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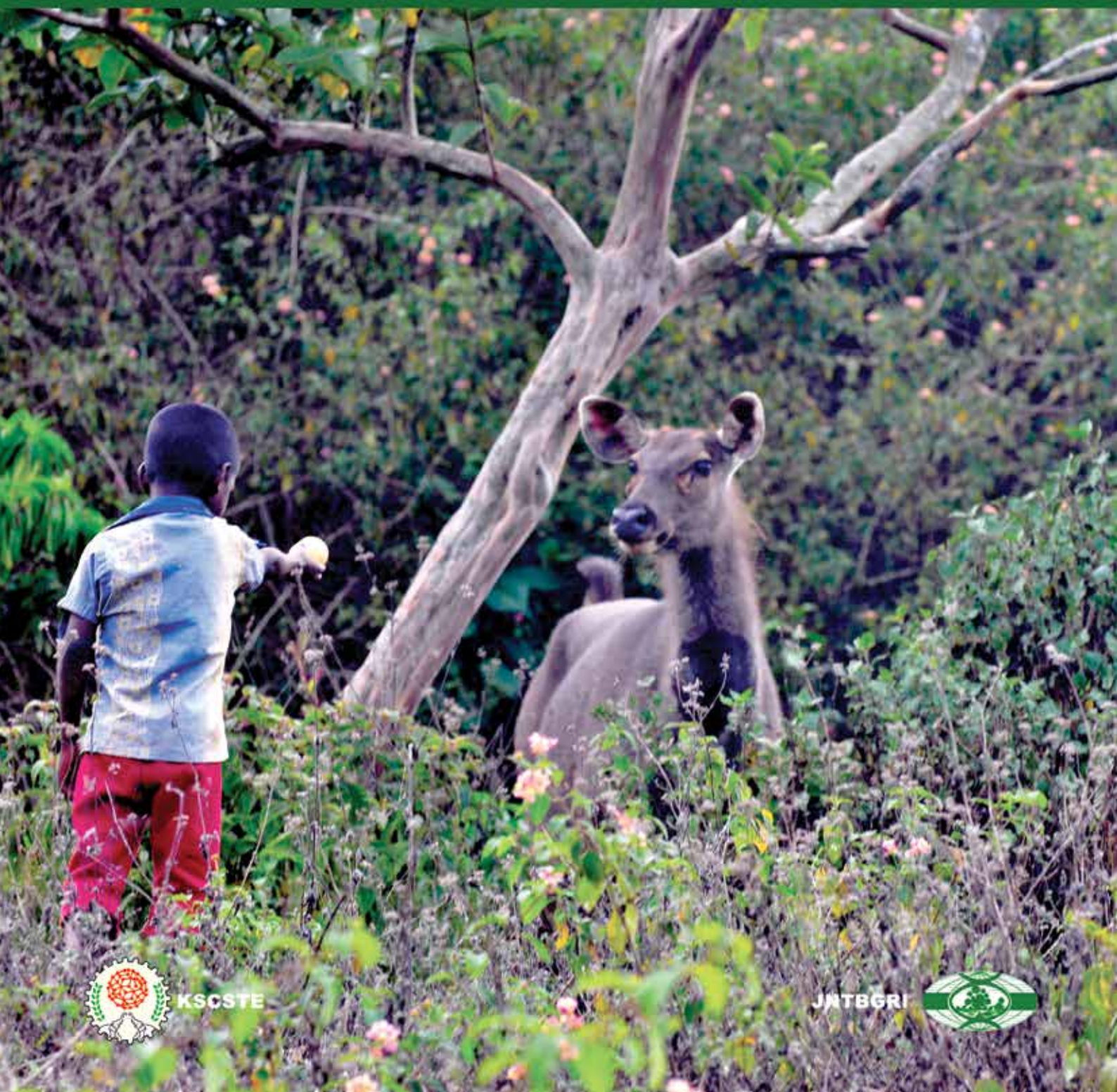

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Hepatoprotective activity of coded plant (222) leaf extract in D-Galactosamine induced hepatotoxicity: Biochemical and histopathological analysis in experimental animals – Part VI

