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RESEARCH ARTICLE

HAEMATO-BIOCHEMICAL AND ANTIOXIDANT EFFECTS OF TRIPHALA (*EMBLICA OFFICINALIS*, *TERMINALIA BELLIRICA* AND *TERMINALIA CHEBULA*) ON JATROPHA (*JATROPHA CURCAS*) DEOILED SEED CAKE INDUCED TOXICITY IN BROILER CHICKEN

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ABSTRACT

A study was undertakento evaluate the haemato-biochemical and antioxidant effects of triphala on *J.curcas* deoiled seed cake induced changes in chicken. Two weeks old, seventy two, vencob broiler chicks maintained on standard broiler diet (BIS, 1992) were randomly divided into four groups of eighteen birds each. Group I was maintained on standard broiler diet (BIS, 1992), group II was given standard diet supplemented with five per cent raw jatropha deoiled seed cake (JSC). Group III was given five per cent untreated JSC as well as four per cent triphala and group IV was given five per cent untreated JSC for first two weeks, four per cent triphala for last four weeks. The experimental birds were maintained on the respective dietary regimes for a period of 42 days. Haemato-biochemical parameters and liver antioxidant levels were estimated at day 28 and at the end of the experiment. Parameters such as haemoglobin (Hb), volume of packed red cells (VPRC), total leucocyte count (TLC), total erythrocyte count (TEC), serum protein, albumin and superoxide dismutase levels (SOD) showed a significant reduction in raw JSC fed group II. Triphala supplemented groups III and IV showed significant reduction in these values, but were lower in magnitude when compared with group II indicating ameliorating effect. A significant increase in aspartate transaminase (AST), creatinine and tissue lipid peroxidation (LPO) was observed in group II. Group III, and IV showed significant increase in these parameters than control birds, but were lower when compared with those of group II. These were due to protective action of triphala on group III and IV, among the groups III and IV, group IV showed highest protection in jatropha toxicity.

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INTRODUCTION

The feed ingredients especially protein sources used in poultry feeds have become too expensive because of their high demand leading to the evaluation of lesser known and underutilized crop seeds for their nutritive values. In this regard, *J. curcas* deoiled seed cake, which is a by-product of bio-fuel industry, rich in crude protein has been proposed as a cheap feed supplement for livestock (Anil kumar *et al*., 2010; Pasaribu *et al*., 2010). But when it is used as such in the poultry feed it produces toxicity because of the presence of antinutritional factors such as phytate, trypsin inhibitors, phenol, tannin, saponin, curcin and phorbol esters (Aderibigbe *et al.,* 1997; Makkar *et al.,* 199; Francis *et al*., 2005). The seeds from *J.curcas* have been reported to be orally toxic to humans, rodents, chickens, ruminants and fish (Wakandigara *et al*., 2013). Triphala is one of the most popular Ayurvedic herbal rasayana formula consisting of equal parts of three myrobalans, taken without seed namely *Emblica officinalis*, *Terminalia bellirica*, and *Terminalia chebula*. It contains several compounds that have been proposed to be responsible for its acclaimed health benefits, including polyphenols, gallic acid, chebulagic acid, and chebulinic acid (Kaur *et al*., 2005; Naik *et al*., 2005 and Nampoothiri *et al*., 2011). It could improve health, immunity and longevity by

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inhibiting lipid peroxidation and scavenging free radicals. So if it proved to be effective in phorbol ester induced toxicity, it could be supplemented with jatropha seed cake for reducing its toxic effect and can also be used to ameliorate the harmful effects of similar toxicants.

