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Amelioration of Ochratoxicosis in Broilers with *Achyranthes aspera*

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A B S T R A C T

Ochratoxinis the mycotoxin that targets mainly kidney and liver. In this experiment, broilers were fed ochratoxin@ 100 ppb and 200 ppb for 30 days daily, it led to depression in haematocrit values and delayed clotting whereas significant increase in serum uric acid, creatinine and serum triglyceride. There was also decrease in serum level of total protein and cholesterol. Immunological status of toxicated birds was diminished as observed through Haemagglutination Inhibition titer (against New Castle disease virus) and skin reactivity test against DNCB (2,4, Dintrochlorobenzene). Addition of 20% aqueous extract of *Achyranthes aspera* @1% of feed into the broiler feed containing ochratoxin showed improved haematological, biochemical, immunological values as well as gross and histopathological changes were comparable to healthy birds. Thus, the experiment manifested ameliorative effect of *Achyranthes aspera* against ochratoxin.

Introduction

Ochratoxin is the most common and dangerous mycotoxin found in poultry feed. The toxin is named after the earliest known fungus to produce it, *Aspergillus ochraceu*s. Other fungi which produce ochratoxin are *Penicillum viridicatum*, *A. westerdijkiae*, and *A. steynii*. Ochratoxin A is the most potent, stable and common oneamongst 4(A, B, C, D) types of ochratoxin. Considering the present trend of using herbomedication in commercial poultry industry, present experimentation was conducted by using *Achyranthes aspera* against induced ochratoxicosis in birds. *Achyranthes aspera* Linn. (*Amaranthaceae*) is commonly found as a weed on way-side and at waste places throughout India. The parts of shoot, stem, root, inflorescence, seed etc. has been variedly used as antimicrobial, anti-inflammatory, immunomodulatory, anti-hyperlipidemic, diuretic, anti-diabetic, anti-fertility, analgesic, antipyretic and anticarcinogenic property. As such, it has multi-use in many ailments in traditional system of medicine.

Nephroprotective and diuretic activity of *Achyranthes aspera* has been proved against many nephrotoxic chemicals such as lead acetate (Jayakumar *et al.*, 2009), ethylene glycol (Aggarwal *et al.*, 2011), furosemide

(Muhammad *et al.*, 2014). Moreover, its anti-oxidant, immunomodulatory, anti-hyperlipidemic and hepatoprotective properties was explored in the present study.

Materials and Methods

Production of ochratoxin

Culture of *Aspergillus ochraceus* obtained from Nagpur Veterinary College, Nagpur was subcultured in Czapek yeast agar for 8 days in 25-30°C. A solution of a loopful of this culture in 5 mL of sterile distilled water was added to flasks containing clean and autoclaved half-ground rice, maize and groundnut (2:1:2 ratio). Such tightly closed flasks containing cereals were incubated at 25-30 ^{0}C for 21 days and shaken twice daily for breaking the formed mycelial mass. Few drops of distilled water was added every 24 hrs for first 3-4 days.

Later, on turning of white/sulphur colour to dark black color, the cereals containing fungus was autoclaved, dried at 50^{0}Cin hot air oven and ground to powder. Two hundred and fifty grams of this substrate sample was sent to for quantification to Animal Feed Analytical and Quality Control Laboratory, Veterinary College and Research Institute, Namakkal (Tamil Nadu).

Collection and preparation of plant material

The plant *Achyranthes aspera* was identified and collected locally. The whole plant was shade dried and reduced to fine powder using electric grinder. Thereafter 200mg of this powder was dissolved in 1000mL of distilled water and kept in refrigeration. The plant extract was then filtered and evaporated at room temperature to obtain 20% aqueous extract of *Achyranthes aspera* which was incorporated in poultry feed in powdered form.

