Ameliorative effect of PartySmart in rat model of alcoholic liver disease

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Received 8 August 2007; revised 23 November 2007

Present study was designed to investigate the effect of poly herbal formulation PartySmart in experimental model of alcoholic liver disease in male Wistar strain rats. Alcohol plus fish oil were administered to animals for 8 weeks to induce liver injury. PartySmart was administered at doses of 250 and 500 mg/kg body weight. After 8 weeks, parameters such as liver weight, liver function serum markers alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) and lipid peroxidation were studied. Livers from all the groups were subjected for histological evaluation. Treatment with PartySmart at the dose of 500 mg/kg body weight showed significant reduction in the levels of serum ALT, AST and ALP with a decrease in liver weight as compared to ethanol-fed rats. A significant decrease was also observed in malondialdehyde levels following treatment with PartySmart at 500 mg/kg body weight. Histological profile of liver tissue in PartySmart-treated animals showed lesser vacuolar degeneration and intactness of hepatic architecture along with improved glycogen deposition as demonstrated by PAS staining. PartySmart ameliorated alcohol-induced liver injury by preventing cell membrane disturbances, reduction of oxidative stress by free radical scavenging and antioxidant activity and normalization of altered intracellular redox status. Thus, PartySmart can be beneficial in the treatment of alcohol-induced liver damage.

Keywords: Alcohol, Hepatoprotective activity, Liver glycogen, Lipid peroxidation, PartySmart

Toxic effect of alcohol involves main organs of the body, especially the liver. Alcoholic liver disease (ALD) remains an important complication and cause of morbidity and mortality from alcohol abuse. ALD is a major health and economic problem in the western world and the treatment of alcoholic liver diseases still remains a challenge for the scientific community. The primary mechanistic factors involved in ALD include acetaldehyde, oxidative stress, immune response, hypoxia and membrane alterations.

In the recent years, plants have become one of the indispensable resources in drug development to treat various diseases. PartySmart, an herbal formulation, containing extracts of Phoenix dactylifera, Cichorium intybus, Andrographis paniculata, Vitis vinifera, Phyllanthus amarus and Emblica officinalis was evaluated to investigate the beneficial effect in alcoholic liver disease.

The individual constituents in PartySmart are well-established for their protective action against diverse hepatotoxins such as ethanol, carbon tetrachloride (CCl₄), anti-tubercular agents, thioacetamide, etc. Fruit extract of Emblica officinalis showed protective effect against anti-tubercular, CCl₄, thioacetamide and ethanol-induced hepatotoxicity. It also showed antioxidant and hypolipidemic actions. Phyllanthus amarus demonstrated hepatoprotective activity in CCl₄- and galactosamine-induced hepatotoxicity and also showed antiviral activity. Oligomeric proanthocyanidins, active principles of Vitis vinifera, have been known to regulate ethanol metabolism and prevent toxic effects. Vitis vinifera is also known to scavenge superoxide and hydroxyl radicals and ethanol-induced lipid peroxidation. Extracts of Phoenix dactylifera fruits showed dose-dependent inhibition of superoxide and hydroxyl radicals, Fe²⁺ / ascorbate system-induced lipid peroxidation and protein oxidation. Antihepatotoxic and antioxidant activities of Cichorium intybus were reported in vivo, in vitro and ex vivo experimental models. Andrographis paniculata demonstrated suppression of nitric oxide production in activated macrophages, and antioxidant activity, anti-inflammatory activity, and hepatoprotection against diverse hepatotoxic agents like CCl₄, paracetamol, galactosamine and benzene hexachloride (BHC)-induced liver injury.

Present study has been designed to investigate the effect of PartySmart in experimental model of ALD.

