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Hepatoprotective Efficacy of *Picrorhiza kurroa* in Experimentally induced Hepatotoxicity in Cockerels

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A B S T R A C T

Hepatoprotective properties of ethanolic and aqueous extracts of *Picrorhiza kurroa* rhizomes were evaluated in cockerels given acetaminophen @ 500 mg/ body weight orally to induce hepatocellular damage. Ethanolic extract given @ 50 mg/kg body wt and acetaminophen helped in 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. Histopathological examination of liver section of treated birds clearly showed normal hepatic cells and central vein thereby confirming hepatoprotective activity. Silymarin used @ 200 mg/kg body weight as reference standard also showed the same results. Aqueous extract revealed the least activity. Phytochemical analysis of ethanolic extract showed presence of alkaloids, flavonoids, glycosides, protein, resin, saponin, sterol and tannins.

Introduction

Many toxins damage the liver and affect its functions resulting in poor health and production. For prevention of hepatocytes, some drugs or chemicals are used which also antagonize the toxins and help to regain its power of metabolism, during early days, liver extract derived from liver of other mammals or fishes was the drug of choice. But such drugs posed serious risk of transmitting infections from animals to animals or to human. Moreover, the cost of liver extract is high specially if economy of the farm and farm products become a matter of concern. Now-a-day herbal liver formulations become more important in treating hepatic diseases. *Picrorhiza kurroa* has been used to treat disorders of the liver and upper respiratory tract, fevers, treat dyspepsia, chronic diarrhoea and scorpion sting (Sood and Chauhan, 2010). *Picrorhiza* has been shown to protect liver cells from a wide variety of toxins including amanita poisoning, carbon tetrachloride (Lee *et al*., 2007), galactosamine (Dwivedi *et al.*, 1992), ethanol (Rastogi *et al.*,

1996), aflatoxin-B1 (Dwivedi *et al*., 1993), acetaminophen (Singh *et al*., 1992), and thioacetamide (Dwivedi *et al*., 1991), in both *in vitro* and *in vivo* experiments. The present study was planned to investigate the activity of *P. kurroa* on liver function markers following experimentally induced hepatotoxicity in cockerel.

Materials and Methods

The rhizomes of *P. kurroa* procured from local market, were identified and authenticated from Department of Biological Sciences of university. These were shade dried and ground in a Willey Grinder at room temperature. For preparation of the ethanolic or aqueous extract, 100 gm each powder of *P. kurroa* was soaked in 1 liter of absolute ethanol or water for 48 hr at 37^0C with continuous stirring, the contents were filtered, concentrated at 45-50°C and reduced pressure using rotatory vacuum evaporator (Singh, 2001), lyophilized to get the final extract residue and stored at 4^0C 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 using methods at Das *et al*. (1964), Harborne (1973), Sofawara (1982) and Arunadevi (2003).

Total 100, three-month-old cockerels of same hatch were procured from IPF university and randomly divided into 5 groups I, II, III, IV and V of 20 each having 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 (Bhar *et al.*, 2009) 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 35^{th} day. In gr IV and V, ethanolic and aqueous extract residues @ 50 mg/kg b wt (Jeyakumar *et al*., 2009) along with acetaminophen for 7 days and thereafter only extract were given upto 35^{th} 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 enzymes AST, ALT, ALP and LDH) using standard methods.

Liver samples were collected in 10% buffered formalin for histopathological examination on 7, 21 and 35 day of treatment. The results were analysed as per method described by Snedecor and Cochran (1994).

Results and Discussion

The ethanolic extract residue was greenish brown in color and oily in consistency while aqueous extract residue was light brown in color and solid dry powder in consistency. Ethanolic and aqueous extract revealed 16.09% and 13.23 % yield. Phytochemical analysis of ethanolic extract of *P. kurroa* showed presence of alkaloids, flavonoids, glycosides, protein, resin, saponin, sterol and tannins, whereas alkaloids, proteins, resin and sterol were absent in aqueous extracts and anthraquinones and triterpenes were present

