

PROTECTIVE EFFECT OF *OPUNTIA ELATIOR* AND *WITHANIA SOMNIFERA* AGAINST LEAD ACETATE INDUCED TOXICITY IN BROILER CHICKENS

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ABSTRACT

The present study was carried out to evaluate the protective effect of *Opuntia elatior* fruit juice (3 ml/kg body weight) and aqua-alcoholic extract of *Withania somnifera* (100 mg/kg body weight) alone and in combination of both against lead acetate (500 ppm in feed for 21 days) induced toxicity in broiler chickens (n=8 each) keeping one group each of lead induced toxicity and healthy control. Clinical symptoms of toxicity were observed in birds received only lead acetate. Haematological parameters, Hb and MCHC in birds treated with lead acetate were found significantly ($P<0.05$) lower as compared to that of control group. Non-significant ($P>0.05$) alterations in PCV, TEC, TLC and MCV levels of birds of all treatment groups were observed compared to that of control birds. Mean values of ALT, BUN, creatinine and total bilirubin were significantly ($P<0.05$) higher in birds treated with lead acetate as compared to control group, whereas mean values of AST, total protein, albumin, globulin, ACP, ALP and LDH did not differ significantly between groups. It was concluded that daily administration of *Opuntia elatior* or *Withania somnifera* at above dose rate may be useful for protection of hepatotoxicity and nephrotoxicity in broilers chickens.

KEY WORDS: *Opuntia elatior*, *Withania somnifera*, Lead acetate, Broiler chickens, Toxicity.

INTRODUCTION

Poultry farming is always prone to a heavy risk of increased disease incidences leading to high mortality due to hepatotoxicity, nephrotoxicity and other major complications in birds after exposure to toxic compounds. Among them metal is one of the ubiquitous environmental pollutants resulting in poor performance and death of animals and birds (Babu *et al.*, 2007).

In recent times complementary and alternative medicines have made significant inroads as an accessory modality in toxicosis, providing a feasible option for toxicosis control, relief of side effects caused by drugs, prevention of recurrence, boosting the immune system, reducing stress and improving quality of life and general well-being (Kantor, 2009). In this context several herbs including *Opuntia ficus indica* and *Withania somnifera* are found to have potential to prevent or treat symptoms of toxicity to major organs (Galati *et al.*, 2003). Dhenge *et al.* (2009) reported a significant increase in Hb on administration of *Withania somnifera* in broiler chicks. Amongst various toxicants, lead may exert toxic effect on several body systems (Patrick, 2006). Therefore, the present study was carried out to evaluate the protective effect of *Opuntia elatior* and *Withania somnifera* plants against lead acetate induced toxicity in broiler chickens.

MATERIALS AND METHODS**Experimental Birds**

The present study was conducted on 40 color broiler chickens. The study plan was approved by the Institutional Animal Ethics Committee (IAEC). Day old broiler chicks were obtained from Intensive Poultry Development Block, Makarba, Ahmedabad, Gujarat. They were kept under constant

observation prior to commencement of the experiment till achieving 3 weeks of age. Vaccination against Newcastle disease and infectious bursal disease was followed as per standard schedule.

Juice and Extract Preparation

Opuntia elatior fruits were collected from local market. Fruits were cleaned properly and seeds were removed and juice was extracted and stored at -20°C for further use in experiment. Powder of roots of *Withania somnifera* was obtained from Ayurvedic pharmacy. The powder was used to prepare aqua-alcoholic extract using double distilled water and methanol (40:60) and then filtered through a Whatman filter paper No.1. The filtrate was reduced to dryness in rotary evaporator under reduced pressure (Singh *et al.*, 2009). Extract obtained was taken in petri plate and then kept in desiccator for drying. The extract was then stored at -20°C till further use.

Experimental Design

In the study, 40 three-weeks old broiler chickens were randomly and equally divided in five groups of eight birds each (C1, C2, T1, T2 and T3). Birds of group C1 were kept as control. Birds of group C2, T1, T2 and T3 were given feed mixed with lead acetate @ 500 ppm for 21 days of experiment. Birds of group T1 and T2 were further administered orally with *Opuntia elatior* fruit juice at dose rate of 3 ml/kg body weight, and aqua-alcoholic extract of *Withania somnifera* @ 100 mg/kg body weight, respectively, while birds of group T3 received both *Opuntia elatior* fruit juice and aqua-alcoholic extract of *Withania somnifera* at the above dose rates.

Sample Collection

After 21 days of experiment, 3 ml of blood sample from each bird was collected from jugular/wing vein in vial containing K₃-EDTA. Blood smears for determination of differential leukocyte count (DLC) were prepared from fresh blood at the time of blood collection. Plasma was harvested by centrifugation at 5000 rpm for 10 minutes at 4°C and stored at -20°C for biochemical analysis which was carried out within two days. Blood glucose level was estimated immediately after collection of samples. All birds were then humanely sacrificed to observe gross pathological changes and to collect the tissue of major organs like liver, kidney, spleen, heart, lungs and small and large intestine in 10% formalin for histopathological evaluation.