Experimental design

Ninety six birds divided into six experimental groups, each comprising of sixteen birds were reared for a period of 30 days. Group I was treated as control in which birds were fed with normal feed and drinking water *ad.lib.* Ochratoxin was fed to birds of Group II @ 100 ppb and to birds of Group III @ 200 ppb through feed. Birds of Group IV were given powder of 20% aq. extract of *Achyranthes aspera* @ 1% of feed. The birds of Group V and VI were given Ochratoxin @ 100 ppb and 200 ppb respectively along with powder of 20% aq. extract of *Achyranthes aspera* @ 1% of feed.

Collection of samples

Blood collection was done from jugular vein on 15th and 30th day of experimental trial for haematology, biochemical and immunological studies. Examination of skin reaction after applying 2,4 Dinitrochlorobenzene (DNCB) cutaneously was done thrice at 24 hrs. interval and then measured with vernier caliper for thickness. The necropsy of representative birds of each group was performed to examine gross changes, and internal organs viz. liver, kidneys, spleen and bursa of Fabricius were collected and weighed to evaluate variation in absolute organ weight of each at both the intervals of experiment i.e. 15th and 30th day of experiment.

Statistical analysis

The data recorded during the study was statistically analyzed by CRD as per Snedecor *et al.*, (1994).

Results and Discussion

Ochratoxin had deleterious effect on haematopoetic system as evident by the results of the study (Table 1). There was

numerical reduction in mean values of Haemoglobin, PCV and TEC of ochratoxicated birds of groups II and III when compared to the values of control group. However, these values of birds of group V and VI showed mild improvement against the values of group II and III though lower than the values of birds of group I (healthy control). A minor decrease was observed in mean TLC of ochratoxicated birds in comparison to values of control and other treatment groups. However, the difference between the values of ochratoxicated birds (groups II and III) and birds of healthy control group was found to be significant($P < 0.05$)on 30th day of experiment. The TLC of birds of groups IV, V and VI were found at par with the birds of group I throughout the experiment. Mean blood clotting time in birds of groups IV, V and VI were comparable with the values of healthy birds whereas birds of groups II and III showed non-significant increase in mean values of blood clotting time.

Table.1 Haematobiochemical changes in birds of all the experimental groups at both intervals of study

