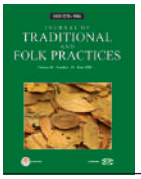

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Coded plant (222) leaf ethanolic extract ameliorates ethanol-induced liver damage and oxidative stress in Wistar albino rats – Part V

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Abstract

In the present study, the hepatoprotective effect of the leaf ethanolic extract of coded plant (Code No. 222**) against ethanol-induced hepatic injury and oxidative stress in Wistar albino rats was evaluated. The animals were divided into different groups and treated with 222 leaf ethanolic extract (125 mg/kg) for twenty days. Silymarin, the standard hepatoprotective compound (100 mg/kg) was administered orally for twenty days. Hepatotoxicity was induced by the oral administration of ethyl alcohol 36.6% (v/v) 30 mL/kg/day and corn oil (10 mL/kg/day) in three divided doses for 20 days. The ethyl alcohol 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. Ethyl alcohol toxicity also resulted in a significant ($P \leq 0.05$) increase in serum cholesterol, triglycerides and creatinine and decrease in total protein 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 ethyl alcohol induced hepatotoxic animals. The reduced levels of hepatic enzymes and serum cholesterol and triglyceride levels exhibited by 222 (125 mg/kg) was comparable to the standard control Silymarin. The decreased antioxidant enzyme status due to ethanol induced heatotoxicity was also normalized by the treatment with 222 (125 mg/kg) dose compared to Silymarin. The histopathological examination also supported the hepatoprotective effect of the extract. The results of the present study indicated that coded plant 222 leaf ethanolic extract (125 mg/kg) can effectively reverse ethyl alcohol induced liver damage and oxidative stress in experimental animals.

Keywords: Hepatotoxicity, Oxidative stress, Ethyl alcohol, Hepatoprotective, Traditional healer

1. Introduction

Hepatotoxicity is one of the common ailments resulting into serious debilities ranging from severe metabolic disorders to even mortality (Jaeschke *et al*., 2002). Reactive Oxygen Species (ROS) are continually generated in the biological system during metabolic processes for the regulation of essential physiological functions. When the production of ROS exceeds the capability of the antioxidant enzymes to detoxify

these reactive intermediates, oxidative stress would be generated and this is the major cause for the pathophysiology of many diseases (Mena *et al*., 2009). Ethyl alcohol is a common cause for ROS insult in the liver and its metabolism results in the generation of excessive free radicals and increase peroxisomal oxidation of fatty acids, which would ultimately affect the antioxidant system functionality in the elimination of ROS (Das and Vasudevan, 2007). Free radicals initiate the damaging process through covalent binding to cell macromolecules resulting in lipid peroxidation, oxidation of protein and DNA causing hepatic damage. Enhanced rate of lipid peroxidation during liver microsomal metabolism of ethyl alcohol may cause hepatitis and cirrhosis (Smucklers, 1975).

Alcohol abuse and alcoholism represents one of the major health, social and economic issues facing the world, and alcoholic liver disease is one of the most serious consequences of chronic alcohol consumption. Ethyl alcohol is widely consumed alcoholic beverage in modern society and it forms one of the major causes of a variety of medical problems and liver diseases worldwide (Cebral *et al*., 1997). Recent studies suggested that ethanol-induced oxidative stress and inflammation generate vast amounts of cytokines, chemokines, nitric oxide, tumor necrosis factor-alpha (TNF-α), transfusing growth factor-beta (TGF-β) and ROS, which play a major role in the pathogenesis and progression of alcoholic liver diseases (Kumar *et al*., 2012).

Currently, treatment modalities for hepatotoxicity are limited and modern medicine has little to offer for the alleviation of hepatic diseases. Therefore, the mechanism to reverse hepatic damage by alcoholic oxidative stress is tightly regulated by antioxidant status of the biological system. The antioxidant medicinal plants can prevent hepatic tissue damage and cell death caused by chronic alcohol consumption (Albano *et al*., 1994). Considerable attention has been given in recent years to the development of complementary and alternative medicines for the treatment of liver diseases and the therapeutic agents developed from natural sources have the potential to reduce the risk of drug toxicity (Batey *et al*., 2005).

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 paracetamol and carbon tetrachloride induced liver damage (Krishnakumar *et al*., 2017 a, b). On verification, no pharmacological studies related to the hepatoprotective potential of the coded plant 222 against ethyl alcohol induced hepatotoxicity had been so far conducted. In the present study, the hepatoprotective property of coded plant 222 leaf ethanolic extract against ethyl alcohol induced liver damage in Wistar rats is reported.

