Antioxidant Activity Of Ajaswagandhadhi Lehyam Against D-Galactosamine Induced Oxidative Stress In Rats

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Abstract

D-Galactosamine is one among the most experimentally used drug to study hepatotoxic effects in experimental animals. The model system of liver damage produced by D-galactosamine in rats is recognized to be much like viral hepatitis in humans from both morphological and functional points of view. It also causes oxidative stress in rats. Ajaswagandhadhi Lehyam has ingredients that reduce oxidative stress. This study deals antioxidant activity of Ajaswagandhadhi Lehyam against D-galactosamine induced oxidative stress in wistar rats.

Key Words D-Galactosamine, Silymarin, Ajaswagandhadhi Lehyam, Antioxidant activity, Oxidative Stress, Bio-chemical Liver parameters and Histopathology

Introduction

Galactosamine has great liver specificity because the hepatocytes have high levels of galactokinase and galactose-1-P-uridylyltransferase mean while; other organs are not affected. Galactosamine causes liver cell injury, with spotty hepatocytes necrosis and prominent portal and parenchymal inflammation.

Galactosamine also causes depletion of uridine diphosphate (UDP) by increasing formation of UDP-sugar derivatives, which results in inhibition of RNA and protein synthesis, leading to deterioration of the cell membranes. The possible antioxidant mechanisms of Ajaswagandhadhi Lehyam have not been reported yet. Therefore, in the present study, antioxidant effects and possible mechanisms of Ajaswagandhadhi Lehyam were examined on the D-galactosamine-intoxicated rats.

SELECTION AND ACCLIMITIZATION OF ANIMALS

Albino rats of wistar strains weighing between 180-220gm were produced from animal experimental laboratory, and used throughout the study. They were housed in micro nylon boxes in a control environment(temp 25±2°C) and 12 hrs dark light cycle with standard laboratory diet and water ad libitum. The study was conducted after obtaining institutional animal ethical committee clearance. As per the standard practice, the rat were
segregated based on their gender and quarantined for 15 days before the commencement of the experiment. They were fed on healthy diet and maintained in hygiene environment in our animal house.

**Treatment protocol**

The acclimatized animals were divided into 5 groups of each 6 animals, designated as

Group 1: Served as normal control and receive normal diet and water.

Group 2: Toxic control received 400mg/kg D-galactosamine for 21 days orally

(400mg/kg)

Group 3: Standard control received 400mg/kg D-galactosamine and 25mg/kg of silymarin orally for 21 Days.

Group 4: Served as a treatment control group and was administered with 400mg/kg D-galactosamine and Ajaswagandhadhi Lehyam at a dose of 100mg/kg suspended with 2ml of 1% CMC through orally.

Group 5: Served as a treatment control group and was administered with 400mg/kg D-galactosamine and Ajaswagandhadhi Lehyam at a dose of 200mg/kg suspended with 2ml of 1% CMC through orally.

**METHODOLOGY**

On day 22 after 24 hrs of Galactosamine administration animals in all the groups were humanely sacrificed using Ketamine HCL and 4ml of blood was withdrawn by cardiac puncture and allowed to clot for 30mins at room temperature. The serum was separated by using refrigerated centrifuge and used for the assay of marker enzymes viz AST, ALT, ALP, TP, TB and LDH. The livers were dissected out immediately, washed with ice-cold saline and 10% homogenates in phosphate buffer solution (PH 7.4) were prepared. Liver homogenate was used for the assay of Lipid per oxidation (LPO) while some fraction of homogenates were centrifuged at 7000rpm for 10 min at 4°C using refrigerated centrifuge, and the supernatants were used for the assay of Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPx).

**STATISTICAL ANALYSIS**

The Statistical analysis was carried out by one way analysis of variance (ANOVA) followed by Newmann Keul’s multiple range tests. The values are represented as Mean ± SEM. Probability value of P <0.01 was determined to be statistically significant.

**Table No: 1** Effect of Ajaswagandhadhi Lehyam and Silymarin pre-treatment on biochemical parameters of the rats intoxicated with D-Galactosamine.