MATERIALS AND METHODS

Seventy-two, Vencobb broiler chicks at the age of two weeks maintained on standard broiler diet (BIS,1992) were randomly divided into four groups of eighteen each. The experimental design and feeding schedule were as follows. Group I - Healthy control birds maintained on standard broiler diet (BIS,1992), Group II - Birds maintained on control diet mixed with 5% raw jatropha deoiled seed cake (JSC), Group III - Birds on control diet supplemented with 5% raw JSC and 4% triphala and Group IV - Birds on control diet supplemented with 5% raw JSC for the first two weeks of experiment followed by control diet supplemented with 4% triphala from the beginning of the third week up to the end of the experiment. Experiments were conducted with the approval of the Animal Ethics Committee, Committee for the Purpose of Control & Supervision of Experiments on Animals (CPCSEA) guidelines. The experimental birds were maintained on the respective dietary regimes for a period of 42 days. All the birds were monitored regularly for clinical symptoms and mortality. Blood samples were collected on 28th day and 42nd day of experiment for the analysis of haemato-biochemical parameters. Two milliliter of blood was collected from each using di-pottassium salt of ethylene diamine tetra acetic acid (EDTA) at the rate of 1 mg/ml of blood as anticoagulant. Hemoglobin concentration was estimated using commercially available kits (AGAPPE Diagnostics).

VPRC was estimated as per standard procedures by Jain (1986). Total red blood cell count and total leukocyte count were determined as per the method described by Sastry (1976). About five millilitre of the blood was collected separately in a sterile test tube without adding anticoagulant for serum separation. Total protein, albumin, creatinine, AST were estimated using commercially available kits (AGAPPE Diagnostics) and the final readings were taken spectrophotometrically. Superoxide dismutase activity in liver was estimated by the method of Minami and Yoshikawa (1979). The levels of lipid peroxidation in liver were estimated by the method of Fraga *et al*. (1988). Data obtained were analyzed by using one way analysis of variance (ANOVA) followed by Duncan's multiple range tests for comparison between groups as described by Snedecor and Cochran (1989). Results were expressed as mean ± standard error. The value of $P \leq 0.05$ was considered statistically significant.

RESULTS

Haematological parameters

On analysis of haemogram values (Table 1), feeding raw JSC at 5% level resulted in significant ($P<0.05$) reduction in the levels of Hb, VPRC, TEC and TLC values in comparison with control group. The amelioration groups with triphala showed improvement in haematological values with respect to raw JSC fed group, in which amelioration in group IV was effective in comparison to group III at the end of the experiment.

Biochemical and Antioxidant Assay

Analysis of serum biochemical values (Table 2) and liver antioxidant effects (Table 3) revealed a significant ($P<0.05$) reduction in mean serum total protein, albumin, globulin and superoxide dismutase levels while a significant increase in AST, creatinine and tissue lipid peroxidation levels in JSC fed group in comparison to control birds. Significant improvement in above parameters was noted in amelioration groups when

Table I. Haematological profile (mean ± S.E.) in different groups of birds at 28th and 42nd day of experiment

Groups	PCV (%)± S.E		Hb (g%)± S.E		TLC(10^3/Cmm± S.E		TEC(10^6/Cmm) ± S.E	
	28 days	42 days	28 days	42 days	28 days	42 days	28 days	42 days
I	30.66 ± 0.66^{a}	30.00 ± 0.73^{a}	10.27 ± 0.36^{a}	9.98 ± 0.20^{a}	30.500 ± 0.639^{a}	30.316 ± 0.437^{a}	3.93 ± 0.06^{a}	3.87 ± 0.09^{a}
II	27.33 ± 0.66^{b}	25.33 ± 0.66^{c}	8.05 ± 0.28^{c}	6.62 ± 0.22^{c}	28.283 ± 0.474^{b}	24.016 ± 0.364^{b}	3.20 ± 0.16^{b}	2.70 ± 0.08^{c}
III	28.00 ± 0.51^{b}	26.67 ± 0.66^{bc}	8.92 ± 0.14^{b}	7.07 ± 0.08^{c}	28.466 ± 0.284^{b}	24.866 ± 0.817^{b}	3.17 ± 0.16^{b}	3.00 ± 0.06^{bc}
IV	28.33 ± 0.61^{b}	27.67 ± 0.61^{b}	8.95 ± 0.31^{b}	8.10 ± 0.21^{b}	28.766 ± 0.282^{b}	28.133 ± 0.105^{b}	3.23 ± 0.16^{b}	3.35 ± 0.20^{b}

Means bearing same superscript within the same column do not differ significantly ($P \leq 0.05$).

