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Effect of *Curculigo orchioides* in experimental hepatotoxicity in cockerels

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Abstract
Ethanolic and aqueous extracts prepared from rhizomes of *Curculigo orchioides* as herbal medicines were evaluated against acetaminophen-induced hepatotoxicity in cockerels. Acetaminophen @ 500 mg/ body weight orally was given to induce hepatocellular damage. Cockerels given with ethanolic extract of *Curculigo orchioides* @ 70 mg/kg body wt and acetaminophen revealed restoration of Hb, PCV, TEC, TLC and lymphocytes and heterophils as well as total protein, albumin and globulin, glucose, cholesterol, bilirubin and activity of AST, ALT, ALP and LDH. The biochemical results were parallel to the histopathological analysis of liver sections as treated birds clearly showed normal hepatic cells and central vein thereby confirming hepatoprotective activity. These findings had suggested that *Curculigo orchioides* is a promising product in protecting the liver against toxic injury via the restoration of haematalogical and biochemical parameter. Aqueous extract showed least activity. Ethanolic extract showed presence of alkaloid, flavonoid, glycosides, Protein, reducing sugars, resin, saponins and sterol.

Keywords: Cockerels, *Curculigo orchioides*, hepatoprotective, phytochemial

Introduction
Poultry meat is one of the fastest growing components of global meat demand and India is experiencing rapid growth in its poultry sector. For optimum productivity in today's intensive nature of poultry farming, it is necessary that the birds remain healthy. Liver is a vital organ involved in various metabolic and detoxification mechanism. The toxins may cause varying degree of damage to the liver and affect liver functions resulting in poor health and production. Liver extract derived from liver of other mammals or fishes are the drug of choice in hepatic abnormilities. However it poses serious risk of transmitting infections to animals and human both as well as is costly. Presently herbal liver formulations are in use in treating cases of primary and secondary hepatic diseases. The rhizome and tuberous roots of *Curculigo orchioides* have been extensively used for the treatment of various diseases, including cancer, jaundice, asthma and diarthrosis wound healing [1]. It is also considered to be tonic, alterative, demulcent, diuretic, and restorative, and is used as a poultice for itch and skin disease [2]. The present study was undertaken to record the effect of *C. orchioides* on liver function markers following experimental hepatotoxicity in cockerels.

Material and Methods
The rhizomes of *C. orchioides* were procured locally and were identified and authenticated from Medicinal research and development center of the university. These were shade dried and ground in a Willey Grinder at room temperature. For preparation of the ethanolic or aqueous extract, 100 grams each powder was soaked in 1000 ml of absolute ethanol or water for 48 hr at 37 ^{0}C with continuous stirring. The contents were filtered and concentrated by evaporation at lower temperature (45-50 °C) and reduced pressure using rotatory vacuum evaporator [3] and lyophilized to get the final extract residue. These were stored at 4 ^{0}C in refrigerator till further use.

The extracts were analysed for major phytochemical groups, viz. alkaloids, anthraquinones, flavonoids, saponins, tannins, sterols, reducing sugars, glycosides, resins, triterpenes, proteins and coumarins by standard methods [4, 5, 6, 7].

One Hundred, 3-month-old cockerels belonging of same hatch were procured from Instructional Poultry Farm of the University and randomly divided into 5 groups I, II, III, IV and V of 20 each. All the groups had almost equal average body weight and maintained under standard deep litter managemental conditions. Gr I served as healthy control while gr II received acetaminophen @ 500 mg/kg body weight orally for 7 days [8] and served as infected control. Gr III received silymarin (as a standard reference) along with acetaminophen for 7 days and thereafter only silymarin was given upto 35th day. In cockerels of gr IV and gr V,

ethanolic and aqueous extracts @ 70 mg/kg b wt [9] along with acetaminophen for 7 days and thereafter only extract were given upto 35th day.

The blood samples were collected on day 0, 7, 15, 21, 28, 35 and 42 of treatment, for haematological (Hb, TEC, TLC, PCV and DLC) and biochemical parameters (glucose, total cholesterol, total protein, albumin, globulin, albumin: globulin ratio, blood urea nitrogen and serum bilirubin) and activities of liver enzymes (aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH)). Liver samples were collected in 10% buffered formalin for histopathological study and examined for any type of gross changes from each group on 7th, 21st and 35th day of treatment. The formalin fixed tissue pieces were serially dehydrated in alcohol and acetone, embedded in paraffin blocks and sections were cut and stained in hematoxyline and eosin stain for histopathological examination by standard procedures. The results were analysed as per the standard method [10].

