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Therapeutic Efficacy of Liv.52 in Paracetamol induced Hepatopathy in Rabbits

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SUMMARY

The therapeutic efficacy of Liv.52 in included hepatopathy was studied in 12 adult New Zealand White rabbits. Hepatopathy was induced by single intravenous (IN) injection of paracetamol @ 400 mg/kg body weighty (B.W.). Group I animals (6 nos.) served as control, whereas Group-II animals (6 nos.) were treated with Liv.52 @ 10 drops orally twice daily for 10 days. Clinical abnormalities were more severe and prolonged in the Group-I animals. A decrease was observed in haemoglobin (Hb), packed cell volume (PCV), total erythrocyte count (TEC, blood glucose level, total serum proteins and albumin:globulin (A:G) ratio, whereas an increase was observed in total leukocyte count (TLC), neutrophils count, total lipids, cholesterol, triglycerides, bilirubin, alanine amino transferase (ALT), aspertate amino transferase (AST) and BSP% retention. These value reversed back to normalcy more rapidly and effectively in the treated animals indicating the therapeutic efficacy of the drug.

Key words: Liv.52, paracetamol-induced hepatopathy, rabbits

INTRODUCTION

Liver possesses a great number and variety of functions essential for survival of the animal. It has the responsibility for normal metabolic functioning of other organs and tissue, which include activities like metabolism and storage of carbohydrates, fats, proteins and vitamins, synthesis of plasma proteins, formation and secretion of bile, erythropoiesis, metabolism and detoxification etc. Obviously, injuries to hepatic parenchyma or interference with hepatic vascular system may have serious and far reaching effects not only on the liver itself but also on other organs and systems. Hence, any disease process involving the liver would adversely affect the health and productivity of the animal.

Primary hepatic diseases in farm animals usually occur as a result of poisoning by toxic agents of chemical or plant origin. Drugs in common use can cause toxic effects on the liver which can mimic almost every naturally occurring liver disease and the drug induced hepatitis resembles an attack of acute viral hepatitis. Hepatotoxicity produced by overdosing of paracetamol may serve as a model for toxic drug interactions with the liver. Usually, liver has tremendous regenerating capacity but does not respond to any available medicinal treatment after reaching a certain stage of degeneration. Indigenous drugs like Liv.52 (The Himalaya Drug Company, Bangalore, India) us reported to be very effective in the treatment of liver disorders in animals. However, there is a paucity of literature regarding the efficacy of Liv.52 in treating experimentally induced hepatopathy in rabbits. The present experiment assessed the therapeutic efficacy of Liv.52 in treating paracetamol-induced hepatopathy in rabbits.

MATERIAL AND METHODS

Twelve (12) adult New Zealand White rabbits of either sex were kept on standard feeding and managemental conditions for 6 weeks before starting the actual experiment. These animals were given anthelmintic and antibacterial coverage. At the end of this 6 weeks period, the animals were examined thoroughly to ascertain their health status. To induce hepatopathy, paracetamol was administered I/V @ 400 mg/kg B.W. These animals were then divided randomly into 2 equal groups (Group I and II) each consisting of 6 animals. Group I animals were not given any treatment and severed as untreated control while Group II animals were given Liv.52 drops @ 10 drops orally twice a day starting from 24 hours after induction of hepatopathy and was continued up to day 10 post-induction. The hepatic toxic effects of paracetamol and therapeutic efficacy of Liv.52 were assessed on the basis of clinical signs, haematological, biochemical and dye (BSP) retention studies. Abnormal clinical signs that were seen in animals were recorded. Haematological parameters were studied as per the methods of Jain [11]. Methods adopted for different biochemical parameters were Folin and Wu [6] for Glucose, Greenberg [9] for total serum proteins and albumin, Frings *et al.* [7] for total lipids, Wootton [23] for serum cholesterol, Gottfried and Rosenberg [8] for teiglycerides, Ducci and Watson [3] for total serum bilirubin, Reitman and Frankel [19] for AST and ALT, and Oser [15] for BSP% retention tests.