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

Sodium dodecyl sulphate (SDS), thiobarbituric acid (TBA), ethylene diamine tetra acetic acid (EDTA), 5,5-dithio-bis-2-nitrobezoic acid, nitro blue tetrazolium (NBT) and hydroxylamine hydrochloride were purchased

from Sigma Aldrich, USA. Commercial kits for the estimation of aspartate aminotransferase (AST), alanine aminotransferase (ALT), serum alkaline phosphatase (ALP), serum bilirubin (SB), serum cholesterol, triglycerides, total protein, creatinine, albumin, urea, uric acid and microprotein 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-01/12/2011/EM & EP - 09).

2.4. Ethyl alcohol-induced hepatotoxicity study

Alcohol has a calorific value of about 7 calories of dietary carbohydrates, so in moderate quantities, it is a food in itself except for vitamins, minerals etc. Hence throughout the experiment, some quantity of glucose (isocaloric to the amount of alcohol) was also administered to all the animals of the control group in order to equalize the calories due to alcohol in other groups. Corn oil is known to contain maximum oleic acid and linoleic acid. Dietary linoleic acid is essential for development of experimentally induced liver damage and Nanji *et al*. (1989) reported that ethanol with corn oil caused severe pathological changes in the rat liver. Hence throughout the experiment, corn oil (10 mL/kg/day) was also administered to all animals of all groups.

Wistar male albino rats were divided into six groups of six animals in each group. Group I served as control and received 1 % Tween-80 in distilled water as vehicle (30 mL/kg/day), corn oil (10 mL/kg/day) and glucose isocaloric to the amount of alcohol in three divided doses. Group II received ethyl alcohol (36.6 % v/v) 30 mL/kg/day and corn oil (10 mL/kg/day) in three divided doses. The coded drug 222 extract at 125 mg/kg dose was found to be effective in paracetamol and carbon tetrachloride induced toxicity studies *in vivo* (Krishnakumar *et al*., 2017 a, b), so that the single dose of the coded extract (125 mg/kg) was fixed in the present study. Group III received the coded drug 222 ethanolic extract at 125 mg/kg/day along with alcohol (30 mL/kg/day) and corn oil (10 mL/kg/day) in three divided doses. Group IV received standard drug Silymarin (100 mg/kg/day) along with alcohol and corn oil as above. All the animals received their respective treatment for 20 days by forced oral administration. On the 21st day, all the animals were sacrificed by carbon dioxide inhalation. Blood samples were collected and serum was separated for the estimation of hepatic marker enzymes and biochemical parameters by standard methods. Liver tissues were collected in ice cold phosphate buffered saline for the determination of antioxidant enzyme status of the liver. The liver tissues were also collected in formalin (10%) for histopathological studies (Jafri *et al*., 1999).

2.4.1. Assessment of liver function and estimation of serum biochemical parameters

The collected blood was allowed to coagulate for 1h 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 hepatic marker enzymes such as aspartate aminotransferase (AST), alanine amino-transferase (ALT), serum alkaline phosphatase (ALP) and serum bilirubin (SB) in the serum samples of all the groups of the animals were estimated according to standard methods. The serum levels of biochemical parameters like cholesterol, triglycerides, total protein,

creatinine, albumin, urea, uric acid and microprotein were also determined.

2.4.2. Determination of antioxidant enzyme status of the liver

2.4.2.1. Evaluation of superoxide dismutase (SOD)

The assay was based on the reduction of nitro blue tetrazolium (NBT) to water insoluble formazan. 0.5 mL of the liver homogenate was taken and added 1 mL of 50 mM sodium carbonate, 0.4 mL of 24 µm NBT and 0.2 mL of 0.1 mM EDTA. The addition of 0.4 mL of 1 mM hydroxylamine hydrochloride initiated the reaction. Absorbance was measured at 560 nm at zero time followed by second measurement after 5 min at 25°C. The control was simultaneously run without liver homogenates. Units of SOD activity were expressed as the amount of enzymes required to inhibit the reduction of NBT by 50% and the specific activity was expressed in terms of units/mg of protein (Rathod *et al*., 2009).