There was significant decrease in Hb, PCV, TEC and lymphocytic values in group II as compared to group I, III, IV and V from 7^{th} day onward up to the end of experiment (Table 1). Ethanolic and aqueous extract, significantly restored these values to

normalcy. Hb values are significantly higher in treated group than untreated and control group at 42nd day of treatment (Table 1). Destruction of RBC, decrease 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 (Kumar *et al*., 2009), thereby allows entry of Ca^{+2} into the cell. The sustained increase in intracellular calcium leads to free-radical generation, which in turn 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 shortened RBC life span and its removal from circulation. Disintegration of erythrocytes in the circulation might have resulted in reduction of haemoglobin content of blood, which in turn was associated with decrease in PCV and TEC (Chauhan *et al*., 2008).

The ethanolic extract *P. kurroa* protected the disintegration of erythrocytes. Mogre *et al*. (1982) found that *P. kurroa* restored Na^{+}-K^{+}-ATPase levels to normal in paracetamol and aflatoxin induced hepatic injury. Neutrophilia and lymphocytopenia in all the 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 (Duncan and Prasse, 1977). Increase in heterophils and decrease in lymphocytes was also reported by Hadau *et al*. (2008). Rukamani *et al*. (1998) also found restoration of TLC with the administration of *P. kurroa*.

Glucose and bilirubin showed marked increase after induction of hepatopathy in untreated group from 7th day till end of experiment (Table 2). There was significant decrease in of total protein, albumin and cholesterol levels and increase in globulin in all the treated groups (Table 2). Hyperglycaemia can be due to the degenerative hepatic lesions and also can follow the metabolic acidosis. Reduction in glucose level after the treatment with extracts was also reported by Talmale *et al*. (2010). Due to the damage of hepatocytes there was decreased elimination of bilirubin and thus an increase was observed. The increase in bilirubin was also observed by Vaidya *et al*. (1996) and Talmale *et al*. (2010). Kaneko (1989) and Mezey (1978) reported that protein synthesized by the liver are frequently decreased in patients with liver diseases and this was manifested clinically by decrease in circulating proteins such as albumin. These values came down to normalcy following therapy indicating the therapeutic values of the drug. Globulins are intermediate proteins which are involved in antibody formation. Jaykumar *et al*. (2008, 2009) and Talmale *et al*. (2010) also observed the same findings. Hepatic cholesterol homeostasis is maintained by equilibrium between the activities of hydroxy methyl glutaryl CoA (HMG-CoA) reductase and that of acyl CoA: cholesterol acyl transferase (Hochgraf *et al*., 2000). Reduction in cholesterol could also be due to the deficient metabolism of lipids in the liver (Gauda *et al*., 1985). Hussain (2009) also noticed decrease in cholesterol level with use of *P. kurroa*.

The activities of ALT, AST, ALP and LDH were elicited in infective group suggesting damage of liver hepatocytes and impairment of liver functions. Use of *P. kurroa* extracts and silymarin significantly reduced the level of these enzymes (Table 2). One of the hallmark signs of hepatic injury or damage is apparent leakage of cellular enzymes into plasma (Kumar *et al.*, 2009). These enzymes are commonly used as marker enzymes in accessing hepatotoxicity (Yanpallewar *et al.*, 2003; Asha *et al.*, 2004 and Yen *et al.*, 2007).

Table.1 The value of Hb, PCV, TEC, TLC, Lymphocytes and Heteropphils in cockerels treated with *Picrorhiza kurroa*

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	86.8±1.135	87.9±1.571[a]	100.2±1.743[ac]	107.0±0.522[c]
Group V	86.8±1.199	84.7±0.505[a]	96.6±1.649[a]	104.2±1.507[c]
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	22.5±0.009	21.5±0.006[a]	31.2±0.016[ac]	32.2±0.014[a]
Group V	22.2±0.011	22±0.007[a]	30.5±0.019[ac]	32.2±0.008[a]
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	2.400±0.080	2.281±0.070[a]	2.668±0.024[a]	2.702±0.034[a]
Group V	2.306±0.049	2.184±0.096[a]	2.694±0.027[a]	2.668±0.024[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	17.989±0.427	18.422±0.465[a]	18.066±0.745[a]	18.456±0.625[a]
Group V	17.715±0.654	17.397±0.685[a]	19.529±0.450[a]	17.326±0.447[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	10.528±0.796	10.988±0.352[a]	11.741±0.440[a]	10.978±0.154[a]
Group V	10.632±0.805	10.067±0.362[a]	10.977±0.353[a]	11.792±0.182[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	4.796±0.242	5.020±0.708[a]	4.948±0.171[a]	5.305±0.096[a]
Group V	4.877±0.207	5.283±0.730[a]	5.133±0.300[a]	5.356±0.094[a]