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Abstract

The hepatoprotective effect of the leaf ethanolic extract of coded plant (Code No. 222**) against D-Galactosamine induced hepatic injury in Wistar albino rats was evaluated in the present study. The animals were divided into different groups and treated with 222 leaf ethanolic extract at different doses (125, 250 and 500 mg/kg) and Silymarin (100 mg/kg) for five days. Hepatotoxicity was induced by the intraperitoneal injection of D-Galactosamine (D-GalN) (400 mg/kg) 2 h after the drug treatments. D-GalN treated rats developed hepatic damage and it is indicated by the significant ($P \leq 0.05$) increase in serum hepatic enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and serum bilirubin (SB) levels. D-GalN toxicity also resulted in a significant ($P \leq 0.05$) increase in other serum biochemical parameter levels in toxin control group compared to the normal control. Pretreatment with coded plant 222 leaf ethanolic extract (125 mg/kg) caused significant ($P \leq 0.05$) reduction of increased serum enzymes and other biochemical parameters in D-GalN induced hepatotoxic animals. The reduced levels of hepatic enzymes and serum bilirubin levels exhibited by 222 (125 mg/kg) was comparable to the standard control Silymarin. The histopathological analysis also supported the hepatoprotective effect of the leaf extract. The results of the present study indicated that coded plant 222 leaf extract (125 mg/kg) can effectively protect against D-Galactosamine (D-GalN) induced liver damage in experimental animals.

Keywords: D-Galactosamine, Hepatoprotective, Hepatotoxicity, Hepatic enzymes, Histopathology

1. Introduction

The primary hepatotoxins including alcohol, aflatoxin, heavy metals and drugs induce liver damage by direct injurious attack to the liver (Zimmerman, 1982). Among these, D-galactosamine (D-GalN) is well-established hepatotoxicant and it is a suitable experimental model of liver injury, which is widely used. The model closely resembles human viral hepatitis in its morphologic and functional characteristics and

therefore considered very useful for the evaluation of hepatoprotection (Langeswaran *et al*., 2012). D-GalN hepatotoxicity is considered as an experimental model of acute hepatitis. It has great liver specificity and does not affect other organs and it is easy and convenient to replicate experimentally (Chaudhary *et al*., 2010). D-GalN is known to selectively block the transcription and indirectly hepatic protein synthesis and as a consequence of endotoxin toxicity, it causes fulminant hepatitis (Tang *et al*., 2004). According to Raj *et al*. (2010), D-GalN has been found to induce extensive liver damage within a period of 24 h following intra-peritoneal administration.

Herbal medicines derived from plant extracts are effective against a wide variety of diseases and drug induced toxicity studies. The researchers paid more attention to the protective effects of natural antioxidants against free radical generation (Frei and Higdon, 2003). Medicinal plants have been valued in developing countries of the world for primary health care due to better cultural acceptability, better compatibility with human body and lesser number of side effects (Shah *et al*., 2011).

The liver diseases are some of the fatal disease in the world today and they pose a serious challenge to international public health. The liver health maintainence is a crucial factor for overall health and well being. Modern medicines have little to offer for the alleviation of hepatic diseases and it is chiefly the plant based preparations which are employed for the treatment of liver disorders. But there is not much drugs available for the treatment of liver disorders (Karan *et al.*, 1999). Therefore, many folk remedies from plant origin are tested for its potential antioxidant and hepatoprotective effects against liver damage in experimental animal models (Rubinstein, 1962). It has been reported that natural compounds from medicinal plants, fruits, vegetables and cereals can protect liver from D-GalN induced toxicity (Sugiyama *et al*., 1999). Antioxidants have been reported to scavenge free radicals by interfering with the oxidation process and chelating metal ions. Thus oxidative stress is prevented by the action of antioxidants. Some investigators have suggested that D-GalN induced liver injury can be suppressed by dietary supplementation with plant extracts containing phytochemical constituents such as catechin, glycoside, oligosaccharide and soluble dietary fiber (Gyamfi *et al*., 1999).