2.4.2.2. Assay of catalase

Catalase was assayed according to the method of Aebi (1974). To 0.9 mL of phosphate buffer (0.01 M, pH 7.0) 0.1 mL of tissue homogenate and 0.4 mL of H_2O_2 (0.2 M) were added. After 60 sec, 2 mL of dichromate - acetic acid reagent (5%) was added. The tubes were kept in boiling water bath for 10 min and the colour developed was read at 620 nm. Standard H_2O_2 in the range of 2-10 µL were taken with blank containing reagent alone. The rate of H_2O_2 reduction was a measure of CAT activity. The activities were expressed as n moles of H_2O_2 consumed/ min/ mg protein. Dichromate in acetic acid was converted to perchromic acid and then chromic acetate, when heated in the presence of H_2O_2. The chromic acetate formed was measured at 620 nm.

2.4.2.3. 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.

2.4.2.4. Estimation of Malondialdehyde

Malondialdehyde in alcohol treated rat liver was estimated by the modified procedure of Ohkawa *et al*. (1979). Liver homogenate (1mL) mixed with 100 µL of 8.1 % SDS and 600 µL of 20% acetic acid solution was kept for 2 min at room temperature. Then 600 µL of 0.8% solution of TBA was added, heated at 95º C for 60 min in water bath and cooled with ice cold water at 4º C. The mixture of n-butanol and pyridine (15:1 v/v) were 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/ mg of protein.

2.4.3. 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 diffe rences between means were determined by Dunnett's multiple comparison test (Raghava, 1987). The computer software employed for the statistical analysis was IBM SPSS Statistics, version 20 (USA).

3. Results and Discussion

High alcohol consumption results in critical problems in the body such as alcoholic liver diseases and it had been associated to a spectrum of liver injuries with varying degrees of severity including acute inflammation, a wide spectrum of lesions, fatty liver, alcoholic hepatitis, fibrosis, steatonecrosis and cirrhosis (Kai, 1995). Ethanol is known to have a profound effect on the metabolism of lipids and lipoprotein. More than 80% of ingested alcohol is metabolized in the liver without feedback mechanism. Accumulation of lipids in the hepatocytes is the most striking initial manifestation of alcohol induced liver injury (Lieber, 2000).

Ethanol is first metabolized to acetaldehyde and then converted to acetic acid, and acetaldehyde is extremely reactive and toxic which binds to phospholipids, amino acid residues and sulphydryl groups resulting in hepatic damage (Rang *et al*., 2003). One of the factors that play a central role in many pathways during alcohol induced damage is oxidative stress. Free radical mediated oxidative stress and induction of cytochrome P_{450} to produce malondialdehyde (MDA) by ethanol plays an important role in the pathogenesis of ethanol induced damage of plasma membrane receptors (Shaw *et al*., 1995). Tuma *et al*. (1996) reported that acetaldehyde and MDA can react together in a synergistic manner and generate highly toxic hybrid adducts which is one of the main reasons for the progression of alcoholic liver damage. Therefore, medicinal plants containing antioxidant compounds attract great attention for the development of herbal drugs against alcohol induced hepatic diseases.

The results of the present study showed that ethanol effectively induced hepatotoxicity in toxin control group as reflected by the increased hepatic marker enzyme levels, decreased antioxidant enzymes of the liver and histopathological architecture of the toxin control group. Elevation of ALT and AST levels implied the disruption of plasma membrane integrity which eventually leads to leakage of hepatic enzymes into the blood stream (Johnston, 1999). The levels of hepatic marker enzymes are increased significantly ($P \leq 0.05$) in ethanol intoxicated animals compared to the normal control group. Administration of the coded plant 222 leaf ethanolic extract (125 mg/kg) significantly ($P \leq 0.05$) prevented ethyl alcohol induced elevation of hepatic marker enzymes compared to the standard control Silymarin (100 mg/kg) (Table 1). This indicated the stabilization of plasma membrane and repair of hepatic tissue damage caused by ethanol.

The increased serum alkaline phosphatase level in ethanol intoxicated animals is due to defective hepatic excretion. The extract treated group normalized the elevated ALP level compared to the standard control (Table 1). The elevated serum total bilirubin level reflected hepatic damage caused by the blockage of bile ducts that obstructed the secretion of bile due to alcohol toxicity. The production of ALP is elevated during pathological conditions such as bile duct obstructions, primary biliary cirrhosis, adult bile ductopenia and metastatic liver diseases. ALP and bilirubin levels measure how well the liver functioned instead of extent of hepatic injury. Treatment of ethanol fed rats with 222 (125 mg/kg) caused significant ($P \leq 0.05$) reduction of serum bilirubin and almost comparable to Silymarin control and normal group (Table 1).

