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Evaluation of Hepatoprotective activity of *Moringa oleifera* in chicken

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ABSTRACT

The present study was carried out to evaluate the hepatoprotective activity of *Moringa oleifera* on paracetamol induced hepatotoxicity in chicken. Twenty-four Giriraja birds were divided into four groups with six birds in each group. Birds in group I served as control and was administered with distilled water for 23 days continuously. Birds of group II were given paracetamol @ 2 g/kg body weight orally on day 17 and continued till the end of the experiment. Birds in group III were given silyamin @ 100mg/kg for 16 days and paracetamol was given from 17th day onwards @ 2 g/kg body weight along with silymarin till the end of the experiment. Birds in group IV were given shade dried powdered leaves of *Moringa oleifera* @ 1000 ppm in feed for 16 days and paracetamol was given from 17th day onwards @ 2 g/kg body weight along with medicinal plant till the end of the experiment. The biomarker enzymes such as alanine aminotransferase, aspartate aminotransferase, gamma glutamyl transferase were tested and expression of mRNA levels of proinflammatory cytokines were screened for hepatoprotective activity of the plant. Histopathological observations were made with liver tissue for analysing hepatoprotective activity of the plant. Based on the results, the study suggests that *Moringa oleifera* has the potential to act as hepatoprotective agent.

Keywords: *Moringa oleifera*, Chicken, Paracetamol, Real Time PCR.

INTRODUCTION

Liver is one of the most vital organs in birds, carrying out a diverse array of functions for the health care of the system. It is accountable for most of the synthesis, biotransformation and detoxification processes in the body [1]. Liver cell damage is induced by toxic chemicals, antibiotics, carbon tetrachloride, thioacetamide, consumption of excess alcohol and microbes [2]. Hepatotoxicity is the very common aliment resulting into serious disorders and it may even lead to mortality in animals and birds. Medicinal plants assist in the management of various liver disorders. Several medicinal herbs have been reported successfully for their hepatoprotective effects in different animal models [3, 4].

Several studies reversed paracetamol induced hepatotoxicity in laboratory animals using medicinal plants [5, 6] and in broiler chicken [7]. *Moringa oleifera* Lam. is one of the most widely cultivated species of the Moringaceae family. Leaves, barks, roots, stems, buds, flowers etc. have been used for different human and animal ailments. The plant possesses activity against several microbial organisms, fungus, inflammation, reactive oxygen species and acts as hypotensive, anaesthetic, anti-ulcer, cardioprotective and antiurolithiatic activity [8]. *Moringa oleifera* has been studied successfully for its hepatoprotective activity in rat [9]. No study experimented hepatoprotective activity of *Moringa oleifera* in chicken model.

It has been demonstrated that various pro-inflammatory cytokines like tumor necrosis factor (TNF)-α, interleukin-6 (IL-6) produced during hepatic injury are involved in promoting tissue damage [10]. The expression of these cytokines is interpretative for both immune response to infection and for supporting internal body homeostasis. Due to the limited specific antibodies against the cytokines of chicken but the presence of their gene sequences, qPCR is often used to specify their expression at the transcriptional level.

Hence the present research work was undertaken to evaluate the hepatoprotective effect of the leaves of *Moringa oleifera* in paracetamol induced toxicity in chicken.

MATERIALS AND METHODS

Twenty four male Giriraja breed of chicken weighing around 500-600 g were purchased from DLF, Tamil Nadu. The birds were kept in an animal house with standard facilities having CPCSEA approval. The birds

were kept in cages with temperature ranging between 30-35° C under 12 h light/dark. Birds were fed with SKM Animal Feed, manufactured by SKM feeds, Erode District, Tamil Nadu. Water was provided ad libitum. They were acclimatized for one week under laboratory conditions. Ethical clearance was obtained from the Institutional Animal Ethical Committee constituted for the purpose.