15th Day						30th Day					
G I	**GII**	**GIII**	**GIV**	**GV**	**GVI**	**G I**	**GII**	**GIII**	**GIV**	**GV**	**GVI**
Haematological parameters:-											
Haemoglobin (g/ dL)											
9.23^{a} ± 0.09	8.80^{a} ± 0.22	8.06^{a} ± 0.19	9.10^{a} ± 0.22	8.93^{a} ± 0.28	8.30^{a} ± 1.31	10.85^{a}± 0.61	10.03^{a}± 0.54	9.26^{a}± 0.57	10.85^{a}± 0.70	10.54^{a}± 0.50	10.12^{a}± 0.64
Packed Cell Volume(%)											
35.00^{a} ± 1.39	31.83^{a} ± 0.98	31.00^{a} ±1.04	35.00^{a} ±1.51	33.67^{a} ± 0.62	32.17^{a} ±1.22	35.50^{a} ± 1.93	33.30^{a} ± 0.96	33.00^{a} ± 1.16	36.80^{a} ± 0.91	35.00^{a} ± 1.51	35.80^{a} ± 0.74
TEC(10^{6}/cu.mm.)											
2.96^{a} ± 0.19	2.43^{a} ± 0.18	2.23^{a} ± 0.21	2.89^{a} ± 0.21	2.62^{a} ± 0.13	2.54^{a} ± 0.15	3.71^{a} ± 0.21	2.61^{a} ± 0.47	2.44^{a} ± 0.29	3.11^{a} ± 0.21	2.70^{a} ± 0.44	2.54^{a} ± 0.41
TLC(10^{3}/cu.mm.)											
20.22^{a} ± 0.37	19.50^{a} ± 0.18	19.00^{a} ± 0.14	20.13^{a} ± 0.33	19.70^{a} ± 0. 48	19.94^{a} ± 0.68	20.98^{a} ± 0.37	18.21^{b} ± 0.95	17.98^{b} ± 0.33	20.00^{ab} ± 0.33	19.64^{ab} ± 0.50	19.82^{ab} ± 0.36
Blood clotting time(sec)											
128.33^{a} ± 1.90	130.17^{a} ± 1.42	131.16^{a} ± 0.95	128.33^{a} ± 0.89	126.83^{a} ± 0.65	127.5^{a} ± 0.93	131.33^{a} ± 1.02	132.0^{a} ± 0.81	132.83^{a} ± 0.95	131.16^{a} ± 0.47	130.67^{a} ± 0.49	130.33^{a} ± 0.56
Biochemical parameters:-											
Serum uric acid (mg/dL)											
5.05^{b} ± 0.26	6.19^{a} ± 0.15	6.43^{a} ± 0.19	4.95^{b} ± 0.53	4.99^{b} ± 0.36	4.91^{b} ± 0.12	5.95^{c}± 0.32	7.27^{ab} ± 0.24	8.14^{a} ± 0.16	5.91^{c} ± 0.54	5.98^{c} ± 0.43	6.15^{bc} ± 0.54
Serum triglyceride(mg/dL)											
100.46^{a}± 4.02	105.6^{a}± 4.23	119.97^{a}± 2.39	101.11^{a} ± 4.98	106.2^{a} ± 5.33	103.63^{a} ± 7.94	98.99^{b}± 2.72	119.96^{a}± 6.90	123.3^{a} ± 6.23	102.16^{b}± 4.11	108.9^{ab}± 5.88	114.13^{ab}± 4.90
Serum cholesterol (mg/dL)											
148.98^{a}± 22.70	111.88^{a}± 29.53	99.93^{a}± 10.80	134.45^{a} ± 28.60	120.87^{a} ± 9.61	120.06^{a} ± 23.12	147.38^{a}± 21.22	118.33^{a}± 33.62	100.62^{a}±2 1.34	132.79^{a}± 13.69	123.2^{a}± 15.86	119.78^{a}± 17.90
Serum total protein(g/L)											
3.62^{a} ± 0.13	2.76^{a} ± 0.22	2.59^{a} ± 0.11	3.23^{a} ± 0.23	2.86^{a} ± 0.34	2.75^{a} ± 0.42	4.24^{a} ± 0.11	3.32^{a} ± 0.52	3.13^{a} ± 0.62	4.01^{a} ± 0.30	3.46^{a} ± 0.47	3.41^{a} ± 0.43

Mean with similar superscripts in column do not differ significantly in a row($P < 0.05$)

Table.2 Mean values of HI titer against ND virus in birds of all the experimental groups at both intervals of study

15th Day						30th Day					
G I	**GII**	**GIII**	**GIV**	**GV**	**GVI**	**G I**	**GII**	**GIII**	**GIV**	**GV**	**GVI**
9.23 [a] ± 0.09	8.80 [a] ± 0.22	8.06 [a] ± 0.19	9.10 [a] ± 0.22	8.93 [a] ± 0.28	8.30 [a] ± 1.31	10.85 [a]± 0.61	10.03 [a]± 0.54	9.26 [a]± 0.57	10.85 [a]± 0.70	10.54 [a]± 0.50	10.12 [a]± 0.64

Mean with similar superscripts in column do not differ significantly in a row($P < 0.05$)

Table.3 Mean thickness of skin observed after application of DNCB cutaneously in birds of all the experimental groups

Group	Duration				Mean thickness
	0hr	**24hrs**	**36hrs**	**72hrs**	
I	0.67[a] ±0.04	2.92 [a] ±0.33	1.87 [a] ±0.06	1.58 [a] ±0.37	**1.76[a] ± 0.24**
II	0.84[a] ±0.72	2.22 [a] ± 0.10	1.72 [a] ± 0.55	1.68 [a] ±0.42	**1.62[a] ± 0.20**
III	0.54[a] ±0.16	1.31[a]± 0.47	1.89 [a] ±0.14	1.46 [a] ±0.18	**1.50[a] ± 0.16**
IV	0.75[a] ± 0.09	2.82[a] ± 0.18	1.94 [a] ± 0.44	1.68 [a] ±0.12	**1.79[a] ± 0.22**
V	0.77[a] ± 0.02	2. 81[a] ± 0.45	1.74 ± 0.31	1.51 [a] ±0.33	**1.70[a] ± 0.23**
VI	**0.62[a] ± 0.07**	**3.21[a] ± 0.22**	**1.90[a] ± 0.31**	**1.35 [a] ±0.14**	**1.77[a] ± 0.26**