Alcohol is metabolized in the liver as a process of detoxification and it occurs mainly via alcohol dehydrogenases, which requires the cofactor NAD^+. The reduced form of NAD^+ (NADH) is attenuated by the excess alcohol concentration and it causes hepatic NADH accumulation. This result in the increased synthesis of fatty acids and triglycerides and β-oxidation of fatty acids will be impeded (Dancygier *et al*., 2010). One of the early signs of excessive ethanol consumption is the liver enlargement caused by the retained water

in the cytoplasm of hepatocytes. According to Gopumadhavan *et al*. (2008), hepatomegaly is a condition after chronic ethanol ingestion and it is mainly due to increased levels of fats and proteins. Elevation of total lipids after ethanol intoxication was mainly due to the increase in cholesterol and triglycerides which leads to develop fatty liver. This may be due to the activation of HMG Co-A reductase enzyme, the rate limiting step in biosynthesis of cholesterol (Suja *et al*., 2014). The cholesterol and triglyceride levels of ethanol control group were significantly ($P \leq 0.05$) higher compared to normal control. The coded plant 222 leaf ethanolic extract (125 mg/kg) significantly ($P \leq 0.05$) reduced the elevated levels of cholesterol and triglycerides in ethanol intoxicated rats (Table 2).

The level of total proteins depends upon the addition of albumin and globulin levels and ethanol administration decreased total protein and albumin levels caused by alcohol induced liver damage. This damage is attributed to the higher concentration of alcohol dehydrogenase enzyme which catalyses alcohol to aldehyde and accumulation of export type proteins due to inhibition of the secretion of the proteins from the liver of alcoholics (Baraona and Lieber, 1982). The level of total protein and albumin in ethanol control group was significantly ($P \leq 0.05$) lower than the normal control group. The leaf ethanolic extract of coded plant 222 (125 mg/kg) was able to restore the levels of total protein and albumin in alcohol intoxicated rats to near the normal value when compared with standard and normal control groups (Tables 2 and 3).

The protective effect of 222 ethanolic extract (125 mg/kg) on ethanol induced toxicity of rat liver may be achieved by preserving the structural integrity of the liver from the toxic effect of alcohol, decreased cholesterol synthesis, decreased absorption from intestine, increased catabolism, decreased fatty acid synthesis, increased utilization or decreased glycerol formation, decreasing the toxicity of ethanol and its metabolic product, acetaldehyde.

A number of *in vivo* experiments demonstrated that either acute or chronic ethanol administration to the experimental animals increases the formation of lipid peroxidation products like malondialdehyde (MDA) and decrease the levels of antioxidant enzymes such as superoxide dismutase (SOD) and reduced glutathione (GSH) (Das and Vasudevan, 2006). The oxidative stress in the liver tissue was assessed by measuring the levels of thiobarbituric acid reactive substances (TBARS) and antioxidant defence enzymes. Binding of acetaldehyde with GSH may contribute to the reduction in GSH level and may lead to increased lipid peroxidation with concomitant changes in membrane permeability and hepatic cellular damage (Prakash, 1998). The ethanol administered groups showed significant increase in MDA level, when compared with normal control group. The treatment with leaf ethanolic extract of coded plant 222 (125 mg/kg) decreased MDA level in a dose dependent manner (Table 4). Standard control Silymarin (100 mg/kg) significantly reduced the rise in MDA level which can be comparable to the normal control. There was a marked decrease in the levels of liver antioxidant defence enzymes such as SOD and GSH in the ethanol treated groups compared to normal control. GSH depletion is an important mechanism in the sensitization of liver to alcohol induced injury (Yuan *et al*., 2007). The mechanism to restore hepatic injury caused by alcoholic oxidative stress is tightly regulated by the antioxidant status of the living system. The depletion of SOD and GSH was inhibited by 222 extract (125 mg/kg) and Silymarin (100 mg/kg). The catalase level showed no significant difference between control and treated groups. (Table 4). Catalase contribution might be enhanced if significant amounts of H_2O_2 become available through β-oxidation of fatty acids. It is capable of oxidizing alcohol *in vitro* in the presence of an H_2O_2 generating system. But, under physiological conditions catalase appears to play no major role (Lieber, 1997). The coded drug extract could normalize the levels of SOD and GSH by increasing the levels by effectively modulating the antioxidant pathways. Coded drug 222 at 125 mg/kg dose was effective in retrieving the normal levels of antioxidant enzymes.