Results and Discussion

The ethanolic extract residue of *C. orchioides* had oily appearance and blackish brown colour with 8.29% yield whereas its aqueous extract was brown in colour and solid dry powder in consistency with 6.47% yield. Ethanolic extract was positive for alkaloid, flavonoid, glycosides, protein, reducing sugars, resin, saponins and sterol. Anthraquinones, couramins and triterpenes were additionally present in aqueous extract with the absence of tannis, saponins and sterols.

A significant decrease in Hb, PCV, TEC & lymphocytes and increase in TLC & heterophils values were observed in gr II as compared to gr I, III, IV and V from 7th day onward up to the end of experiment (Table 1). Ethanolic and aqueous extract of *C. orchioides* significantly restored these values to normalcy. Hb value in ethanolic extract treated birds was significantly higher than aqueous extract treated birds from 28th day to the end of experiment (Table 1).

Table 1: The value of Hb (mg/dl), PCV (%), TEC(x10^6), TLC(x10^3), Lymphocytes (%) and Heteropphils (%) in cockerels treated with *Curculigo orchioides*

Haematological	0 day	7th day	28th day	42nd day
Haemoglobin				
Group 1	89.7±0.892	89.2±0.778^a	97.8±0.443^a	99.6±1.771^a
Group II	87.5±0.638	71.2±0.704^b	73.6±1.185^b	80.1±1.336^b
Group III	89.1±0.842	87.1±0.678^a	101.7±1.731^c	109.7±0.622^c
Group IV	88.6±0.982	87.6±1.341^a	99.8±0.548^c	107.8±1.491^c
Group V	89.4±1.017	82.9±0.784^a	94.1±1.647^d	100.4±0.899^d
PCV				
Group 1	22.5±0.957	22.75±0.629^a	28.25±0.854^a	29.75±1.493^a
Group II	22.25±0.479	17±0.707^b	18.25±0.629^b	19.25±0.479^b
Group III	23±0.707	21.75±0.479^a	32.5±0.289^c	32.25±0.854^a
Group IV	21.5±0.006	21.2±0.006^a	31.±0.009^a	30.5±0.011^a
Group V	21±0.004	20.5±0.006^c	27.7±0.011^a	27.5±0.002^c
TEC				
Group 1	2.283±0.149	2.400±0.103^a	2.682±0.016^a	2.646±0.018^a
Group II	2.238±0.115	1.771±0.096^b	2.292±0.051^b	2.403±0.102^b
Group III	2.414±0.048	2.368±0.123^a	2.659±0.014^a	2.727±0.031^a
Group IV	2.403±0.066	2.254±0.097^a	2.727±0.031^a	2.717±0.025^a
Group V	2.225±0.099	2.173±0.052^a	2.689±0.038^a	2.688±0.028^a
TLC				
Group 1	17.150±0.552	19.069±0.387^a	18.267±0.238^a	18.223±0.379^a
Group II	17.405±0.185	24.849±0.913^b	22.854±0.913^b	23.254±0.465^b
Group III	17.853±0.592	18.377±0.648^a	18.305±0.606^a	18.589±0.360^a
Group IV	17.479±0.210	17.949±0.558^a	17.701±0.545^a	19.565±0.469^a
Group V	18.912±0.269	17.526±0.789^a	18.639±0.334^a	19.427±0.643^a
Lymphocytes				
Group 1	10.898±0.723	10.744±0.393^a	11.026±0.385^a	11.183±0.530^a
Group II	10.168±0.449	8.901±0.527^b	9.012±0.427^b	9.619±0.286^b
Group III	10.036±0.447	10.956±0.531^a	11.038±0.683^a	11.883±0.471^a
Group IV	10.956±0.405	10.859±0.140^a	11.079±0.457^a	10.921±0.394^a
Group V	9.621±0.206	10.449±0.454^a	11.457±0.290^a	10.886±0.308^a
Heterophils				
Group 1	4.943±0.459	4.916±0.567^a	5.060±0.226^a	5.438±0.166^a
Group II	4.701±0.514	7.630±0.599^b	6.676±0.250^b	6.100±0.159^b
Group III	4.577±0.238	5.087±0.676^a	4.911±0.415^a	5.398±0.166^a
Group IV	4.906±0.230	5.100±0.516^a	5.125±0.292^a	5.280±0.151^a
Group V	5.294±0.220^a	5.556±0.650^a	5.057±0.169^a	4.981±0.645