The present ethnopharmacological study was carried out based on the traditional claim related to a coded medicinal plant (Code No. 222) disclosed by a traditional healer from Karunagappally, Kollam District, Kerala and the claim was that he was using the particular medicinal plant species against diabetes and related complications and it also provides hepatoprotection. We have already reported the hepatoprotective activity of coded plant 222 leaf ethanolic extract against various hepatotoxicants such as paracetamol, carbon tetrachloride and alcohol induced liver damage (Krishnakumar *et al*., 2017 a, b; Krishnakumar *et al*., 2018). On verification, no pharmacological studies related to the hepatoprotective potential of the coded plant 222 leaves against D-galactosamine (D-GalN) induced hepatotoxicity had been so far conducted. The hepatoprotective activity of coded plant 222 leaf ethanolic extract against D-GalN induced liver damage in Wistar rats is reported in the present study.

2. Materials and Methods

2.1. Preparation of the plant leaf extract

The coded plant 222 leaves were collected from the Traditional Healer at Karunagappally, Kollam District, Kerala. They were authenticated by Dr. Mathew Dan, the plant taxonomist of the Institute and a voucher specimen (TBGT 57057 dated 16/10/2009) was deposited at the Institute's Herbarium. The leaves were washed thoroughly, shade dried, powdered and 100 g of the leaf powder was extracted with 1000 mL of ethanol overnight, at room temperature with constant stirring (200 rpm). The extract was filtered, concentrated and the solvent evaporated completely in a rotary evaporator at 40°C under reduced pressure, dried in a desiccator and it was reconstituted in 0.5% Tween-80 to required concentrations and used for the experiments.

2.2. Chemicals and commercial kits

D-Galactosamine, Silymarin, Sodium Dodecyl Sulphate (SDS) and Thiobarbituric acid (TBA) were purchased from Sigma Aldrich, USA. Commercial kits for the estimation of biochemical parameters such as Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Serum

alkaline phosphatase (ALP), Serum bilirubin (SB), Serum cholesterol, Triglycerides, Creatinine, Urea and Uric acid were purchased from Coral Clinical System, Goa, India.

2.3. Animals

Wistar albino rats, males (200–250 g) obtained from the Institute's Animal House were used for the present study. They were housed in poly-acrylic cages with three animals per cage and maintained under standard laboratory conditions (temperature 24-28 ± 1°C, relative humidity 60 ± 5% and 12 h light/dark cycles), fed commercial rat feed (Lipton India Ltd., Mumbai, India) and boiled water *ad libitum*. All the experiments involving animals were carried out according to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, after getting the approval of the Institute's Animal Ethics Committee (B-form No. B-04/12/2013/EM & EP - 09).

2.4. D-Galactosamine (D-GalN) induced hepatotoxicity study

Wistar albino male rats (200-250 g) were divided into different groups each containing 6 animals. Group I, the normal control group and Group II, D-Galactosamine (D-GalN) control group were administered 1 ml of 0.5 % Tween-80, p. o. Group III, IV and V received varying doses of the crude ethanolic leaf extracts of coded drug 222 (125, 250 and 500 mg/kg), while Group VI was treated with silymarin (100 mg/kg, p. o). All the treatments were continued for five days. On the 6th day, Groups II-VI received D-GalN (400 mg/kg in saline, i. p) 2 h after the respective treatments. 24 h after D-GalN intoxication, the animals were sacrificed by carbon dioxide inhalation. Blood samples were collected for the estimation of hepatic marker enzymes and biochemical parameters. Liver tissues were collected in ice cold phosphate buffered saline for the determination of reduced glutathione (GSH) level in hepatocytes. The liver tissues were also collected in formalin (10%) for histopathological studies (Lin *et al*., 1995).

2.4.1. Biochemical estimation

The collected blood was allowed to coagulate for 1 h at room temperature and centrifuged at 1500 rpm for 15 min at 37°C to separate the serum. . The serum was then used for the assay of marker enzymes, namely aspartate aminotransferase (AST), alanine aminotransferase (ALT), serum alkaline phosphatase (ALP) and serum bilirubin (SB) according to standard methods. Other serum biochemical parameters such as serum cholesterol, triglycerides, creatinine, urea and uric acid levels were also estimated.