<table>
<thead>
<tr>
<th>Group No.</th>
<th>TREATMENT DOSE (mg/Kg)</th>
<th>AST (IU/mL)</th>
<th>ALT (IU/mL)</th>
<th>ALP (IU/mL)</th>
<th>TP (gm/dl)</th>
<th>TB (mg/dl)</th>
<th>LDH (U/L)</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Group No.</th>
<th>TREATMENT DOSE (mg/Kg)</th>
<th>SOD (U/mg) Protein</th>
<th>CATALASE (U/mg) Protein</th>
<th>GPx (U/mg) Protein</th>
<th>LIPID PEROXIDATION (nmoles OF MDA/g liver weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal control 10ml/kg normal saline</td>
<td>9.20±0.90</td>
<td>0.198±0.07</td>
<td>10.9±0.90</td>
<td>114.90±4.20</td>
</tr>
<tr>
<td>II</td>
<td>Toxic control 25mg/kg D-galactosamine</td>
<td>2.78±0.14 *a</td>
<td>0.115±0.06 *a</td>
<td>2.70±0.25 *a</td>
<td>167.90±7.94 *a</td>
</tr>
<tr>
<td>III</td>
<td>Standard control Silymarin 25mg/kg</td>
<td>7.90±0.66 *b</td>
<td>0.162±0.05 *b</td>
<td>7.86±0.65 *b</td>
<td>119.85±5.30 *b</td>
</tr>
<tr>
<td>IV</td>
<td>Treatment control Ajaswagandhadhi Lehyam 100mg/kg</td>
<td>6.32±0.50 *b</td>
<td>0.150±0.04 *b</td>
<td>6.25±0.52 *b</td>
<td>151.35±6.40 *b</td>
</tr>
</tbody>
</table>

➢ Values are expressed as Mean ± SEM.
➢ Values are found out by using one way ANOVA followed by Newmann keul’s multiple range tests.
➢ *a – values are significantly different from Normal control at P< 0.01.
➢ *b – values are significantly different from Toxic control at p< 0.01.

Table No:2 Effect of Ajaswagandhadhi Lehyam and Silymarin pre-treatment on biochemical liver parameter of the rats intoxicated with D-Galactosamine.
### Treatment control

<table>
<thead>
<tr>
<th>V</th>
<th>Treatment control</th>
<th>6.82±0.55</th>
<th>0.155±0.05</th>
<th>6.50±0.60</th>
<th>152.35±6.60</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ajaswagandhadhi Lehyam 200mg/kg</td>
<td>*b</td>
<td>*b</td>
<td>*b</td>
<td>*b</td>
</tr>
</tbody>
</table>

- Values are expressed as Mean ± SEM.
- Values are found out by using one way ANOVA followed by Newmann keul’s multiple range tests.
- *a – values are significantly different from Normal control at P< 0.01.
- *b – values are significantly different from Toxic control at p< 0.01.

### RESULT

#### BIOCHEMICAL OBSERVATIONS

Significant increase in (P< 0.01) Serum Aspartate Transaminase (AST), Alanine Transaminase (ALT), Alkaline phosphatase (ALP), Total bilirubin (TB) and Lactate dehydrogenase and significant decrease in (P< 0.01) Total protein levels were observed in animals treated with galactosamine 400mg/kg (Group II) as compared to normal control group (Group I).

Pretreatment with Ajaswagandhadhi Lehyam at a dose of 100mg and 200mg/kg, orally for 21 days decreased the levels of above indices like AST, ALT, ALP, TB, LDH, and increased levels of TP significantly (P <0.01) in group VI.

Silymarin pretreatment produced significant decrease in (P< 0.01) serum AST, ALP, TB, LDH and significant increase in TP at (P< 0.01) in group III.

#### BIOCHEMICAL OBSERVATION IN LIVER HOMOGENATE TISSUE

In liver homogenate, there was significant decrease in SOD, CAT and GPx levels and increase in LPO levels were observed in animals treated with galactosamine 400mg/kg (group II) as compared to normal control group (Group I).

Pretreatment with Ajaswagandhadhi Lehyam at a dose of 100mg/kg and 200mg/kg orally for 21 days increase the levels of above indices like SOD, CAT and GPx levels and decrease levels of LPO significantly (P<0.01) in group VI.

Silymarin pretreatment produced significant increase in (P< 0.01) Liver homogenate enzyme such as SOD, CAT, GPx levels and decrease the levels of LPO significantly (P<0.01) in group III.

### DISCUSSION

The main objective of this study was to obtain a better understanding of the mechanism responsible for the D-GalN induced hepatotoxicity and to study the effect of Ajaswagandhadhi Lehyam against D-GalN induced hepatotoxicity. The changes associated with D-GalN induced liver damage are similar to that of acute viral hepatitis. Hence, D-GalN mediated hepatotoxicity was chosen as the experimental model. D-GalN dose of 400 mg/kg body weight was finalized after acute toxicity studies and following standard protocol.