Mean with similar superscripts in column do not differ significantly in a column($P < 0.05$)

Table.4 Mean absolute weights of kidneys, liver and bursa of Fabricius of birds of different experimental groups at different intervals of time

Parameters	15th Day						21st Day					
	G I	**GII**	**GIII**	**GIV**	**GV**	**GVI**	**G I**	**GII**	**GIII**	**GIV**	**GV**	**GVI**
Kidney	9.23 [a] ± 0.09	8.80 [a] ± 0.22	8.06 [a] ± 0.19	9.10 [a] ± 0.22	8.93 [a] ± 0.28	8.30 [a] ± 1.31	10.85 [a]± 0.61	10.03 [a]± 0.54	9.26 [a]± 0.57	10.85 [a]± 0.70	10.54 [a]± 0.50	10.12 [a]± 0.64
Liver	12.24[a] ± 0.33	13.51 [a] ± 0.36	14.21[a] ± 0.77	12.25 [a] ± 0.31	12.78 [a] ± 0.31	13.21 [a] ± 0.63	27.56 [a] ± 0.69	28.87 [a] ± 0.94	30.93 [a] ± 0.85	27.58 [a] ± 0.76	27.74 [a] ± 0.81	27.98 [a] ± 0.92
Bursa of Fabricius	5.51 [a] ± 0.25	5.09 [a] ± 0.41	4.89 [a] ± 0.38	5.54 [a] ± 0.22	5.31 [a] ± 0.39	5.26 ± 0.23	3.26[a] ± 0.19	2.59[bc] ± 0.19	2.15[c] ± 0.14	3.17[a] ± 0.08	2.83[ab] ± 0.17	3.07[a] ± 0.13

Mean with similar superscripts in column do not differ significantly in a column($P < 0.05$)

Presumably production of ROS (Reactive oxygen species) under influence of ochratoxin leading to RBC destruction and also its function is the cause behind the reduced mean levels of Hb, PCV and TEC as stated by Mohiuddin *et al.,* (1992) and Rama Devi *et al.,* (1998). The marginal improvement in haemoglobin level of ochratoxicated birds treated with *Achyranthes aspera* could be attributed to antioxidant potential of *A. aspera* in preventing oxidative damage. This theory is supported by the works of Edwin *et al.,* (2008), Gayathri *et al.,* (2009) and Malarvili *et al.,* (2009). Lea and Fredick (1989) reported ochratoxin has lymphotoxic activity through their experiment. The immunostimulatory effect of plant against the lymphotoxic activity of ochratoxin has resulted in elevated levels of mean TLC in treatment groups against the leucopenia observed in ochratoxicated birds. Impairment of blood coagulation system due to

hypofibrinogemia by ochratoxinas proven by the studies of Doerr *et al.,* (1982) might have been overcome by stimulatory and protective effect of plant on liver and haematopoetic system.

The serum samples of the experimental birds of group II when subjected for biochemical investigation showed significant rise in levels of serum uric acid only in birds of group II and III at 15th day and 30th day of experiment when compared to values of birds of group I. There was non-significant decrease in mean serum Total Protein values on 15th and 30th day of experiment.

The mean values of Serum total protein (STP) of group V and VI were numerically higher when compared to mean values of ochratoxicated birds of group II and III respectively but were lower than values of birds of group I and IV. Meanwhile, values of mean serum triglyceride in birds of group II and group III were numerically increased at 15th day, whereas, these values at 30th day of study increased significantly when compared with the values of birds of control group. The values of mean serum tryglyceride of birds fed with plant extract were lower than the groups of ochratoxicated birds. At both the studied interval of experiment, birds of group V and VI showed marginally higher mean serum cholesterol values than that of ochratoxicated groups of birds, however lower than the values of birds of groups I and IV.