Table II. Serum biochemical profiles (mean ± S.E.) in different groups of birds at 28th and 42nd day of experiment

Groups	AST(IU/L)		Creatinine(mg%)		Serum total protein(g%)		Serum albumin(g%)		Serum globulin(g%)	
	28 days	42 days	28 days	42 days	28 days	42 days	28 days	42 days	28 days	42 days
I	110.32 ± 5.11^{b}	107.69 ± 2.49^{c}	0.83 ± 0.02^{c}	0.92 ± 0.02^{c}	3.29 ± 0.20^{a}	3.18 ± 0.11^{a}	1.65 ± 0.08^{a}	1.60 ± 0.06^{a}	1.63 ± 0.14^{a}	1.58 ± 0.06^{a}
II	129.20 ± 1.85^{a}	138.46 ± 4.32^{a}	1.37 ± 0.05^{a}	1.47 ± 0.02^{a}	2.62 ± 0.16^{b}	2.22 ± 0.06^{d}	1.44 ± 0.06^{b}	1.25 ± 0.04^{b}	1.17 ± 0.10^{b}	0.97 ± 0.06^{c}
III	123.82 ± 3.03^{a}	131.52 ± 2.60^{a}	1.29 ± 0.03^{ab}	1.42 ± 0.03^{a}	2.83 ± 0.11^{ab}	2.68 ± 0.10^{bc}	1.43 ± 0.04^{b}	1.48 ± 0.01^{a}	1.40 ± 0.07^{ab}	1.20 ± 0.10^{bc}
IV	107.46 ± 2.28^{b}	115.58 ± 2.63^{bc}	1.20 ± 0.03^{b}	1.25 ± 0.02^{b}	2.90 ± 0.13^{ab}	2.91 ± 0.06^{ab}	1.52 ± 0.04^{ab}	1.49 ± 0.04^{a}	1.38 ± 0.11^{ab}	1.42 ± 0.07^{ab}

Means bearing same superscript within the same column do not differ significantly ($P<0.05$).

Table III. Liver lipid peroxidation and superoxide dismutase levels (mean ± S.E.) in different groups of birds at 28th and 42nd day of experiment

Groups	LPO(nMol MDA/g of tissue)		SOD(U/mg of protein)	
	28 days	42 days	28 days	42 days
I	114.83 ± 4.55^{d}	118.67 ± 2.81^{d}	4.27 ± 0.14^{a}	4.19 ± 0.15^{a}
II	286.17 ± 10.01^{a}	323.50 ± 8.59^{a}	1.88 ± 0.11^{d}	1.46 ± 0.06^{d}
III	215.83 ± 6.63^{b}	234.33 ± 4.72^{b}	2.20 ± 0.05^{cd}	2.32 ± 0.01^{c}
IV	175.00 ± 6.63^{c}	176.50 ± 9.53^{c}	3.11 ± 0.14^{b}	3.10 ± 0.12^{b}

Means bearing same superscript within the same column do not differ significantly ($P<0.05$).

compared with raw JSC fed group. Among the amelioration groups, group IV showed significant improvement over group III.