In histopathological studies, the liver slices of normal control rats showed normal hepatic architecture and no fatty changes, whereas rats treated with ethyl alcohol showed moderate to marked fatty changes, liver cell degeneration, hepatic necrosis and active Kupffer cells. The coded drug 222 extract and Silymarin treated groups produced a marked degree of protection against ethyl alcohol induced alterations, and liver sections from this group were almost comparable to those from unchallenged control rats (Fig. 1).

Table 1. The effect of the coded drug 222 leaf ethanolic extract on rat serum parameters after ethanol (EA) induced hepatotoxicity

Groups	ALT (IU/L)	AST (IU/L)	ALP (KA units/100 mL)	Bilirubin (mg/dL)
Normal control	44.03±2.70	41.70 ±1.56	28.46±3.76	0.42±0.03
Ethanol (EA) toxin control	86.22±1.68	83.56 ±5.80	65.87±2.60	1.75±0.02
EA + 222 ethanolic extract (125 mg/kg)	48.14±3.54**	48.74±2.46**	36.21±5.31**	0.68±0.05**
EA + Silymarin (100 mg/kg)	46.37±3.85**	46.22±0.96**	33.95±3.51**	0.52±0.05**

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 ethanol (EA) induced hepatotoxicity

Groups	Cholesterol (mg/dL)	Triglycerides (mg/dL)	Total protein (g/dL)	Creatinine (mg/dL)
Normal control	34.42±1.61	58.93±2.90	6.82±0.20	0.91±0.01
Ethanol (EA) toxin control	71.98±1.90	151.85±4.99	2.89±0.14	1.06±0.01
EA+ 222 ethanolic extract (125 mg/kg)	50.74±2.11**	100.00±1.22**	4.52±0.12**	0.95±0.04**
EA + Silymarin (100 mg/kg)	45.82±2.49**	69.02±1.88**	5.34±0.06**	0.95±0.02**

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 drug 222 leaf ethanolic extract on rat serum parameters after ethanol (EA) induced hepatotoxicity

Groups	Albumin (g/dL)	Uric acid (mg/dL)	Urea (mg/dL)	Microprotein (mg/dL)
Normal control	1.50±0.05	2.39±0.55	22.27±0.53	18.80±5.56
Ethanol (EA) toxin control	0.48±0.14	5.05±1.13	30.10±0.43	6.51±2.36
EA + 222 ethanolic extract (125 mg/kg)	1.68±0.14**	3.57±0.07**	25.05±0.67	15.30±5.89**
EA + Silymarin (100 mg/kg)	1.55±0.01**	2.91±0.20**	23.37±0.89	17.38±3.77**

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 4. Effect of leaf ethanolic extract coded drug 222 on malondialdehyde (MDA), superoxide dismutase (SOD), reduced glutathione (GSH) and catalase (CAT) after ethanol (EA) induced hepatotoxicity

Groups	MDA (nmol/ mg wet liver)	SOD (U/mg protein)	GSH (nmol/ mg protein)	CAT (U/mg protein)
Normal control	0.461±0.001	16.22±0.87	0.766±0.002	59.00±1.09
Ethanol (EA) toxin control	1.229±0.003	4.13±0.35	0.230±0.006	56.21±4.87
EA +222 ethanolic extract (125 mg/kg)	0.512±0.001**	13.38±0.51**	0.569±0.072**	57.30±5.89
EA + Silymarin (100mg/kg)	0.457±0.009**	15.87±0.64**	0.616±0.010**	60.93±2.24

Values are mean ±SD, n=6, in each group, analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. ** Significance, $P \leq 0.01$, compared to toxin control group