Materials and Methods

Animals—Laboratory-bred Wistar male rats weighing between 300-350 g and 12-14 weeks old were used for the study. The animals were housed and
acclimatized to a constant temperature of 22° ± 3°C with 30-70% RH and were exposed to 12:12 hr light : dark. Pelleted rat feed (M/s. Amrut Feed, Pranav Agro Industries Ltd., Sangli, India) and water were provided ad libitum. The study protocol was approved by the Institutional Animal Ethics Committee (IAEC) and the animals used for this study were maintained in accordance with the guidelines recommended by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Preparation of PartySmart—Each gram of PartySmart contains dried aqueous extracts of Phoenix dactylifera (fruit : 188 mg), Cichorium intybus (seeds : 188 mg), Andrographis paniculata (aerial part : 188 mg), Vitis vinifera (fruit : 188 mg), Phyllanthus amarus (aerial part : 124 mg), and Emblica officinalis (fruit : 124 mg). The constituents of plant material were procured from M/s. Abhirami Botanical Corporation, Tuticorin, Tamil Nadu, India and identified by Dr. R. Kannan, Botanist, R&D Center, The Himalaya Drug Company and voucher specimens were preserved at R&D Center. Such two or more batches of preparations from raw materials of different origin were standardized by fingerprint analysis for characterization using high performance thin layer chromatography (HPTLC).

HPTLC analysis—One gram of PartySmart was extracted by refluxing on a water bath with 15 ml of dichloromethane. Extract was filtered and concentrated to 2 ml. 10 µl of concentrate was spotted on pre-coated silica gel plate. Plate was developed using dichloromethane:methanol (97:3). Developed plate was scanned using densitometer at 254 nm. HPTLC fingerprint of PartySmart is shown in Fig. 1.

Experimental design—Laboratory-bred rats (40) were divided into 4 groups of 10 each. Rats of Group 1, received 2.5 ml/kg of fish oil along with isocaloric dextrose intragastrically to serve as control. Rats of group 2, received ethanol at a dose of 6 g/kg/day for the first week along with 2.5 ml/kg of fish oil. The dose of ethanol was progressively increased during week 1 to a maintenance dose of 8 g/kg/day, which was continued for further 7 weeks. Rats of groups 3 and 4, received PartySmart as an aqueous suspension orally at 250 and 500 mg/kg body weight respectively. In addition, both groups of the rats also received fish oil and ethanol one hour after administration of PartySmart as in group 2, for the same duration.

At the end of the experimental period, i.e. after 8 weeks, blood was collected from the retroorbital plexus for the estimation of alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP) levels (Boehringer Manheim, Germany). The animals were euthanized with anaesthetic ether and the liver was excised, washed in saline and weighed. A portion of the liver was excised and homogenized in ice-cold 0.15 M KCl for the estimation of thiobarbituric acid reactive substances (TBARS). The remaining portion of liver was fixed in 10% neutral buffered formalin and processed for histopathological evaluation using Hematoxylin & Eosin (H&E) and Periodic Acid Schiff’s (PAS) staining for glycogen.

Statistical analysis—The values are expressed as mean ± SE and were analyzed statistically using One-way ANOVA followed by Dunnet’s Multiple Comparison test using GraphPad Prism software package (Version 4.0) to find out the level of significance. The minimum level of significance was fixed at P<0.05.

Results

Effect of PartySmart on serum ALT, AST and ALP—Intoxication with ethanol + fish oil showed a significant elevation of serum ALT, AST and ALP as compared to animals treated with fish oil alone. Treatment with PartySmart at 250 and 500 mg/kg body weight orally ameliorated alcohol-induced toxic changes in ALT, AST and ALP levels (Table 1). The observed changes were found to be significant at 500 mg/kg body weight dose of PartySmart treatment.

Effect of PartySmart on liver weight and lipid peroxidation—Administration of ethanol showed a significant increase in liver weight and malondialdehyde levels as compared to the fish oil-treated group.
Fig. 2—Liver section of rat (a) control group showing structural intactness and normal architecture (400×; H&E); (b) ethanol + fish oil-treated group showing ballooning of hepatocytes with vacuolar degeneration, necrosis and prominent Ito cells (arrows) with clear cytoplasm and flattened nuclei in the hepatic sinusoids (400×; H&E); (c) PartySmart treatment + ethanol-intoxicated group showing less vacuolar degeneration and intactness of hepatic architecture (400×; H&E); (d) glycogen deposition in control rats (400×; PAS) (e) ethanol + fish oil-fed rats showing severe depletion of glycogen stores of (450×; PAS); (f) PartySmart treatment + ethanol-intoxicated rats showing marked improvement in glycogen deposition (450×; PAS)
A significant reversal was observed in both parameters following treatment with 500 mg/kg body weight dose of PartySmart (Table 1).