Haemato-Biochemical Estimations

Haemoglobin (Hb) estimation was carried out by Sahli's haemoglobinometer, while packed cell volume (PCV) was estimated by micro-haematocrit method (Coles, 1986). Total leukocytes count (TLC), erythrocytes count (TEC) and differential leukocytes counts (DLC) were made as per the method described by Jain (1986).

Biochemical parameters like alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), creatinine, glucose, total protein (TP), albumin, globulin, total bilirubin, uric acid, alkaline phosphatase (ALP), acid phosphatase (ACP) and lactate dehydrogenase (LDH) were estimated by using assay kits (Merck Specialties Pvt. Ltd., GOA, India and Biosystems SA, Barcelona) on semi-automatic biochemistry analyzer (Microlab-300, ELITech Group, France).

Data were analyzed statistically by one way ANOVA and different treatment means were compared by Duncan's multiple range tests.

RESULTS AND DISCUSSION

No signs of toxicity were observed in birds of any group up to first 10 days of study period. Thereafter the clinical symptoms like mild depression, reduced feed intake and dullness were observed in birds receiving only lead acetate (500 ppm in feed). Ibitoye *et al.* (2011) also did not observe symptoms of lead toxicity in broiler birds at dose level of 200 ppm in feed for seven weeks.

Mean values of haematological and biochemical parameters are presented in Table 1 and 2, respectively.

Table 1: Effect of daily oral administration of *Opuntia elatior* juice (3 ml/kg/day, T1) and *Withania somnifera* extract (100 mg/kg/day, T2) and its combination (T3) on haematological parameters in broiler chickens treated with lead acetate (500 ppm in feed) for 21 days

Parameters	Treatment groups				
	C1	C2	T1	T2	T3
Hb (g/dL)	12.81±0.27 ^b	9.73±0.25 ^a	13.00±0.51 ^b	12.51±1.01 ^b	12.18±0.033 ^b
RBC(10 ⁶ /μl)	2.82±0.07	2.82±0.14	2.73±0.19	2.80±0.21	2.76±0.05
WBC(10 ³ /cmm)	239.04±0.61	240.36±0.45	235.09±4.71	231.45±8.86	234.30±3.72
PCV(%)	36.98±0.98	36.16±1.54	35.66±2.39	35.89±2.73	35.40±0.55
MCV (fl)	131.04±0.89	128.32±1.14	131.14±1.31	130.08±1.24	128.64±2.23
MCHC (%)	34.64±0.45 ^b	27.07±0.83 ^a	37.22±1.80 ^b	34.76±0.25 ^b	34.76±1.14 ^b
Lymphocyte (%)	51.13±0.95	51.63±0.38	52.13±0.55	51.75±0.45	52.25±0.82
Heterophils (%)	41.63±0.98	40.38±0.26	39.88±0.55	39.88±0.30	40.00±0.71
Eosinophil (%)	2.88±0.35	3.13±0.35	3.13±0.30	3.38±0.18	3.13±0.40
Monocytes (%)	3.00±0.27	3.38±0.18	3.50±0.19	3.63±0.18	3.13±0.30
Basophils (%)	1.38±0.18	2.00±0.19	1.38±0.18	1.38±0.18	1.50±0.19

Means bearing uncommon superscripts within the row differ significantly ($P < 0.05$) between groups.

The mean value of Hb and MCHC in birds treated with lead acetate only (C2) were found significantly ($P < 0.05$) lower as compared to that of control group (C1). Whereas, the mean values of Hb (g/dl) and MCHC in other treatment groups (T1, T2, T3) were not significantly different from that of control group. No significant alterations in PCV, TEC, TLC and MCV levels of birds of any treatment group were observed compared to that of control birds. Hanan and Riham (2012) reported significant decrease in RBCs count, Hb concentration and PCV in lead treated Albino rats. Lead acetate is known to inhibit the heme synthesising enzymes as aminolevulinic acid (ALA), dehydratase enzyme and conversion of coproporphyrinogen III to protoporphyrin IX, and inhibit heme synthesis leading to reduced haemoglobin production and shortened life span of erythrocytes (Klassen, 2001). The reduced level of MCHC (%) found in birds exposed to lead acetate only in the present study is in agreement with reports of Helmy *et al.* (2000) and Suradkar *et al.* (2009).

Alimi *et al.* (2013) revealed no significant changes in haematological parameters when animals were given both *Opuntia ficus indica* juice and ethanol, demonstrating a defensive effect of the cactus fruit juice. In the present study, both *Opuntia elatior* juice and *Withania somnifera* alone and in combination significantly mitigated haematological turbulences in lead treated birds. Protective effect of *Opuntia* spp. might be due to presence of polyphenols, flavonoids, ascorbic acid, carotenoids, and betalains. Such antioxidant compounds might confer to preventive effect of *Opuntia* spp. on blood cells from lead acetate induced damage. The non-significant difference noted in Hb in *Withania somnifera* along with lead acetate treated group may be due to the stem cell proliferation stimulus activity of *Withania somnifera*. Further, no additive or synergistic protective effects of combination of *Opuntia elatior* juice and *Withania somnifera* was observed against lead acetate induced

alterations in haematological parameters.