2.4.2. Determination of reduced glutathione (GSH)

Glutathione was estimated by the procedure of Ellmann (1959). To measure the reduced glutathione (GSH) 0.2 mL of tissue homogenate was mixed with 1.8 mL of EDTA solution. To this 3.0 mL precipitating reagent (1.67 g of meta phosphoric acid, 0.2 g of EDTA disodium salt, 30 g sodium chloride in 1 L distilled water) was added, mixed thoroughly and kept for 5 min before centrifugation. To 2 mL of the supernatant, 4.0 mL of 0.3 M disodium hydrogen phosphate solution and 1.0 mL of DTNB (5,5-dithio-bis-2-nitrobenzoic acid) reagent were added and absorbance was read at 412 nm. Absorbance values were compared with a standard curve generated from known GSH.

2.4.3. Lipid peroxidation study (*in vivo*)

Lipid peroxidation was estimated by the method of Ohkawa *et al*. (1979). Liver homogenate was mixed with 100 µL of 8.1% sodium dodecyl sulphate and 600 µL of 20% acetic acid solution, kept for 2 min at room temperature, then 600 µL of 0.8% solution of thiobarbituric acid (TBA) was added, heated at 95°C for 60 min in water bath and cooled with ice cold water at 4°C. Then n-butanol was added, shaken vigorously and centrifuged at 10,000 rpm for 5 min. The absorbance of the organic layer was measured at 532 nm. Lipid peroxidation was expressed as n moles of MDA/g of wet liver tissue.

2.4.4. Histopathological studies

Liver specimens obtained from the control and the treated groups of the experiment were trimmed to small pieces and preserved in formalin (10% solution) for 24 h. They were subjected to dehydration with acetone of strength 70, 80 and 100% respectively, each for 1 h. The infiltration and impregnation was done by treatment with paraffin wax, twice each time for 1 h. Specimens were cut into sections of 3-7 µm thickness and stained with haematoxylin and eosin and mounting

of the specimens was done using Distrene Phthalate Xylene (DPX). The specimens were observed under high resolution microscope (magnification × 400) with camera and attachment (Carl Zeiss, Germany).

2.5. Statistical analysis

All the analyses were carried out in triplicate. The statistical significance between the samples were determined by the Analysis of Variance (ANOVA) and the data were recorded as mean ± Standard Deviation (SD). $P \leq 0.05$ was considered to be statistically significant. Significant differences between means were determined by Duncan's Multiple Range tests (Raghava, 1987). The computer software employed for the statistical analysis was IBM SPSS Statistics, version 20 (USA).

3. Results and Discussion

D-Galactosamine (D-GalN) induces diffuse type of liver damage closely resembling human viral hepatitis and acute self-limiting hepatitis with necrosis, inflammation and regeneration, resembling drug induced disease in human. The metabolism of D-GalN may deplete several uracil nucleotides including UDP-glucose, UDP galactose and UTP, which trapped in the formation of uridine-diphosphogalactosamine. Accumulation of UDP-sugar nucleotide may contribute to the change in the rough endoplasmic reticulum and to the disturbance of protein metabolism. Intense galactosamine of the membrane structure is thought to be responsible for loss in the activity of ionic pumps. The impairment in the calcium pumps with consequent increase in the intracellular calcium is considered to be responsible for cell death (Nakagiri *et al*., 2003). Evidences support the fact that the release of reactive oxygen species (ROS) and cytokines such as tumour necrosis factor (TNF-α) and interleukin-1 (IL-1) by Kupffer cells in the liver also contribute towards the hepatocyte damage in D-GalN hepatotoxicity (Padmanabhan and Jangle, 2014).

Keppler *et al*. (1974), suggested that the toxicity of D-GalN is mainly related to the depletion of uridine pools that are associated with limited ribonucleic acid (RNA) and protein synthesis through a decrease in the cellular UTP concentration, thus altering hepatocellular functions. According to Palanisamy *et al*. (2007), rapid depletion of uridine diphosphate glucose appears to be the first biochemical lesion, followed by defects in glycoprotein synthesis which is secondary biochemical lesion. This leads to eventual damage of cellular membranes and ultimately to spotty liver cell necrosis. Then the cellular damage provokes inflammatory reaction or tertiary reactions, resulting in a picture closely resembling viral hepatitis

D-Galactosamine intoxication is known to cause marked elevation in liver enzyme levels (Jonker *et al*., 1992). D-GalN administration in rats disrupts the membrane permeability of the plasma membrane, causing leakage of the enzymes from the cell and damage to hepatocytes which lead to elevation in levels of serum enzymes (Mitra *et al*., 2000). In the present study, the levels of hepatic marker enzymes AST, ALT, ALP and other biochemical parameters such as total cholesterol, bilirubin and triglycerides increased significantly in the group treated with D-GalN (400 mg/kg b.wt) compared to normal control (Table 1 and Table 2). Hepatocellular necrosis also leads to an increase in serum levels of both AST and ALT which are released from the liver into the blood stream (Wu *et al*., 2012).

