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Hepatoprotective activity of *Basella rubra* on paracetamol induced toxicity in chicken

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Abstract

The present study was conducted to evaluate the hepatoprotective activity of medicinal plant, *Basella rubra* on paracetamol induced hepatotoxicity in White LegHorn cockerels. Birds were divided into five groups of six birds each and birds in group I served as untreated control and was given distilled water for 23 days continuously. The group II birds were administered with paracetamol @ 2 g/kg body weight orally from 17th day onwards and till the end of the experiment. Birds in the group III were given silyamin @ 100mg/kg for 16 days followed by paracetamol @ 2 g/kg body weight till the end of the experiment. Birds in group IV and V were administered with *Basella rubra* @ 500mg and 2500 mg/kg body weight, respectively for 16 days followed by paracetamol @2 g/kg body weight till the end of the experiment along with the medicinal plant. Hemato-biochemical observations were recorded in all the groups treated. Pro inflammatory cytokines, IL6 and TNF- α were quantified using real time PCR. ALT, AST, GGT enzyme levels were found to be reversed in paracetamol intoxicated birds pre-treated with silymarin and *Basella rubra*. Fold changes in real time PCR were found to be increased in paracetamol treated birds as compared to other groups. The study suggests that *Basella rubra* has the potential in reversing the paracetamol induced hepatotoxicity in chicken.

Keywords: *Basella rubra*, chicken, paracetamol, real time PCR

Introduction

Liver is considered to be one of the most vital organs that functions as a centre of metabolism of nutrients such as carbohydrates, proteins, lipids and excretion of waste metabolites (Kumar, 2012) [6]. Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects. Several herbs have been used traditionally to prevent and treat human and animal diseases. Recently, scientific evaluation of plants and preparations of plant origin medications have received more attention. *Basella rubra* is annual or perinial herb situated wildly in many countries including India. However, no research work has been carried out on the hepatoprotective activity of this plant against paracetamol induced hepatotoxicity in White LegHorn chicken. The present study was undertaken to find out the effect of *Basella rubra* leaves on the paracetamol induced hepatic injury in White LegHorn chicken model.

Materials and Methods

Thirty male White LegHorn cockerels weighing between 500-600 g were purchased from District Livestock Farm, Orathanadu, Thanjavur District, Tamil Nadu, India. The birds were maintained in an animal house with standard facilities having CPCSEA approval. The birds were housed in cages and maintained at 30-35° C under 12 h light/dark. They were fed with SKM Animal Feed, manufactured by SKM feeds, Erode District, Tamil Nadu. Water was provided ad libitum. The birds were acclimatized for one week under laboratory conditions. Ethical clearance for handling the animals was obtained from the ethic committee constituted for the purpose.

Birds were divided into five groups of six birds each and birds in group I served as untreated control and was given distilled water for 23 days continuously. The group II birds were administered with paracetamol @ 2 g/kg body weight orally from 17th day onwards and till the end of the experiment. Birds in the group III were given silyamin @ 100mg/kg for 16 days followed by paracetamol @ 2 g/kg body weight along with silymarin till the end of the experiment. Birds in group IV and V were administered with shade dried powdered leaves of *Basella rubra* @ 500mg and 2500 mg/kg body weight, respectively for 16 days followed by paracetamol @2 g/kg body weight till the end of the experiment along with the medicinal plant.

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Initial and final body weights were measured. Blood samples were collected from the jugular vein of birds per replicate into a set of sterilized glass tubes containing ethylene diamine tetra acetic acid (EDTA) for determination of haematological parameters and into a set of glass tubes without anticoagulant for serum separation. Hemato-biochemical observations were made at the end of the experiment to assess the potential of medicinal plant in reversal of paracetamol induced toxicity. Blood samples were analyzed as per Schalm (1975) [10]. The levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), gamma glutamyl transpeptidase (GGT) were assayed by the standard kit methods using Systronics colorimetry. The results were analysed as per the method of Snedecor and Cochran (1994) [11].

Assessment of Relative Gene Expression of IL-6 and TNF- α in hepatocytes

RNA was isolated from pro inflammatory cytokines IL-6 and TNF- α and the levels were quantified in treated and control samples as prescribed by Kaiser *et al.* (2000) [5] with some modifications. Real-time PCR (Eppendorf, Germany) was utilized for the quantification. The target gene expression was normalized to the relative expression of Glyceraldehyde-3-phosphate dehydrogenase, a house keeping gene in the same sample (Yiqun *et al.*, 2016) [13] to get Δ Ct. The Δ Ct of the test sample was compared with the Δ Ct of control sample (calibrator gene) to get $\Delta\Delta$ Ct value. The fold changes/relative expression was derived using the formula $2^{-\Delta\Delta Ct}$. Real time PCR was operated with the following cycle profile: 95 °C for 2 min followed by 40 cycles of 95 °C for 5 s, 60 °C for 5 s and 72 °C for 25s. Each sample was tested in three technical replicates.

Results and Discussion

The results of the haemato-biochemical studies are depicted in Table 1. The ALT, AST and GGT levels in paracetamol treated group were significantly higher (48.94%, 91.84%, 104.09%) than the control group. ALT, AST, GGT enzyme levels were found to be reversed in paracetamol intoxicated birds pre-treated with silymarin (by 37.64%, 33.47%, and 42.40, respectively). *Basella rubra* in lower dose could reverse the levels of ALT, AST, GGT by 14.00%, 12.23%, 27.70% and in higher dose by 12.24%, 14.27%, 32.10%, respectively.