Blood samples were collected for haematological studies in clean vials containing EDTA as anticoagulant, with the exception of clotting time, which were carried out at the time of collection. Blood samples were collected for different biochemical studies in clean vials without any anticoagulants for separation of serum, with the exception of blood glucose for which blood samples were collected in vials containing sodium fluoride. All these collections were made before administration of paracetamol (0 day) and thereafter on day 1, 5, 10, 15 and 20. BSP% retention studies were conducted before paracetamol administration (0 day) and then on day 1, 10 and 20.

RESULTS

Some of the animals started showing struggling even during administration of the drug. After administration of the drug the animals started showing symptoms of toxicity like struggling, jumping, increased respiratory rate, frequent defecation and micturition, and in-coordination in movement. Most of the animals preferred to rest on sternum with stretched limbs. The conjunctival mucous membrane of most of the animals became congested and the episode lasted for about 2 hours.

On day 1 almost all animals were dull, anorectic and disinclined to move even on compulsion. Diarrhoea, serous nasal discharge, paleness of mucous membranes, weakness and roughness of body coat were also seen. These changes were of severe magnitude and longer duration in the animals of Group I than in Group II. Almost similar clinical findings have been reported by Dwivedi [5] in rabbits. The haemoglobin value decreased significantly ($P<0.05$) in both the groups following paracetamol administration. The values of 10.46 ± 0.28 g/dl on zero day which decreased to 8.10 ± 0.29 g/dl on day 1, increased to 9.90 ± 0.22 g/dl on day 20 in Group II animals. PCV and TEC values also decreased in both the Groups following paracetamol administration. But PCV value of Group II animals on zero day was 32.66 ± 0.88% and on day 5 the value decreased to 27.66 ± 0.80% which again increased significantly to become 32.33 ± 0.71% on day 20. TEC value of 7.54 ± 0.14 x 10^6/µl on zero day in Group II animals decreased to 6.20 ± 10.11 x 10^6/µl on day 1

and subsequently increased to become 7.42 ± 0.12 x 10^6/µl, on day 20. The values of these three parametes remained low till the end of the experiment in Group I animals. TLC values of 8.48 ± 0.14 x 10^3/µl on zero day in Group II animals increased to 9.93 ± 0.24 x 10^3/µl on day 1. This value showed decrease towards normalcy on day 20. The TLC value of 8.53 ± 0.13 x 10^3/µl on zero day in Group I animals remained increased to 10.28 ± 0.02 x 10^3/µl on day 20.

A marked increase in clotting time in the experimental animals was observed. Clotting time of 5.91 ± 0.45 minute on zero day in Group I animals remained increased to 6.97 ± 0.37 minute till day 20. In case of Group II animals the clotting time of 5.25 ± 0.49 minute though increased following paracetamol administration, decreased considerably to 5.37 ± 0.44 minute on day 20.

Total serum proteins, albumin and A:G ratio showed a marked reduction after induction of hepatopathy in both the groups. These values reversed to normalcy following therapy in Group II animals while the values remained more or less altered in Group I animals. There was a significant ($P<0.05$) elevation of total lipid concentration in serum of all the rabbits after paracetamol administration. The value of 183.99 ± 4.64 mg/dl in Group II animals on zero day increased to 307.07 ± 8.95 mg/dl on day 5 which then reduced to 205.61 ± 8.51 mg/dl on day 20. Similarly, the serum cholesterol and triglyceride values, which increased following paracetamol administration, decreased in treated animals (Group II) whereas animals of Group I showed considerably higher values till the end of the experiment.

AST values of 47.18 ± 1.01 RFU/ml on zero day which increased to 143.12 ± 12 RFU/ml on day 5 in Group II animals came down to 69.29 ± 1.80 RFU/ml on day 20. In case of Group I animals the AST value of 46.36 ± 0.98 RFU/ml on zero day which increased to 251.84 ± 3.56 RFU/ml on day 10 remained as high as 171.30 ± 2.37 RFU/ml on day 20. ALT values of both the Groups also increased following paracetamol administration. But these values decreased to normalcy in Group II animals whereas the values remained high in the Group I animals till day 20.