Alkaline phosphatase (ALP) is mainly produced in bile duct and its release is enhanced in conditions such as cholestasis. The bile duct obstruction may cause more pronounced elevation in ALP level. D-GalN treated group showed an increase in serum ALP compared to normal control and the rise is due to the disturbance in the secretory activity or in the transport of metabolites or may be due to altered synthesis of certain enzymes (Kumar *et al*., 1978). ALP activity is related to the hepatocyte function and an increase in its activity may also be due to the elevated enzyme synthesis in presence of increased biliary pressure (Sureshkumar *et al*., 2006).

The administration of coded drug 222 (125 mg/kg) resulted in a significant ($P \leq 0.05$) reduction of AST, ALT and ALP towards normal values (Table 1) and it is an indication of stabilization of plasma membranes, protecting the liver by restoring the altered levels in rats as well as repair of damage tissues caused by D-GalN. The standard drug siymarin treated group also significantly reduced the elevated D-GalN-induced hepatic enzymes to a near normal level (Table 1). The serum bilirubin level is an index of hepatic

function and any deformity found in the levels of the same suggested abnormal hepatocellular function. The elevated levels of total bilirubin in D–GalN intoxicated rats were in agreement with previous reports (Shivashangari *et al*., 2006). Coded drug 222 leaf ethanolic extract (125 mg/kg) mediated suppression of the increased bilirubin levels in the treatment groups (Table 1) suggest that the extract was able to alleviate the hepato-biliary dysfunction induced by D-GalN.

Marked alterations in lipid metabolism have been reported in D-GalN induced hepatitis in rats (Shivashangari *et al*., 2006). D-GalN, a well-known hepatotoxin that produces non-zonal lesions, has been used as a model of massive hepatic inflammation limited to hepatocytes. The results showed increased levels of cholesterol and triglycerides levels in D-GalN injected rats (Table 2). The increased cholesterol level may be due to increased HMG-CoA reductase activity, which is the rate-limiting step in cholesterol biosynthesis. A number of agents that produce liver injury also cause the accumulation of abnormal amounts of fat, predominately triglycerides, in the parenchymal cells. Cartwright *et al*. (1982) reported the increased accumulation of triglycerides during D-GalN-induced hepatitis in rats. Pretreatment with coded drug 222 leaf ethanolic extract significantly ($P \leq 0.05$) lowered serum cholesterol and triglyceride levels compared to D-GalN control (Table 2).

According to Javlé *et al*. (1998), D-GalN-induced liver injury is associated with the development of renal failure. The results from the present study showed that D-GalN induced rats significantly increased the levels of creatinine, urea and uric acid compared to the normal control (Table 2). Urea is the major nitrogen containing metabolic product of protein metabolism; uric acid is the major product of purine nucleotides; creatinine is endogenously produced and released into body fluids and its clearance is measured as an indicator of glomerular filtration rate. Coded drug 222 (125 mg/kg) treated group exhibited a significant ($P \leq 0.05$) decrease in the elevated levels of serum creatinine, urea and uric acid in D-GalN toxicated animals and can be comparable to the standard drug Silymarin treated group (Table 2).

Zhou *et al*. (2008) reported that, treatment with D-GalN decreased hepatic antioxidative enzyme activities especially that of GSH. It is known that GSH plays a crucial role in detoxification and cellular defense and the loss in GSH content reflects oxidant defense mechanism. It also provides hepatocytes with resistance to oxidative stress (Irita *et al*., 1994). Intracellular free radical production gradually increases with D-GalN concentration in rat hepatocytes. It had been reported that administration of a hepatotoxic dose of D-GalN induced a significant decrease in hepatic GSH levels in rats due to oxidative stress (Ohta *et al*., 2007). In the present study, the results showed that GSH was depleted in the liver of rats after D-GalN administration compared to the normal control group (Fig. 1). GSH content was expected to be consumed by enhanced radical reactions. Coded drug 222 (125 mg/kg) administration reversed the condition by a significant ($P \leq 0.05$) increase in GSH level and it was almost comparable to Silymarin group (Fig.1).