**Histological evaluation of liver**—The liver sections of control group animals treated with fish oil showed normal structure and architecture (Fig. 2a). Sections of the liver in the fish oil plus ethanol gavage caused ballooning of hepatocytes with vacuolar degeneration, necrosis and prominent vacuolated Ito cells (fat storing cells) with clear cytoplasm and flattened nuclei in the hepatic sinusoids (Fig. 2b). Treatment with PartySmart reduced the hepatopathological manifestations of ethanol intoxication as indicated by lesser vacuolar degenerations and intactness of the hepatic architecture (Fig. 2c). Liver sections stained with PAS stain showed severe depletion of glycogen stores in fish oil plus ethanol-fed rats as compared to control (Fig 2d and e). Treatment with PartySmart replenished the reduced glycogen stores induced by ethanol intoxication (Fig. 2f).

**Discussion**

ALD is as a result of complex pathophysiological events involving various types of cells. In the experimental model of ALD apart from administration of alcohol, an absolute control of nutrient intake by rodents has depicted the critical role of nutrition in determining sensitization and priming of the liver to ethanol-induced hepatic injury. The concept of “sensitization” and “priming” is currently considered for elucidation of pathogenetic mechanisms of ALD. Studies have demonstrated profound effects on ethanol-induced liver injury by intake of nutrients such as polyunsaturated fatty acid. In the present study, fish oil was used along with ethanol, which formed the basis for the experimental model of ALD in rats.

Hepatomegaly is a common finding after chronic ethanol ingestion. Hepatomegaly is mainly due to an increase of fat and protein content as well as accompanying water, resulting in an increase in cell size. The increase in cell size could be the basis for ballooning of the hepatocytes. Present observations in ethanol-intoxicated animals were in concurrence with the earlier findings. Treatment with PartySmart normalized the liver weight. Histopathological evaluation also showed vacuolar degeneration, necrosis and prominent vacuolated Ito cells in the hepatic sinusoids in chronic ethanol-intoxicated rats, which was ameliorated following PartySmart treatment. These findings suggest that PartySmart prevents the eventual decrease in the release of lipoproteins from the liver after prolonged alcohol intake coincident with the development of liver dysfunctions.

The present study on chronic alcohol intoxication showed significant increase in serum transaminases and ALP levels, the indices of liver injury. The elevated levels are primarily due to the leakage of cellular enzymes into the blood stream leading to their increase in serum. As regards ALP, extracellular discharge was also recorded. Protection of ethanol-induced intoxication in PartySmart-treated animals could be a manifestation of reduction in cell membrane disturbances.

Oxidative stress plays an important role in the pathogenesis of ethanol toxicity. The close relation between ethanol and liver is due to the fact that more than 80% of ingested alcohol is metabolized in the liver without feedback mechanism. Acetaldehyde, a metabolite of ethanol, in excess markedly alters the intracellular redox status, induces fat deposits, and triggers the inflammatory and immune responses. Excessive production of reactive oxygen species (ROS), formation of adducts with acetaldehyde and lipid peroxidation markers are well documented in alcoholics. In the present study, ethanol intoxication showed a significant increase in the hepatic
malondialdehyde levels, which is an indicator of lipid peroxidation and the same was corrected following treatment with PartySmart. The antioxidant activity of PartySmart could be attributed to various individual ingredients, which are established for its antiperoxidative and free radical scavenging activity. In addition, studies revealed that PartySmart hastens the metabolism of ethanol and acetaldehyde, thereby alleviating the toxic effects following chronic ingestion of ethanol.

In the present study, chronic intoxication with ethanol showed depletion of glycogen stores in the liver. It is well-documented that excessive ethanol intake impairs carbohydrate metabolism. This liver. It is well-documented that excessive ethanol ethanol showed depletion of glycogen stores in the liver. Treatment with PartySmart improved the glycogen stores in the liver as compared to ethanol-intoxicated rats as depicted by PAS stained sections.

In conclusion, this study demonstrated PartySmart ameliorates alcohol-induced injury and the mechanism may involve the prevention of cell membrane disturbances, and reduction of oxidative stress by free radical scavenging and antioxidant activity, this in turn prevents Kupffer cell activation and pro-inflammatory mediators and normalization of the altered redox state in addition to hastened elimination of ethanol and acetaldehyde from the blood. Further studies on the expression of pro-inflammatory factors in the liver are warranted to elucidate the precise mechanism of action.

References

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