Giriraja birds were divided into four groups with six birds in each group. Birds in group I served as control and was administered with distilled water for 23 days continuously. Birds of group II were given paracetamol @ 2 g/kg body weight orally on day 17 and continued till the end of the experiment. Birds in group III were given silyamin @ 100mg/kg for 16 days and paracetamol was given from 17th day onwards @ 2 g/kg body weight along with silymarin till the end of the experiment. Birds in group IV were given shade dried powdered leaves of *Moringa oleifera* @ 1000 ppm in feed for 16 days and paracetamol was given from 17th day onwards @ 2 g/kg body weight along with medicinal plant till the end of the experiment.

Initial and final body weights of birds were measured. Blood was collected from the jugular vein of birds per replicate into a set of sterilized glass tubes without anticoagulant for serum separation. Serum samples were stored in at -20 °C for further analysis. Biochemical observations were made at the end of the experiment to evaluate the potential of medicinal plant in reversal of paracetamol induced toxicity. The levels of liver biomarker enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma glutamyl transferase (GGT) were assayed by the standard kit methods using using Double beam UV visible Spectrometer. The results were analysed by suitable statistical method [11]. Liver samples were collected from all groups and stored at -80°C for conducting histopathology and also for analysing expression of proinflammatory cytokines, IL-6 and TNF-α.

Histopathological studies

The collected liver was preserved in 10% formalin for histopathological studies. The representative liver blocks were selected and processed for paraffin embedding by standard microtechnique. Section (5 µm) of liver tissue stained with hemotoxylin and eosin was observed microscopically for histopathological changes.

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

Isolation of total RNA was carried out from rat liver [12]. The quantity and intactness of RNA were periodically tested by bio photometer. Levels of mRNA of pro inflammatory cytokines IL-6 and TNF-α were quantified in treated and control samples [13]. Real-time PCR (Eppendorf, Germany) was utilized for the quantification. The published primers were purchased from Qiagen, USA [14]. The expression of target gene was normalized to the relative expression of Glyceraldehyde-3-phosphate-dehydrogenase (GAPDH), a house keeping gene in the same sample [15] to get ΔCt. The ΔCt of the test sample was compared with the ΔCt of control sample (calibrator gene) to get ΔΔCt value. The fold changes/relative expression was derived using the formula $2^{-\Delta\Delta Ct.}$ Real time PCR was operated by the following cycle: 95°C with 2 min followed by 40 cycles of 95°C with 5 s, 60°C with 5 s and 72°C with 25s. Each sample was tested in three technical replicates.

RESULTS

In the body weight, no significant difference was found between all groups treated in the experiment. The ALT, AST and GGT levels were found to be significantly increased and the levels of haemoglobin and WBC were found to be significantly decreased in birds treated with paracetamol as compared to control (Fig.1). Levels of ALT, AST and GGT enzymes were reduced in paracetamol intoxicated birds treated with *Moringa oleifera* by 23.23%, 40.80% and 44.17% respectively, as compared to paracetamol intoxicated birds.

The liver of chicken treated with paracetamol revealed severe congestion, periportal fibrosis and mononuclear cell infiltration (Fig.2) where as these lesions were reversed by groups treated with *Moringa oleifera* (Fig.3). Results of relative mRNA expression of cytokine IL-6 and TNF-α in hepatocytes of chicken are depicted in Fig.4. In the parameter of fold changes of IL-6, the largest change was noticed in paracetamol alone treated group (8.40 fold). Fold changes in group III and IV were 0. 61 and 0.46 folds, respectively. In the parameter of fold changes of TNF-α, the largest change was noticed in paracetamol alone treated group (12.06 fold). Fold changes in group III and IV were 0.57 and 0.54 folds, respectively.