Oxidative stress and lipid peroxidation are mediated by oxygen free radicals leading to chronic liver damage and hepatic fibrosis (DiSario *et al*., 2005). Hepatocytes are well recognized as being continuously exposed to reactive oxygen species in various liver diseases. It includes various forms of activated oxygen and nitrogen which lead to generation of free radicals. These free radicals are continuously formed as a result of exposure to exogenous chemicals and endogenous metabolic reactions involving bioenergetic electron transfer and redox enzymes. It has been reported that oxygen derived free radicals released from activated hepatic macrophages are the primary cause of D-GalN induced liver damage (Hu and Chen, 1992). In the hepatic injury caused by D-galactosamine, it has been suggested that reactions involving excessive production of free radicals generated in response to D-GalN intoxication can damage macromolecules such as lipids by lipid peroxidation. Increased level of hepatic tissue malondialdehyde (MDA) is detected in rats treated with D-GalN which is an indication of enhanced lipid peroxidation (Hu and Chen, 1992).

In free radical mediated tissue injury, lipid peroxidation leads to increase in the level of phospholipids and alteration in membrane fluidity, which is essential for liver cell function. Phospholipids are vital components

of biomembranes, and their composition greatly affects the properties and functions of the membrane, including signal transduction. This alteration in the membrane composition might be one of the reasons for the toxic effect caused by D-GalN and the coded drug 222 extract administration decreased the levels of free fatty acid and phospholipids, thereby preventing the toxic complication produced by increased levels of these lipids.

Several plants significantly prevented the D-Galactosamine-induced alterations in respiration and oxidative phosphorylation of liver mitochondria (Anandan *et al*., 1999). It is known that a variety of antioxidants scavenge free radicals to prevent oxidative damage in the cell. Hepatic MDA levels were found to be increased in D-GalN treated rats in comparison to normal control rats and the difference was statistically significant ($P \leq 0.05$). The pretreatment with 222 (125 mg/kg) probably inhibited oxidative liver injury through its antioxidant effect as evidenced from the significant ($P \leq 0.05$) decrease (48.85 %) in the MDA level compared to the toxin control (Table 3). The biochemical findings were also in line with the decreased MDA level.

The standard hepatoprotective drug, Silymarin reduces free radical production and lipid peroxidation in the setting of hepatotoxicity as it has powerful antioxidant effects. It may act through the ability for toxin blockade by binding to the hepatocyte cell membrane receptor and inhibits the binding of toxins to these receptors. The increased D-GalN-induced MDA level was significantly ($P \leq 0.05$) reduced in Silymarin control group (67.78 %) (Table 3).

D-Galactosamine is reported to produce intensive inflammatory infiltration in the liver parenchyma and peripheral areas (El-Mofty *et al*., 1975).In histopathological studies, the normal control group exhibited no evidence of any microscopic abnormalities. The histopathological studies in the transverse liver sections showed normal parenchymal architecture hepatocytes in the normal control group with no cell injury or cirrhosis found. D-GalN group showed vasculitis, represented by congested blood vessels and perivascular edema and few lymphocyte infiltrations, ballooning, degeneration of the hepatocytes as well as focal necrosis within lobules is noted. D-GalN showed complete damage to hepatocytes with hepatocellular vacuolization, focal hepatic necrosis and congestion of hepatic sinusoids. Pretreatment with 222 at dose 125 mg/kg and standard drug Silymarin control appeared to significantly prevent the D-galactosamine toxicity as revealed by the hepatic cells with were preserved cytoplasm (Fig. 2).