There was significant decrease in PCV values in groups V as compared to treated and control groups on 7th and 42nd day post-treatment. Reduction in TEC and Hb may be due to oxidative damage-mediated removal of affected erythrocyte induced by acetaminophen. Increased generation of free radicals can cause cell membrane damage, which in turn inactivate membrane Na^+-K^+-ATPase [11], thereby allows entry of Ca^{+2} into the cell. The sustained increase in intracellular calcium leads to free-radical generations, which in turn further inhibit Na^+-K^+-ATPase. Thus the acetaminophen mediated generation of free-radicals and consequent oxidative damage to erythrocytes can cause mechanical fragility of plasma membrane, thereby shortening RBC life span and its removal from circulation. Disintegration of erythrocytes in the circulation might have resulted in Hb reduction, which in turn was associated with decrease in PCV and TEC [12]. Ethanolic extract of *C. orchioides* protects the disintegration of erythrocytes. Gupta and Mishra (2008) [13] found that *C. orchioides* restored Na+K+ATPase levels to normalcy in paracetamol and aflatoxin induced hepatic injury. Neutrophilia and lymphocytopenia were prominent in animals subjected to

hepatopathy. This might be due to stress coupled with inflammatory changes in body tissue, which is responsible for phagocytosis of toxic substances and neutrophilia was induced by tissue demand for phagocytic function [14]. An increase in heterophil count and decrease in lymphocyte count was also reported by Rajalakshmi and Sasikala (2010) [15] as was observed in this study. Roa and Mishra (2004) [16] and Rajalakshmi and Sasikala (2010) [15] also found the restoration of PVC and Hb with the administration of *C. orchioides*.

Glucose and bilirubin values were increased after induction of hepatopathy in untreated group from 7th day till end of experiment (Table 2). Treated groups revealed significant reduction in total proteins, albumin and cholesterol than untreated groups (Table 2). Significant increase in the globulin was recorded following treatment with ethanolic and aqueous extracts and silymarin as compared to gr 2 (Table 2). The changes in blood urea nitrogen were non-significant.

Table 2: The value of Glucose (mg/dl), Cholestrol (mg/dl), Total Protein (g/L), Albumin (g/L), Globulin (g/L), and A: G ratio in cockerels treated with *Curculigo orchioides*

Biochemical	0 day	7th day	28th day	42nd day
Glucose				
Group 1	10.633±0.225	9.302±0.202^{a}	9.846±0.214^{a}	9.705±0.331^{a}
Group II	9.737±0.216	20.290±1.746^{b}	17.085±1.419^{b}	13.717±1.037^{b}
Group III	10.635±0.583	11.144±1.105^{a}	10.014±0.236^{a}	9.699±0.305^{a}
Group IV	10.365±0.403	11.928±1.255^{a}	9.650±0.264^{a}	9.676±0.331^{a}
Group V	9.835±0.229	13.199±0.960^{a}	10.251±1.077^{a}	9.935±0.313^{a}
Cholestrol				
Group 1	4.326±0.137	4.368±0.038	4.287±0.106^{a}	4.372±0.037^{a}
Group II	4.336±0.062	4.579±0.097	5.261±0.113^{b}	4.937±0.252^{b}
Group III	4.342±0.055	4.480±0.059	4.282±0.064^{a}	4.415±0.055^{a}
Group IV	4.364±0.118	4.328±0.074	4.331±0.107^{a}	4.250±0.074^{a}
Group V	4.212±0.077	4.322±0.067	4.377±0.100^{a}	4.255±0.102^{a}
Total protein				
Group 1	58.665±2.666	62.843±2.188^{ac}	61.918±2.453^{a}	62.408±1.371^{a}
Group II	58.615±2.147	45.878±1.575^{b}	47.993±1.842^{b}	50.783±2.053^{b}
Group III	59.243±2.579	63.438±2.500^{a}	68.870±1.193^{a}	67.513±2.794^{a}
Group IV	57.848±1.07	60.465±1.05^{a}	67.365±1.78^{a}	69.183±0.94^{a}
Group V	61.125±2.02	58.228±1.58^{a}	64.165±3.42^{a}	63.813±2.37^{a}
Albumin				
Group 1	34.553±2.305	35.955±1.482^{a}	35.425±1.697^{a}	35.470±0.921
Group II	33.473±1.057	27.510±1.472^{b}	27.600±1.177^{b}	30.268±1.919
Group III	35.173±1.531	34.323±2.420^{a}	35.620±1.264^{a}	36.058±1.397
Group IV	34.928±1.362	32.615±0.813^{a}	34.028±0.774^{a}	36.193±1.378
Group V	34.643±1.067	32.173±1.501^{a}	34.600±1.925^{a}	33.160±2.108
Globulin				
Group 1	24.113±0.825	26.888±1.046^{a}	26.493±1.229^{a}	26.938±1.590^{a}
Group II	25.143±1.394	18.368±0.747^{b}	20.393±1.170^{b}	20.515±0.719^{b}
Group III	24.070±1.204	29.115±2.049^{a}	33.250±1.456^{c}	31.455±1.866
Group IV	34.643±1.067	32.173±1.501^{a}	34.600±1.925^{a}	33.160±2.108^{a}
Group V	26.483±1.030	26.055±0.900^{a}	30.653±1.550^{ac}	29.565±2.124^{a}
A:G ratio				
Group 1	1.435±0.095	1.340±0.055	1.342±0.070^{ab}	1.334±0.100^{ab}
Group II	1.340±0.059	1.506±0.099	1.463±.083^{b}	1.481±0.106^{b}
Group III	1.464±0.045	1.200±0.125	1.080±0.072^{c}	1.144±.059^{c}
Group IV	1.536±0.109	1.178±0.064	1.029±0.059^{c}	1.144±0.083^{c}
Group V	1.310±0.026	1.240±0.074	1.182±0.084^{c}	1.091±0.093^{c}