DISCUSSION

Levels of ALT, AST and GGT enzymes in groups III and IV indicated that *silymarin* and *Moringa oleifera* have the potential to modify paracetamol intoxication in birds. The enzyme ALT is rich in liver cells in the body and is used as a module for hepatic damage mainly in small animals and primates [16]. The increased levels of these enzymes in liver injury may be the result of excessive release into the serum by fractious hepatic parenchymal cell membrane [17]. The results indicated that the degree of hepatic cell injury was of lesser magnitude in group of birds treated with *Moringa oleifera*. The reduction of histopathological lesions in the intoxicated birds treated with *Moringa oleifera* indicates the ability of this plant to act as a hepatoprotective agent. This histopathological result is in line with the result of ALT activity, a bio marker of cell damage which was ameliorated by the administration of *Moringa oleifera.* It appears as a hepatoprotective activity by reinstituting the antioxidant defense, preventing the incidence of oxidative stress and subsequently checking mitochondrial malfunction and inflammation, as well as restricting the resultant necrotic cell death [18]. The findings were corroborated with the study [19] which reported similar hepatoprotective effect with *Moringa oleifera* against CCl4 induced hepatic damage in rats. The reversal of these lesions in intoxicated birds in these groups indicates the prospective of this plant as a hepatoprotective agent.

Groups III and IV have been found to show down regulation of mRNA expression of IL-6. Groups treated with silymarin and *Moringa oleifera* have also been found to show down regulation of mRNA expression of TNF-α. The results were in line with another study which also reported the reduction in pro inflammatory cytokines in CCl_4 intoxicated rats when treated with Curcumin [10].

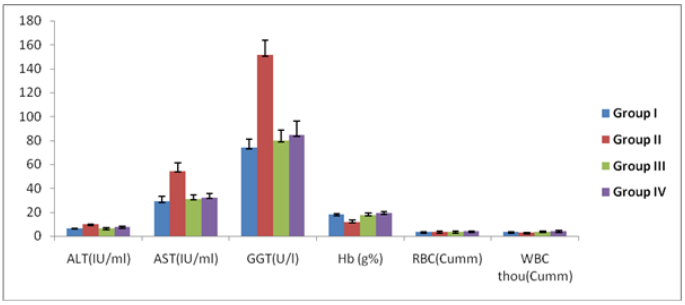

Figure 1: Effect of *Moringa oleifera* on serum biochemical parameters in paracetamol induced hepatotoxicity in chicken

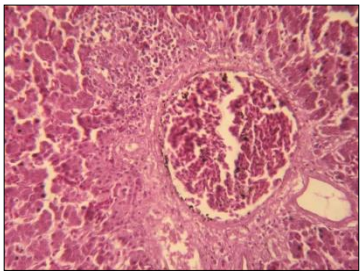

Figure 2: Paracetamol treated group liver showed severe congestion and periportal fibrosis hepatocytes H&EX 400x

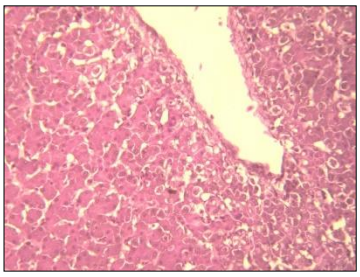

Figure 3: *Moringa oleifera* and Paracetamol treated group liver showed apparently normal architecture of hepatocytes H&EX 400x

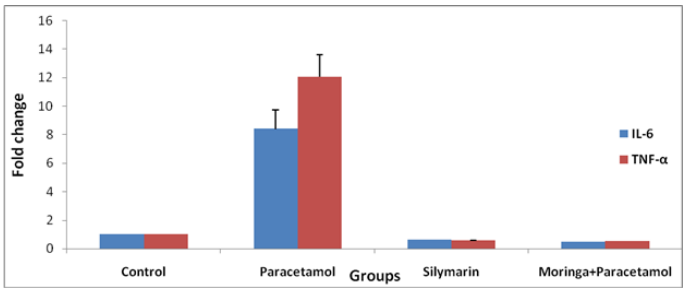

Figure 4: Expressions of proinflammatory cytokines in chicken to evaluate hepatoprotective activity of *Moringa oleifera*

CONCLUSION

The results of the study suggested that *Moringa oleifera* has the potential to act as hepatoprotective agent in paracetamol induced hepatotoxicity in chicken.

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