.52 [c]	5	53.29±9.98 [b]	5	47.65±10.91 [b]	7	82.60±13.45 [a]	7	104.72±29.47 [a]

[a, b, c.] The mean values within the same row with different letters are statistically significant ($p<0.05$).

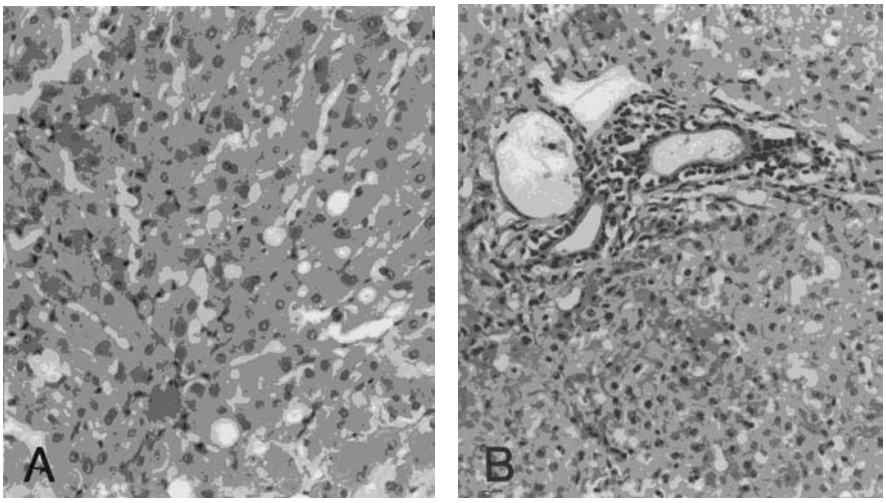

Fig. 2. Group 3 (given aflatoxin + 250 mg/kg NAC). (A) Lipid vacuoles in some of the hepatocytes. H x E, x200. (B) Slight fibrosis and restricted lymphoid cell infiltration in the portal interspace and lipid vacuoles in some of the hepatocytes. H x E, x100.

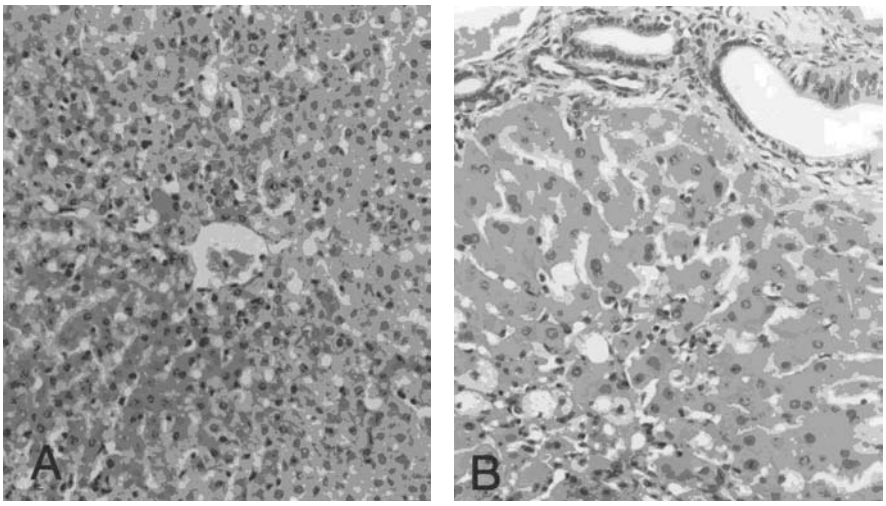

Fig. 3. Group 4 (given aflatoxin + 500 mg/kg NAC). (A) Lipid vacuoles in some of the hepatocytes in the acini and a few number of lymphocytes in the sinusoids. H x E, x100. (B) Slight fibrosis and restricted lymphoid cell infiltration in the portal interspace, lipid vacuoles in some of the hepatocytes. H x E, x200.

severity of aflatoxicosis. On the other hand, the tendency in favour of the approximation of WBC, RBC and platelet counts, hct, hgb, MCV and MPV values of Groups 3 and 4 treated with NAC to those of the control group might be attributed to the reduction of the negative effects of AF on haemopoetic tissue resulting from treatment.

Increased serum AST and ALT activities due to hepatocellular destruction and serum ALP and GGT activities due to cholestasis are frequently observed in aflatoxicosis in rabbits [4, 8, 14]. The changes in the activities of the above-mentioned enzymes were found to be less severe in the groups treated with NAC compared to the Group 2 given only AF. The histopathological findings supported the view that liver damage was less severe in the groups treated with NAC. According to the results of this study, NAC was considered to reduce the severity of hepatic destruction. Variations in the effect of AFB_1 on transaminase activity might be related to differences in gender and lines of the animals [34]. In this study, the decrease of AST activity was observed in AF-given groups on day 1. The histopathological changes observed in the rabbits given AF were also in accordance with previous reports [5, 8, 27, 35, 36].

It has been reported that serum total protein level may decrease due to impairment of protein synthesis arising from the inhibitory effect of AFs on DNA and RNA synthesis [3, 15, 37, 38]. In this study, a decrease in serum total protein level might be considered resulting from impairment of protein synthesis due to similar effects of AF. It is possible that the decrease in serum glucose level might be related to the severity of hepatic damage and the increase in urea level might be attributed to dehydration. According to histopathological examination, these

changes were found to be less severe in treated groups compared to Group 2 administered only AF. This was attributed to the reduction of hepatic destruction resulting from administration of the drug.

In aflatoxicosis cases some researchers [38] have reported a decrease while some [4, 7, 14] detected an increase in serum total cholesterol and triglyceride levels. Since these changes may be affected by factors including individual sensitivity, breed, age, sex, species and nutrition, they are also considered to related to the dose of AF administered to animals [38]. While an increase in serum total cholesterol and triglyceride levels was determined in animals administered low dose of AF [4, 14], a decrease in the mentioned parameters was found in animals administered high doses of AF [38]. In this study, animals administered AF at the dose of 0.4 mg/kg bw reported as LD_{50} for rabbits [6] displayed a decrease in serum total cholesterol and triglyceride levels. The effects of AF on serum cholesterol and triglyceride levels suggest that lipid metabolism and transport were disrupted due to hepatic damage induced by AF [38, 39]. Changes in serum total cholesterol and triglyceride levels were found to be less severe for the animals in Group 3 and 4 given AF and treated with NAC ompared to Group 2 and this was attributed to the effect of NAC which might lead to a reduction in the severity of lipid degeneration in the liver.

Conclusions

The results of the present study indicate that changes were determined in clinical, some haematological and biochemical parameters in experimentally induced aflatoxicosis in rabbits. Liver was the most affected organ according to histopathological findings. It might be said that administrations of NAC at doses of 250 and 500 mg/kg bw for 5 days to rabbits with aflatoxicosis partly reduced the toxic effects of AF and both doses of NAC exhibited similar effects.

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