Table 2: Effect of daily oral administration of *Opuntia elatior* juice (3 ml/kg/day, T1) and *Withania somnifera* extract (100 mg/kg/day, T2) and its combination (T3) on biochemical parameters in broiler chickens treated with lead acetate (500 ppm in feed) for 21 days

Parameters	Treatment groups				
	C1	C2	T1	T2	T3
ALT (IU/L)	16.25±0.70 ^a	20.90±1.1 ^b	16.50±0.98 ^a	16.63±0.94 ^a	16.75±0.96 ^a
AST (IU/L)	156.75±5.44	178.00±17.67	157.25±5.00	157.38±4.82	154.25±2.85
BUN (mg/dL)	5.74±0.50 ^a	7.41±0.96 ^c	5.66±0.465 ^a	6.05±0.60 ^b	6.14±0.51 ^b
Creatinine (mg/dL)	0.14±0.01 ^a	0.63±0.05 ^b	0.16±0.02 ^a	0.15±0.02 ^a	0.12±0.02 ^a
Glucose (mg/dL)	156.10±6.40	151.30±3.10	165.80±3.00	161.40±5.40	167.10±4.60
TP (g/dL)	10.82±0.23	10.47±0.29	10.10±0.37	9.83±0.49	10.33±0.35
Albumin (g/dL)	2.48±0.15	2.75±0.14	2.61±0.06	2.75±0.06	2.79±0.04
Globulin (g/dL)	8.34±0.25	7.72±0.28	7.48±0.33	7.08±0.49	7.54±0.35
Bilirubin (mg/dL)	8.86±0.51 ^a	10.62±0.24 ^b	8.99±0.29 ^a	8.75±0.27 ^a	8.54±0.25 ^a
Uric acid (mg/dL)	5.82±0.28	8.30±0.69	5.25±0.88	5.19±1.01	6.84±1.47
ALP (IU/L)	828.25±78.62	719.13±68.66	657.00±72.67	763.63±74.03	729.75±77.96
ACP (IU/L)	2.80±0.10	3.20±0.20	2.60±0.20	2.80±0.20	2.70±0.20
LDH (IU/L)	1045.13±18.55	919.13±31.63	932.00±28.32	892.25±43.01	928.13±18.56

Means bearing uncommon superscripts within the row differ significantly ($P < 0.05$) between groups.

The mean values of ALT, BUN, creatinine and total bilirubin were significantly ($P < 0.05$) higher in birds treated with lead acetate only compared to that of control group (Table 2). Whereas mean values of AST, total protein, albumin, globulin, ACP, ALP and LDH did not change significantly between groups. Significant ($P < 0.05$) increase in the value of ALT in individual lead acetate treated birds might be due to increased cell membrane permeability or cell membrane damage of hepatocytes caused by lead acetate toxicity. In accordance to our findings, dose dependent significant increase in level of ALT due to lead acetate in broiler chickens was also reported by Suleman *et al.* (2011). Increased level of ALT due to lead acetate was returned to normal range when birds were treated with fruit juice of *Opuntia elatior* and *Withania somnifera*. This finding was in close agreement with previous reports (Thangavel and Perumal, 2010; Alimi *et al.*, 2013; Shimmi *et al.*, 2011).

Hossain *et al.* (2014) reported significant increase in the levels of BUN and creatinine in lead acetate treated broiler chickens at dose rate of 100 ppm. In the present study, increased levels of BUN and creatinine due to lead acetate were attenuated with fruit juice of *Opuntia elatior* and *Withania somnifera*. Similar finding was observed in rats treated with ethanol along with *Opuntia elatior* fruit juice (Alimi *et al.*, 2013). This effect might be due to the antioxidative properties of *Withania somnifera*, which protect the outer membrane of mammalian cells. Thangavel and Perumal (2010) also found that *Withania somnifera* when given at dose of 500 mg/kg reduced level of BUN and creatinine that were elevated by gentamicin in rats. Nephroprotective effect of *Withania somnifera* might

be due to the significant inhibition of lipid peroxidation and generation of free radicals.

Upon gross examination, liver of experimental birds treated with lead acetate only (group C2) shown paleness, enlargement and fatty changes. Whereas, macroscopic view of kidneys shown haemorrhage and atrophy while liver showed varying degree of degenerative changes as well as vascular changes in group C2. Intestine of birds from group C2 showed denaturation and necrosis of tips of intestinal villi. No appreciable histopathological lesions have been observed in the spleen and heart of birds in all treatment groups.

Opuntia elatior and *Withania somnifera* have been found with ameliorating effect on haematological and biochemical alterations caused by low level exposure of lead acetate in chickens. However, controlled study to validate the protective effect of both plants in toxicity after chronic exposure is needed.

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