DISCUSSION

Haemato-biochemical changes in raw JSC fed group could be attributed to capillary damage and increased red blood cell fragility produced by the active constituents in the jatropha seeds, Gadir *et al*. (2003). Chivandi *et al.* (2006) also observed severe anaemia in jatropha fed pigs due to haemorrhagic internal blood losses as well as damage to gastro-intestinal tract as indicated by persistent diarrhoea and ulceration, leading to loss of nutrients required for erythropoiesis. The reduction in total leukocyte count in the group II could be due to the sequestration of leukocytes in various organs. Improvement of haematological parameters observed in group III and group IV birds in levels of haemoglobin, VPRC, TEC, TLC with respect to toxin fed group could be due to the inhibition of haemolytic factors in JSC by the ameliorating agents in triphala, which is in accordance with the findings of Kaleem *et al.* (2014) who observed that tannins derived from *Emblica officinalis,* a component of triphala possessed immunostimulatory properties in broilers. Similar improvement was also observed by Indu (2009) by triphala supplementation in rabbits against aflatoxin induced changes. Increased levels of serum AST and creatinine and decreased levels of serum total protein, albumin and globulin observed in JSC fed group could be attributed to the hepatotoxic and nephrotoxic effects of the components present in JSC. Increased creatinine level could be due to renal dysfunction in chickens fed *Jatropha curcas* and the increased levels of serum liver enzymes were an indicator of liver damage and cytotoxic effect of raw jatropha seed on liver cells leading to leakage of these enzymes from damaged hepatocytes to blood stream Johnson *et al.* (2013). Awasthy *et al.* (2010) also observed similar findings. The interaction of phorbol ester with protein kinase C affects activities of several enzymes, biosynthesis of proteins, DNA, polyamines, cell differentiation processes, and gene expression (Goel *et al*., 2007). Anil kumar *et al*., 2010 also observed a reduction in overall mean values of VPRC, Hb, TEC, TLC, total protein, albumin, globulin, and an increase in ALT, AST, creatinine, bilirubin, total cholesterol and triglycerides levels on incorporation of five per cent JSC in broiler chicken.Phorbol esters, one of the major toxic components in JSC brings about a wide range of biochemical and cellular changes, alter cell morphology, serve as lymphocyte mitogens and induce platelet aggregation (Becker and Makkar, 1998). The extensive liver damage could have potentially impaired the synthesis of proteins and other anabolic products as was evidenced by a significantly reduced level of total protein and albumin levels at all times of observation. The hypoproteinemic effect of *Jatropha curcas* seeds may be traced to the presence of antinutrients such as trypsin inhibitor and tannins in the jatropha seeds.

Increased level of serum creatinine and AST could be attributed to the injury induced by toxic components present in the JSC on kidney. Phorbol esters present in jatropha seeds, bring their effect by acting on the cellular membrane receptors with modification of their activities leading to release of the different inflammatory mediators, including histamine, which led to vascular disturbances such as congestion and oedema of different organs (Azzaz *et al*., 2011). Decreased serum AST and liver lipid peroxides and increased serum protein, albumin, globulin and liver superoxide dismutase level observed in group III and group IV compared with group II, clearly indicated that triphala could effectively ameliorate the deleterious effects of toxic components of jatropha. These observations are in accordance with the findings of Indu (2009) who observed that triphala supplementation at four per cent level could effectively counteract the toxic effects produced by aflatoxin in the liver of rabbits. Supplementation of *Terminalia belliricia* fruit extract could impart regenerative and reparative capacity to liver and kidneys of rats exposed to carbon tetrachloride induced elevation of ALT, AST, ALP and lipid peroxidation levels (Jadon *et al*., 2007). Sreelakshmi (2012) also observed similar type of findings in triphala treated group in oxytetracycline induced hepatotoxicity.

There was an increase in the level of liver lipid peroxides but decreased level of superoxide dismutase in JSC fed group which clearly indicated the oxidative damage of the hepatocytes. A reduction in liver superoxide dismutase enzyme could be due to the increased utilization of the enzyme to scavenge free radicals in oxidative stress produced by the toxic components of jatropha on liver. An elevation in the liver lipid peroxide level could be due to the increased oxidative damage of the hepatocytes. At the same time, reduced lipid peroxide level and increased superoxide dismutase level was observed in the liver of triphala fed groups. This could be attributed to the antioxidant effects of polyphenols present in triphala. Methanolic extracts of *Terminalia belliricia* and *Emblica officinalis* fruits were shown to possess scavenging activity against superoxide, hydroxyl and nitric oxide radicals revealing showing their good antioxidant potential (Nampoothiri *et al.,* 2011).

Naik *et al*. (2005) also observed the antioxidant property of triphala which was found to be an excellent scavenger of hydroxyl and superoxide radicals. The authors also stated that the antioxidant property of triphala was considered to be due to polyphenols, which would reduce the oxidative stress by converting the reactive oxygen free radicals to non-reactive products.The present study revealed that *J. curcas* at five per cent in broiler chicken feed could produce significant adverse haemato-biochemical changes. When JSC was withdrawn from the feed and triphala was administered, the adverse effects were found to be ameliorated, especially those resulted from hepatotoxicity. Thus supplementation of JSC containing feed with triphala could effectively reduce the effects of toxic components in JSC fed broiler chicken.

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