The primary targetorgan of ochratoxin being kidney, the compensation of its functionality has led to increase in serum concentration of uric acid (Elaroussi *et al.*, 2008). However, the studies conducted by Jayakumar *et al.*, (2009), and Muhammad *et al.,* (2014) has proven the diurectic effect of *A. aspera* which might have resulted in reduction in nephrotoxicity caused due to ochratoxin. The present findings of increase in serum triglyceride, lowered serum cholesterol and total protein are in close agreement with findings of Huff *et al.,* (1988), Kalorey *et al.,* (2005) and Sakhare *et al.,* (2007). Hepatoprotective effect of *A. aspera* as observed by Bafna and Mishra (2004) may have resulted in protein level nearer to normal.

Results of assessment of HI titer against New-Castle disease virus revealed non-significant depression in humoral immunity in ochratoxicated birds whichwas improved in experimental groups which were fed plant extract (Table 2). To assess the effect of ochratoxin on cell mediated immunity, DNCB was applied cutaneously on the area under the wing of experimental birds of all groups. The skin thickness of the area was increased with moderate inflammatory reaction only in birds of treatment and control groups. The thickness of affected skin in ochratoxicated birds was not remarkably changed as depicted in Table 3.

Apart from leucotoxic activity (Lea and Fredick, 1989) and hypoproteinemia, especially hypoglobulinemia (Sakhare *et al.*, 2007) ochratoxin leads to improper antigen processing due to impaired phagocytosis (Singh *et al.*, 1990). Similar inference was reflected from the assessment of skin thickness after challenge of DNCB cutaneously. Ochratoxin weakens cell mediated immunity through cytotoxic and inhibitory action on macrophages and neutrophilsis also observed by Politis *et al.,* (2005). Instead, the active principles of *A. aspera* was found to be immunomodulatory and stimulatory to immunoglobulins as well as splenic and thymus function as derived from observations of Vasudeva *et al.,* (2002) and Gupta *et al.,* (2010).

The mean values of absolute kidney weight of group II and III showed non- significant rise than that of mean absolute kidney weight of

group I (healthy control group) at 15th day but the rise was significant in group III at 30th day. Absolute weights of kidneys in birds of all other experimental groups were comparable with each other.

However, mean absolute weight of liver showed non-significant increase among the birds of ochratoxicated groups in comparison to the liver of healthy birds and birds treated with plant extract. At 15th day, the values of mean absolute weight of bursa of Fabricius were non-significantly reduced in case of group II and III when compared to that of group I. But at 30th day, this reduction in absolute weight of bursa of Fabricius in these groups was significant. The values of group IV, V and VI remained comparable with the value of healthy control at both the intervals of study.

The increase in absolute weight of liver and kidney and bursa of Fabricius of ochratoxicated birds was also observed by Huff *et al.,* (1988), Kalorey *et al.,* (2005) and Elaroussi *et al.,* (2008). However, the aqueous extract of plant has been proven to have hepatoprotective (Bafna and Mishra, 2004) and nephroprotective (Muhammad *et al.*, 2014) ingredients which might have led to comparable weights of liver and kidney of treated birds with that of normal group. The immunostimulatory effect of the plant has compensated the lymphotoxic activity of ochratoxin in plant extract fed groups of birds.

The results of the present study lead to the conclusion that the 20% aqueous extract of *A. aspera* at inclusion rate of 1% of feed showed ameliorative effect against 100ppb and 200ppb of ochratoxin over a period of 30 days.

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References

Aggarwal A., S.K. Singla, M. Gandhi and C. Tandon (2011) Preventive and curative effects of *Acharynthes aspera Linn.* extract on experimentally induced nephrolithiasis. Indian Journal of Experimental Biology 50:201-208.

Bafna A.R. and S.H. Mishra (2004) Ars Pharmaceutica 45(4): 343-351.

Doerr J.A., M.L. Campbell Jr. and W.E. Huff (1982) Interaction between dietary citrinin and ochratoxinA in broiler chickens. Poult. Sci. 61(7): 1453.