Table 1. The effect of the coded drug 222 leaf ethanolic extract on rat serum parameters after D-Galactosamine (D-GalN) induced hepatotoxicity

Groups	ALT (IU/L)	AST (IU/L)	ALP (KA units/100 mL)	Bilirubin (mg/dL)
Normal control	33.30 ± 1.34	63.00 ± 1.41	15.20 ± 0.63	0.16 ± 0.01
Toxin control D-Galactosamine(400 mg/kg)	97.62 ± 4.17	112.45 ± 1.81	67.50 ± 0.63	1.69 ± 0.01
222 ethanolic extract (125 mg/kg)	44.49 ± 0.72**	73.52 ± 0.91**	27.80 ± 3.40**	0.63 ± 0.01**
222 ethanolic extract (250 mg/kg)	56.06 ± 0.63	85.19 ± 0.94	42.41 ± 5.58	1.03 ± 0.01
222 ethanolic extract (500 mg/kg)	69.41 ± 0.84	92.29 ± 0.81	57.35 ± 3.55	1.09 ± 0.02
Standard control Silymarin (100 mg/kg)	39.24 ± 0.56	68.40 ± 0.98**	23.80 ± 0.67**	0.65 ± 0.01**

Values are the mean ± SD, n=6 in each group, analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. **Significance $P \leq 0.05$ compared to the toxin control.

Table 2. The effect of the coded drug 222 leaf ethanolic extract on rat serum parameters after D-Galactosamine (D-GalN) induced hepatotoxicity

Groups	Cholesterol (mg/dL)	Triglycerides (mg/dL)	Creatinine (mg/dL)	Urea (mg/dL)	Uric acid (mg/dL)
Normal control	45.14±1.33	60.22±1.45	0.84±0.03	18.11±0.30	2.11±0.09
Toxin control D-Galactosamine (400 mg/kg)	92.26±1.21	132.70±2.17	2.92±0.95	35.44±1.18	5.82±1.73
222 ethanolic extract (125 mg/kg)	60.10±1.08**	73.43±1.25**	0.90±0.04**	19.61±0.47**	2.27±0.20**
222 ethanolic extract (250 mg/kg)	78.77±1.38	89.55±1.74	1.15±0.59	27.54±0.66	3.11±0.72
222 ethanolic extract (500 mg/kg)	80.54±1.42	94.38±1.82	1.28±0.66	29.28±0.82	3.43±0.86
Standard control Silymarin (100 mg/kg)	56.11±1.15**	69.02±1.88**	0.86±0.04**	18.45±0.37	2.18±0.17

Values are the mean ± SD, n=6 in each group, analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. **Significance $P \leq 0.05$ compared to the toxin control.

Table 3. The effect of the coded plant 222 leaf ethanolic extract on lipid peroxidation *in vivo* after D-Galactosamine (D-GalN) intoxication

Groups	MDA (n mol/g wet liver)	MDA inhibition (%)
Normal control	3.25 ± 0.02	----------
Toxin control D-Galactosamine (400 mg/kg)	16.54 ± 1.22	----------
222 ethanolic extract (125 mg/kg)	8.46 ± 0.57**	48.85 %
222 ethanolic extract (250 mg/kg)	11.67 ± 0.81	29.44 %
222 ethanolic extract (500 mg/kg)	12.33 ± 0.93	25.45 %
Standard control Silymarin (100 mg/kg)	5.33 ± 0.27**	67.78 %

Values are the mean ± SD, n=6 in each group, Analysis of Variance (ANOVA) followed by Duncan's multiple range test. **Significance $P \leq 0.05$, compared to D-GalN control.