The BSP% retention values which increased in both the Groups following induction of hepatopathy also decreased after medication in Group II animals. The value of 3.40 ± 0.20 on zero day in Group I animals which increased to 8.70 ± 0.10 on day 1 remained increased to 8.25 ± 0.025 on day 20. The value of 3.60 ± 0.20 on zero day in Group II animals though increased to 8.50 ± 0.10 on day 1, this showed decline to come down to 4.30 ± 0.10 on day 20.

DISCUSSION

Dullness, in-coordination and other nervous signs might be attributed to biochemical alterations such as hypoglycaemia, accumulation of excess amino acids or of acetylcholine due to failure of the usual hepatic detoxification mechanism [12]. Anorexia and diarrhoea might be due to partial or complete absence of bile and irregularities in normal digestion. Low oxygen carrying capacity of the erythrocytes might have resulted in increased respiration rate.

Reduction in Hb, PCV and TEC values also reported [17], Venna [22], Dwivedi *et al.,* [5]. This reduction may be attributed to the inability of the damaged hepatic parenchyma to produce erythropoietinogen and partly to the reduced feed intake, decreased absorption and metabolism of nutrients. 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. The improvement in these values was observed in the treated animals which confirms the findings of Dwivedi *et al.* [5] in rabbits and Singh [21] in goats.
Increased TLC values have also been reported by Piperno *et al.* [17], Gupta [10] and Dixit [2] in experimental animals. Neutrophilia and lymphocytopenia were prominent in differential leukocyte count 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 [4]. All these value reversed back towards normalcy in the animals of Group II following treatment indicating the efficacy of the drug. Almost all the clotting factors are produced in the liver and damage of hepatic cells might have led to reduced production of these factors which in turn resulted in delayed clotting of blood. Vitamin K requires adequate amount of bile salts for its absorption from the intestine and also helps in the normal synthesis of some of the clotting factors, which are said to be vitamin K dependent factors. In hepatic damage less amount of bile might have hampered the normal absorption of vitamin K, which subsequently resulted in decreased production of these factors. Prescott [18] and Seef *et al.,* [20] also reported prolongation of clotting time due to hepatic damage caused by paracetamol.

Decrease in blood glucose level has been observed by (Dwivedi *et al.,* [5]) in rabbits following carbon tetrachloride administration. The reduction might be due to failure of the damaged hepatic parenchyma to perform their normal mechanism of glucose production. The blood glucose levels returned to normalcy following therapy with Liv.52 in the animals of Group II which is in agreement with the findings of Peer [16] and Singh [21].

Mezey [14] reported that proteins 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 regenerating ability of the drug. Similar observations were recorded by Gupta [10] and Dixit [2] in paracetamol-induced hepatopathy in goats.

Hyperlipidaemia in this study might be due to increase in level of cholesterol and other lipid components, and decreased secretion of bile, bile salts and total biliary fatty acids. Treatment of the animals in Group II resulted in decrease in these values towards normalcy. This confirms the finding of Dwivedi *et al.* [5] in rabbits. The increase in the triglycerides might be attributed to inhibition of hepatic cells to produce lipoproteins and lipases which are essential for triglyceride metabolism. Total serum bilirubin level showed similar changes. These findings are agreement with Peer [16] and Singh [21]. The initial elevation in the bilirubin levels might be as a result of improper uptake, metabolism and excretion of bile by the damaged hepatic cells.

The elevation of the AST and ALT values might be due to release of the enzymes from disrupted hepatic cells either due to necrosis or altered membrane permeability due to toxic effects of paracetamol. Similar elevations and reduction of these values in the treated animals confirms the findings of Dwivedi *et al.,* [5] in rabbits. This might be attributed to the regenerating ability of the drug.

The increase in the percent retention of BSP in this study may be attributed to the inability of the damaged hepatic parenchymal cells to excrete the dye at normal rates. Decrease in percent retention following therapy with Liv.52 indicates the regenerative efficacy of the drug. These are in agreement with the findings of Dixit [2].

Liv.52 has hepatoprotective and stimulating actions [1] and also has anabolic effects [13]. The drug has been used with success in cases of liver diseases in animals by various workers [16,21]. Dwivedi *et al.,* [5] reported good efficacy of Liv.52 in treating experimental liver damage in rabbits. The overall observation of the present experiment is that Liv.52 was found to be effective in treating cases of paracetamol-induced hepatopathy in rabbits.

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