Edwin S., E. Jarald, D.L. Edwin, A. Jain, H. Kinger, K.R. Dutt and A.A. Raj (2008) Pharmaceutical Biology 46(12): 824-828.

Elaroussi M.A., F.R. Mohamed, M.S. Elgendy, E.M. El Barkouky, A.M. Abdou and M.H. Hatab (2008) Ochratoxicosis in broiler chickens: Functional and histological changes in target organs. International Journal of Poultry Science 7(5): 414-422.

Gayathri D.S, A. Archanah, P. Abiramasundari, V. Priya, K. Uma, T. Abirami (2009) Indian journal of nutrition and dietetics 46(12):485-490.

Gupta V., C.M. Krishna, P. Bansal, S. Kumar, G.P. Prasad, D.V. Ravi Krishna (2010) Phytochemistry and pharmacological potential of *Achyranthes aspera*- A review. International Journal of Ayurvedic Medicine 1(1):1-11.

Huff W.E., L.F. Kubena and R.B. Harvey (1988) Progression of ochratoxicosis in broiler chickens. Poultry Science 67: 1139 -1146.

Jayakumar T., M.P. Sridhar, T.R. Bharathprasad, M. Ilayaraja, S.

Govindaswamy and M.P. Balasubramanian (2009) Experimental studies of *Achyranthes aspera* (L.) preventing nephrotoxicity induced by Lead in Albino rats. Journal of Health Science 55 (5): 701-708.

Kalorey, D.R., N.V. Kurkure, J.S. Ramgaonkar, P.S. Sakhare, S. Warke and N.K. Nigot (2005) Effect of polyherbal feed supplement "Growell" during aflatoxicosis, ochratoxicosis and combined mycotoxicosis in broilers. Asian-Aust.J.Anim.Sci.18 (3):375-383.

Lea T. S. and C. S. Fredick (1989): Mechanism of ochratoxin induced immunosuppression. Mycopathologia. 107: 153-159.

Malarvili, T. and N. Gomathi (2009) Biosciences Biotechnology Research Asia, 6(2): 659-664.

Mohiuddin S.M., M.V. Reddy and S.R. Ahmed (1992) Studies on ochratoxicosis in broiler chicks. Indian Vet. J. 69:1011-1014.

Muhammad A., Q. Jabeen, M. Atif, A.M.S.A Majid and M. Qamar-Uz-Zaman(2014) Diuretic activity of *Achyranthes aspera* Linn crude aqueous extract in Albino rats. Tropical Journal of Pharmaceutical Research 13(12): 2039-2045.

Politis I., K. Fegeros, S. Nitsch, G. Schatzmayr and D. Kantas (2005) Use of *Trichosporon mycotoxinivorans* to suppress the effects of ochratoxicosis on the immune system of broiler chicks. British Poultry Science46: 58-65.

Rama Devi V., N.R. Gopal Naidu and P.K. Shreeraman (1998) Pathology of ochratoxicosis in broilers.Indian J. Vet. Path. 22(2):93-95.

Sakhare, P. S., S. D. Harne, D. R. Kalorey, S. R. Warke, A. G. Bhandarkar and N. V. Kurkure (2007) Effect of Toxiroakpolyherbal feed supplement during induced aflatoxicosis, ochratoxicosis and combined mycotoxicoses in broilers. Vet. arhiv 77(2): 129-146.

Singh G.S.P., H.V.S. Chauhan, G.J. Jha and K.K. Singh (1990) Immunosuppression due to chronic ochratoxicosis in broiler chicks. J. Comp. Path. 103:399-410.

Snedecor, G.M. and W.C. Cochran (1994) Statistical Methods,6thedn., Oxford and IBN Publishing Co., Kolkata,16.

Vasudeva R. Y., G. R. Duddukuri, B. G. Sunil and R. R. Athota (2002) Immunomodulatory activity of *Achyranthes aspera* on the elicitation of Antigen-Specific Murine Antibody response. Pharm. Biol. 40(3): 175-178.

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