Table.2 The value of Glucose, Cholestrol, Total Protein, Albumin, Globulin and A: G ratio in cockerels treated with *Picrorhiza kurroa*

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	9.583±0.295	12.100±1.164[a]	9.823±0.227[a]	9.580±0.324[a]
Group V	10.060±0.529	11.247±0.319[a]	9.926±0.898[a]	10.226±0.140[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	4.379±0.123	4.352±0.116	4.388±0.025[a]	4.362±0.095[a]
Group V	4.374±0.021	4.326±0.109	4.361±0.095[a]	4.277±0.114[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[c]	67.513±2.794[a]
Group IV	60.548±3.18	61.96±01.56[a]	68.543±3.46[c]	67.990±3.16[a]
Group V	63.430±0.23	58.438±1.16[a]	67.223±1.67[ac]	63.013±1.14[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	35.395±2.191	34.203±1.993[a]	35.053±2.405[a]	35.480±0.926
Group V	35.968±1.275	32.408±0.902[a]	34.818±1.239[a]	31.995±1.192
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[c]
Group IV	25.153±1.944	27.758±1.260[a]	33.490±1.855[c]	32.510±2.345[c]
Group V	27.463±1.183	26.030±0.972[a]	32.405±1.656[c]	31.018±0.964[ac]
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	1.428±0.126	1.247±0.126	1.053±0.074[c]	1.103±0.056[c]
Group V	1.324±0.111	1.251±0.064	1.085±0.077[c]	1.036±0.061[c]

Table.3 The activities of AST, ALT, ALP and LDH in cockerels treated with *Picrorhiza kurroa*

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	402±13.279	415±20.046[a]	408±11.540	401±17.093
Group V	397±7.223	417±9.721[a]	403±6.178	411±10.008
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	99±3.488	112±0.816[a]	100±3.979[a]	111±1.080
Group V	101±2.483	118±5.447[a]	111±4.378[a]	113±2.345
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	125±5.323	134±7.594[a]	132±4.813	120±3.391
Group V	121±3.582	140±3.109[a]	128±5.066	132±5.148
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	481±10.591	504±5.354[a]	491±4.528	488±2.677
Group V	498±23.611	522±17.762[c]	496±7.106	499±6.916

Recovery towards normalization of the enzymes following *P. kurroa* treatment suggested that the plant extract have role in preserving structural integrity of hepatocellular membrane, thus prevented enzymes leakage into circulation (Bhar *et al*., 2005, Singh *et al*., 2005 and Talmale *et al*., 2010).

There was significant decrease in feed consumption and body weight in group II as compared to group I, III, IV and V from 14th day onward till end of experiment. A significant increase in body weight was observed in the group IV at 35th day of treatment as compared to control group which might be due to increase in function of hepatocyte and increased palatability of feed.

The biochemical findings were supported with histopathological observations of liver sections. The healthy control group (Fig. 1) showed normal cellular architecture with sinusoidal spaces and central veins while intoxicated cockerels revealing centrilobular hepatic necrosis. The hepatic cords were irregularly distributed and distorted and the cells were rounded with opaque cytoplasm and showed mild vacuolated cells that suggested the fatty degeneration (Fig 2). In treated birds, hepitocellular changes could be restored towards normalcy.

These results indicated that *Picrorhiza kurroa* has hepatoprotective action. It increases the Hb, PCV, TEC, lymphoctes, total protein, albumin and globulin levels and decreases

glucose, total cholesterol, bilirubin, AST, ALT, ALP and LDH values to normalcy in intoxicated bird.

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