The enzyme ALT is abundant in liver cells in the body and is primarily used as a specific module for hepatic damage mainly in small animals and primates (Cornelius, 1989) [2]. The levels of these enzymes in liver damage may be the result of excessive release of enzyme into serum by disruptive hepatic parenchymal cell membrane (Hoe and Wilkinson, 1973) [3]. The results apparently indicated that the degree of hepatic cell damage was of lesser magnitude in *Basella rubra* pre-treated birds. Similar observations have been reported by Chattopadhyay *et al.*, (1992) [1] with *Azadirachta indica* (neem) in rats in reversing the levels of ALT, AST and GGT during paracetamol intoxication.

There was significant decrease in Hb level in paracetamol intoxicated birds (34.90%) as compared to control group where as significant reversal of Hb was found in the groups pre-treated with *Basella rubra* with lower and higher dose by 39.20% and 46.74%, respectively. Silymarin could reverse the Hb level by 66.89% (Table 1). There were no significant changes in total RBC levels in all the groups studied where as significant decrease was noticed in paracetamol treated birds and the level was reversed in groups pre-treated with *Basella rubra* and silymarin. Many plants have been observed to stimulate the levels of leukocytes (Jafarian *et al.*, 2012, Raphael *et al.*, 2003) [4, 9]. The observed effect may be attributed to the effect of *Basella rubra* as an anti-inflammatory agent (Yanadaiah *et al.*, 2011) [12]. Flavonoids have been tested successfully as hepatoprotective agents against CCl₄ induced hepatotoxic activity in HepG2 cells (Nguyen *et al.*, 2017) [7]. *Basella rubra* plants have also been reported to contain flavonoids (Ramesh Kumar *et al.*, 2018) [8]. The results of the hepatoprotection may be attributed to the presence of flavonoids in the plant.

Results of relative mRNA expression of cytokine IL-6 and TNF- α in hepatocytes of chicken are depicted in Figure.1. In the parameter of fold changes of IL-6, the largest change was noticed in paracetamol alone treated group (6.15 fold). Fold changes in group III, IV and V were 0.37, 0.91 and 0.29 folds, respectively. Groups III, IV and V have been found to show down regulation of mRNA expression of IL-6. In the parameter of fold changes of TNF- α , the largest change was noticed in paracetamol alone treated group (18.51 fold). Fold changes in group III, IV and V were 0.30, 0.52 and 0.27 folds, respectively. Groups III, IV and V have also been found to show down regulation of mRNA expression of TNF- α .

Table 1: Effect of treatments on Hemato-biochemical observations and body weight gain in chicken

Parameters	Group I	Group II	Group III	Group IV	Group V
Body wt gain (%)	91.40	82.00	90.20	85.00	90.25
ALT (IU/ml)	6.62 ^c ± 0.03	9.86 ^a ± 0.20	6.44 ^c ± 0.34	8.47 ^b ± 0.51	8.86 ^b ± 0.34
AST (IU/ml)	25.14 ^d ± 3.32	48.23 ^a ± 7.02	31.12 ^c ± 3.34	41.22 ^b ± 2.45	40.96 ^b ± 7.50
GGT (U/L)	74.12 ^c ± 7.17	151.27 ^a ± 12.71	88.62 ^b ± 8.85	112.27 ^b ± 12.70	104.38 ^b ± 28.47
Hb (g%)	18.14 ^a ± 0.79	11.81 ^c ± 1.46	19.71 ^b ± 1.44	16.44 ^a ± 1.67	17.33 ^a ± 0.33
RBCm (Cumm)	04.04± 0.30	4.02± 0.72	4.04± 0.63	4.22± 0.18	4.21± 0.40
WBC thou(Cumm)	04.75 ^a ± 0.22	2.99 ^c ± 0.02	4.21 ^b ± 0.11	3.87 ^b ± 0.76	3.96 ^b ± 0.33

Values (mean ± S.E.M., n=6) in the same rows bearing no superscript common vary significantly ($P \leq 0.05$)

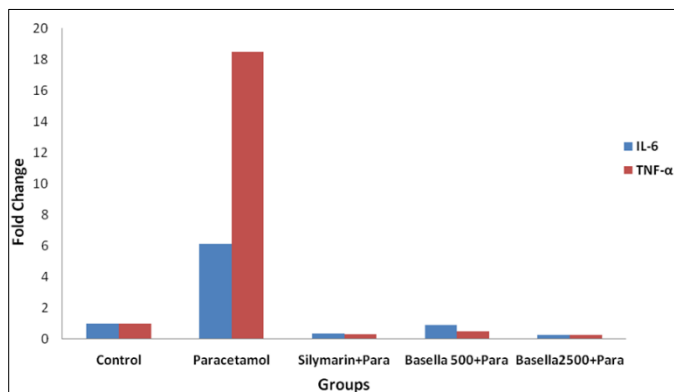


Fig 1: Evaluation of fold changes (Relative mRNA expression) of IL-6 and TNF- α in hepatocytes of chicken

Conclusion

The results suggest that the plant *Basella rubra* @ 500 mg and 2500 mg /Kg body weight has the potential to reverse the paracetamol induced liver toxicity in chicken.

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