Hyperglycaemia may be due to the degenerative hepatic lesions and also can follow the metabolic acidosis. Reduction in glucose level after the treatment with extracts was reported by Irsad *et al*. (2006) [17], Madhavan *et al*. (2007) [18] and Aritajat *et al*. (2008) [19] also**.** Due to the damage of hepatocytes there was decreased elimination of bilirubin and thereby its level was increased as observed by Gupta and Mishra (2008) [13] and Rosa *et al*. (2009) [20] also Kaneko (1989) [21] and Mezey (1978) [22] reported that proteins synthesized by the liver are frequently decreased in patients with liver diseases and decrease in circulating proteins such as albumin occur. These values came to normalcy following therapy indicating the regenerating ability of the drug. Globulins are intermediate proteins which are involved in antibody formation. Venukumar and Latha (2002) [9] and Rao and Mishra (2004) [16] also observed the same findings. Hepatic cholesterol homeostasis is maintained by an equilibrium between the activities of hydroxy methyl glutaryl CoA (HMG-CoA) reductase and that of acyl CoA: cholesterol acyl transferase [23]. Reduction in cholesterol could also be due to the deficient metabolism of lipids in the liver [24].

ALT, AST, ALP and LDH activities were significantly elevated in infected group as compared to the treated groups (Table 3). Increased activities of ALT, AST, ALP and LDH reflect the damage of liver hepatocytes and indirectly impairment of liver functions. Extracts of *C. orchioides* significantly reduced the elevated levels of these enzymes and it was also similar to the birds treated with silymarin. One of the hallmark signs of hepatic injury or damage is apparent leakage of cellular enzymes into plasma [11]. ALT, AST, ALP and LDH are commonly used as marker enzymes in accessing hepatotoxicity [25, 26, 27]. Hepatoprotective effect of *C. orchioides* is evidenced by the improvement of ALT, AST, ALP and LDH levels. Venukumar and Latha (2002) [9], Irshad *et al*. (2006) [17], Gupta and Mishra (2008) [13] and Rosa *et al*. (2009) [20] also recorded similar observations.