Galactosamine has great liver specificity because hepatocytes have high levels of galactokinase and galactose-1-uridylytransferase. Galactosamine does not affect other organs. Galactosamine causes hepatic injury with spotty hepatocytes necrosis and marked portal and parenchymal infiltration. Galactosamine also causes depletion of uridine diphosphate (UDP) by increasing the formation of UDP-sugar derivatives, which results in inhibition of RNA and protein synthesis leading to cell membrane deterioration. Galactosamine administration in rats disrupts the membrane...
permeability of the plasma membrane causing leakage of the enzymes from the cell, which leads to elevation in levels of serum enzymes. Elevated serum enzymes are indicative of cellular leakage and loss of functional integrity of the cell membrane in liver. Hence significant rise in the transaminases levels could be taken as an index of liver damage. In our study the rise in AST, ALT, ALP, LDH levels induced by galactosamine administration was significantly reduced by Ajaswagandhadhi Lehyam pre-treatment suggesting that its antioxidant and hepatoprotective activity might be due its effect against cellular leakage and loss of functional integrity of the cell membrane in hepatocytes. Estimation of serum bilirubin is used for the assessment of hepatic function in order to diagnose the hepatobiliary diseases and severe disturbance of hepatocellular functions Increased level of bilirubin in this study is in agreement with previous reports showing that Galactosamine induced hepatitis is characterized by increased levels of bilirubin in serum.

The pretreatment of Galactosamine –intoxicated rats with Ajaswagandhadhi Lehyam produced significant Suppression of increased bilirubin level suggests the ability of Ajaswagandhadhi Lehyam being to counteract biliary dysfunction. Several reports have shown an increase in hepatic lipid peroxidation with liver injury development in galactosamine-treated rats. In the present study treatment of rats with Galactosamine elicited a significant increase in the LPO. Ajaswagandhadhi Lehyam treatment prevented the increase in LPO indicating the protective effect of Ajaswagandhadhi Lehyam against lipid peroxidation. Moreover, it has been observed that Ajaswagandhadhi Lehyam significantly decreased lipid peroxidation and increased endogenous antioxidants such as SOD,CAT, and GPx. Since administration of Ajaswagandhadhi Lehyam prevented the hepatic GPx depletion, it appears that the protective effect of Ajaswagandhadhi Lehyam involves the maintenance of antioxidant capacity in protecting the hepatic tissue against oxidative Stress. Excessive production in hydroxyl radicals in the blood and liver has previously been demonstrated in rats , which demonstrated that circulating pro-inflammatory cytokines ,such as TNF-α, which triggers hepatic injury , were increased , at least in part , by a free-radical- mediated apoptotic.

In this study, treatment with Ajaswagandhadhi Lehyam probably ameliorated oxidative liver injury through its antioxidant effect which further alleviated hepatic injury as shown by biochemical findings. In addition, several reports showed that hepatic antioxidant defense system associated with antioxidant enzymes such as SOD, CAT and GPx are disrupted in galactosamine treated rat. In rats treated with Galactosamine, neutrophil infiltration in to the liver cells increased with the formation and progression of liver injury and that reactive oxygen species , such as superoxide radical, released from activated neutrophils infiltrating into the liver of Galactosamine- treated rats caused the extension of liver cell necrosis. Rats treated with Galactosamine showed that, neutrophil infiltration into liver cells occurs at an early stage of the injury. Accordingly, the reactive oxygen species scavenging action of Ajaswagandhadhi Lehyam if it is administered after galactosamine intoxication may contribute to attenuation of the disruption of hepatic antioxidant defense system in Galactosamine-treated rats. Therefore, it may be possible that post administration of Ajaswagandhadhi Lehyam exerts a preventive effect on liver injury progression in Galactosamine-treated rats through its indirect antioxidant action to system in addition to its direct antioxidant action to maintain antioxidant defense systems to scavenge ROS and to inhibit lipid peroxidation. As galactosamine induced-hepatotoxicity has striking resemblance with the human viral hepatitis. The present study confirms the Antioxidant efficacy of Ajaswagandhadhi Lehyam against Galactosamine –induced hepatitis in rats.

Histopathological Observations:
FIG.NO:1 NORMAL CONTROL

FIG.NO:2 TOXIC CONTROL 400mg/kg
IP D-galactosamine

FIG.NO:3 STANDARD CONTROL
25mg/kg of silymarin

FIG.NO:4 TREATMENT CONTROL
Lehyam at a dose of 100mg/kg

FIG.NO:5 TREATMENT CONTROL Lehyam at a dose of 200mg/kg

**CONCLUSION**

The Ajaswagandhadhi Lehyam found to have significant hepatoprotective activity by D-galactosamine induced hepatic toxicity. The effect is almost comparable to silymarin or slightly less. Ajaswagandhadhi Lehyam in the doses of 100mg/kg body wt, reduced the levels of serum AST, ALT, ALP, LDH and Total bilirubin significantly. These observations highlight that Ajaswagandhadhi Lehyam is one of the promising herbal formulation for improving defense mechanisms in the physiological systems against oxidative stress caused by the D-galactosamine induced
oxidative stress in rats. The probable action could be due to: stabilizing the hepatocellular membrane, inhibiting neutrophil infiltration into the liver cells, preventing the process of lipid peroxidation, preventing inactivation of antioxidant enzymes. It might be used in the treatment of viral hepatitis.

REFERENCES