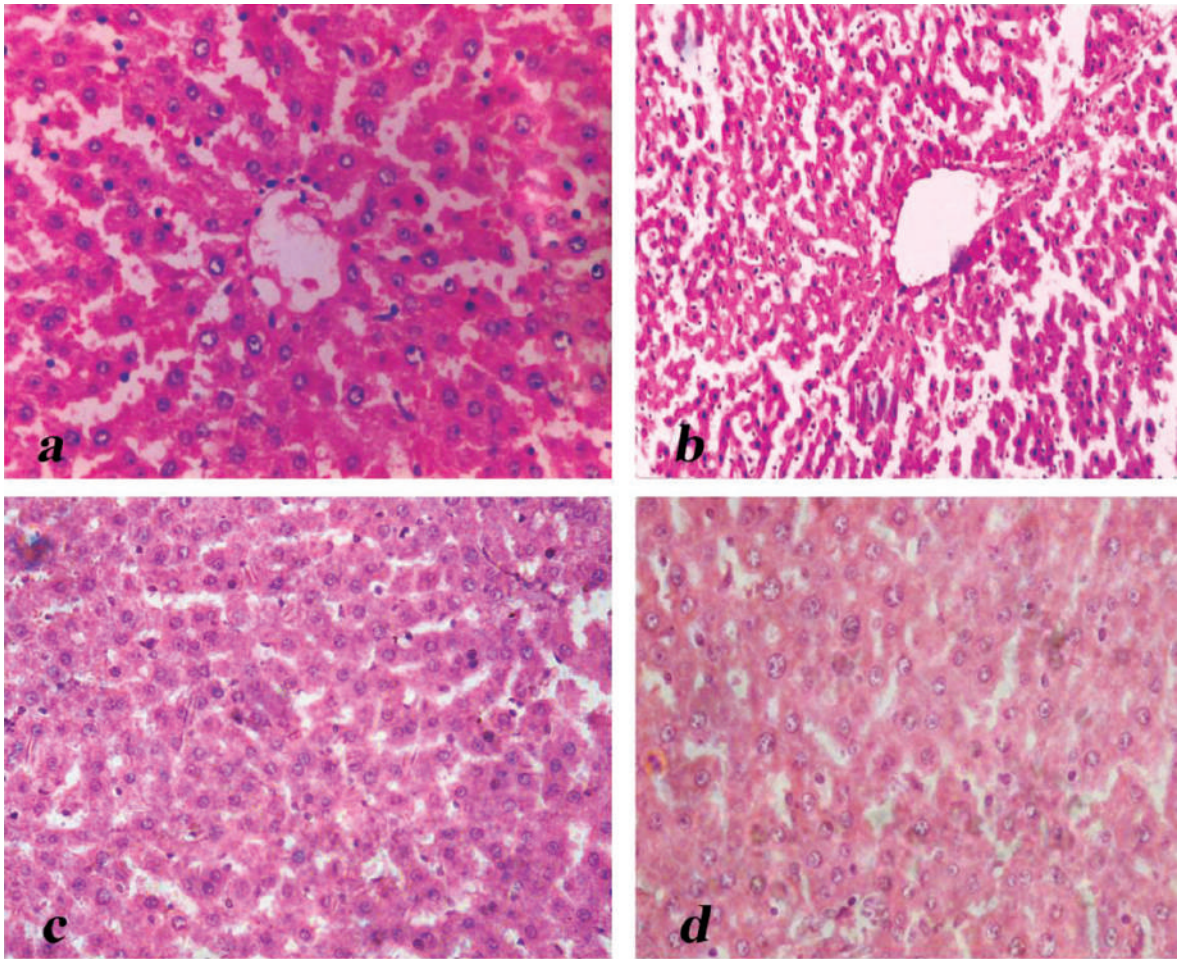

Fig. 1. Effect of coded drug 222 ethanolic extract on alcohol-induced liver damage in Wistar rats. **a)** Normal control rat liver showing normal hepatic architecture (x 400). **b)** Alcohol treated rat liver showing hepatic necrosis (x 400). **c)** Liver treated with 222 extract at 125 mg/kg dose showing normal hepatocytes with minimal necrosis (x 400). **d)** Silymarin treated rat liver showing normal hepatocytes (x 400).

4. Conclusion

In conclusion, the coded plant 222 leaf ethanolic extract exhibited significant hepato protective effect by reducing the elevated serum enzymes levels and biochemical parameters in ethanol-induced hepatotoxic rats. The extract also showed protection against ethanol-induced lipid peroxidation and oxidative stress by significantly reducing the formation of malondialdehyde (MDA) or by scavenging the free radicals by antioxidant activity and stimulating antioxidant mechanism. Histopathological studies support the biochemical estimation of serum parameters and antioxidant enzyme status indicating hepatoprotective activity of the extract. The ability of the extract to protect liver from ethanol induced liver damage might be attributed to its antihepatotoxic effect or may be due to its ability to restore the activity of antioxidant enzymes.

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