Table 3: The activities of AST (U/L), ALT (U/L), ALP (U/L) and LDH (U/L) in cockerels treated with *Curculigo orchioides*

Biochemical	0 day	7th day	28th day	42nd day
AST				
Group 1	391±15.138	402±19.399[a]	404±11.453	401±7.692
Group II	385±16.350	621±12.754[b]	463±16.361	441±22.587
Group III	397±17.093	411±16.366[a]	404±8.554	419±14.646
Group IV	400±10.472	411±18.686[a]	402±16.941	422±15.422
Group V	404±16.718	485±9.354[a]	421±16.618	414±15.519
ALT				
Group 1	98±3.697	100±4.882[a]	101±1.080[a]	110±2.828
Group II	99±1.472	309±8.256[b]	128±9.704[b]	122±7.494
Group III	100±1.683	113±4.601[a]	101±2.582[a]	113±3.488
Group IV	101±2.449	111±1.780[a]	101±3.240[a]	112±2.483
Group V	99±3.416	148±9.806[a]	112±6.124[a]	112±3.342
ALP				
Group 1	123±5.196	126±8.287[a]	124±4.378	122±2.799
Group II	121±7.106	343±4.708[c]	148±5.115	141±3.391
Group III	124±4.378	135±8.175[a]	130±5.066	125±4.491
Group IV	124±4.301	136±10.124[a]	128±5.050	125±5.958
Group V	124±2.739	246±14.849[b]	126±7.269	134±7.083
LDH				
Group 1	479±16.010	482±14.872[a]	494±1.080	486±9.018
Group II	484±19.506	773±12.891[b]	509±12.457	493±3.082
Group III	482±11.225	502±8.784[a]	483±3.559	486±4.848
Group IV	477±4.601	503±13.058[a]	491±5.523	479±8.765
Group V	481±6.868	553±14.680[c]	497±11.195	497±5.307

There was significant decrease in feed consumption and body weight in gr II as compared to gr I, III, IV and V from 14th day onward till end of experiment. Body weight increased significantly in the gr IV at 35th day of treatment as compared to other groups which might be due to increase in function of hepatocyte and increased feed intake.

The biochemical results were comparable to the histopathological analysis of the liver sections as gr I (Fig. 1) showed normal cellular architecture with sinusoidal spaces and central veins. Severe histopathological changes were clearly observed in intoxicated cockerels revealing centrilobular hepatic necrosis. At 1st week, moderate to mild leukocytic infiltration into the portal areas was evident. Occasionally clumps of hepatic cells were hazy in appearance and the sinusoids were wide but narrow (Fig 2). When *C. orchioides* were given with acetaminophen, significant decrease in hepitocellular changes was observed. Similar changes were seen in silymarin treated group. These results suggest that *Curculigo orchioides* has hepatoprotective action. It increases the Hb, PCV, TEC, Lymphocytes, total protein, albumin, globulin and decreases glucose, total cholesterol, bilirubin, AST, ALT, ALP and LDH values to normalcy.

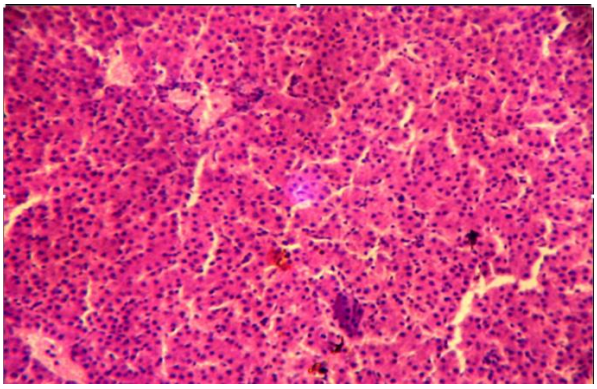

Fig 1: Normal hepatic lobulations with normal hepatocytes

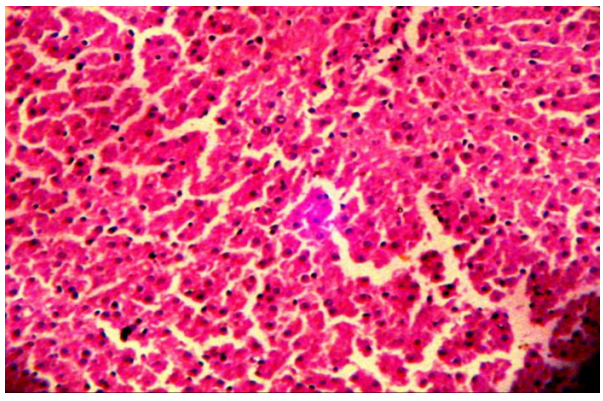

Fig 2: Intracytoplasmic cloudy swelling pyknotic nuclei and vacuoles representing fatty degeneration and lymphocytic inflitration

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