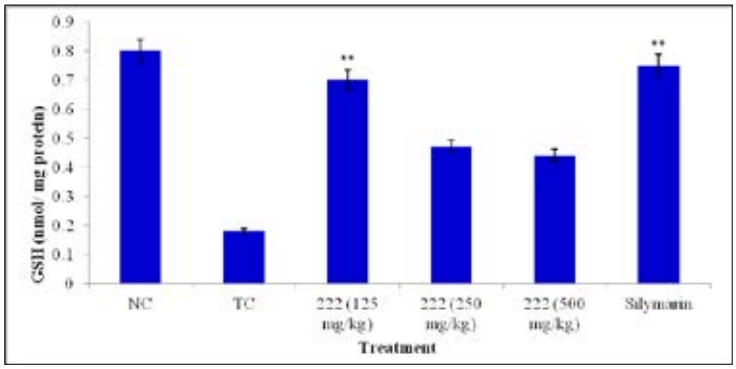
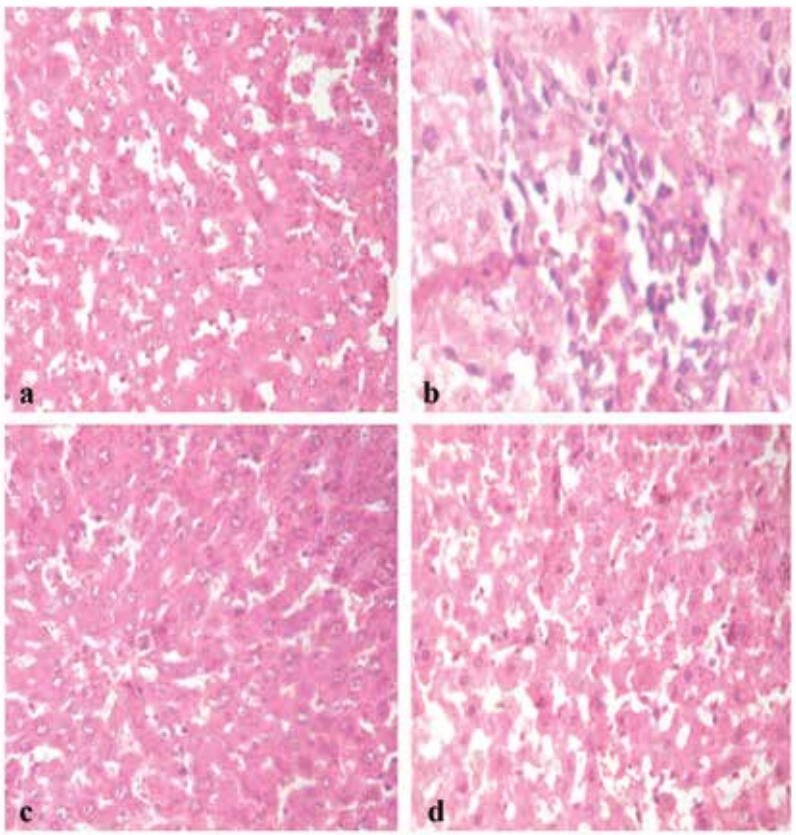


Fig. 1. Effect of leaf ethanolic extract coded drug 222 on reduced glutathione (GSH) after D-Galactosamine (D-GalN) intoxication (NC: Normal Control, TC: Toxin (D-GalN) Control)

Values are the mean ± SD, n=6 in each group, Analysis of Variance (ANOVA) followed by Duncan's multiple range test. **Significance $P \leq 0.05$, compared to D-GalN control.



Histopathological architecture of the liver treated with D-galactosamine. a) Liver of normal control group illustrating normal hepatic architecture (x 400). b) Liver of D-gal treated group showing hepatocyte damage (x 400). c) Liver of 222 ethanolic extract (125 mg/kg) treated group with near normal appearance (x 400). d) Liver of silymarin treated group showing normalcy in hepatocyte arrangement (x 400).

4. Conclusion

The present study demonstrated that the hepatic marker enzymes and serum bilirubin levels were significantly decreased by 222 at 125 mg/kg dose to the normal range. The elevated levels of other biochemical parameters such as serum cholesterol, triglycerides, creatinine, urea and uric acid after D-GalN intoxication were significantly lowered in the coded drug 222 leaf ethanolic extract (125 mg/kg) and standard drug Silymarin control. The hepatic antioxidant enzyme status of coded drug 222 extract was almost comparable to Silymarin group. The increased D-GalN-induced MDA level was also significantly ($P \leq 0.05$) reduced in both coded drug 222 (125 mg/kg) and Silymarin control group. These findings are supported by the histopathological studies. The protective action of 222 ethanolic extract at 125 mg/kg dose may be due to the inhibition of UDP-sugar derivatives, enhancing the biosynthesis of glycoprotein, stabilizing the hepatocellular membrane. The extract may also inhibit neutrophil infiltration into the liver cells, preventing the process of lipid peroxidation.

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