HEPATOPROTECTIVE AND ANTIOXIDANT POTENTIAL OF WITHANIA SOMNIFERA AGAINST PARACETAMOL-INDUCED LIVER DAMAGE IN RATS

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ABSTRACT

Objective: The aim of this study was to evaluate the hepatoprotective and antioxidant effects of Withania somnifera against Paracetamol-induced liver injury in rats.

Methods: In the present study, the protective effect of Withania somnifera was investigated against Paracetamol-induced hepatotoxicity and compared with Silymarin, a standard hepatoprotective reference drug. The rats received a single dose of paracetamol (900 mg/kg body weight, i.p.); Withania somnifera (500 mg/kg body weight and 1000mg/kg body weight, p.o.) and Silymarin (25 mg/kg body weight, p.o.) were administered 30 min after the injection of paracetamol. Liver marker enzymes (Aspartate Transaminase, Alanine Transaminase and Alkaline Phosphatase), Total Protein content, Bilirubin, Antioxidant status (Reduced Glutathione, Superoxide Dismutase, Catalase and Glutathione-S-Transferase) were evaluated and histopathological analysis was done for the control and experimental rats.

Results: Paracetamol treatment leads to elevated levels of liver marker enzymes and bilirubin and there was deterioration in total protein content, histological observations and antioxidant status. However, treatment with Withania somnifera significantly reversed (p < 0.05) the above changes compared to the control group as observed in the paracetamol-challenged rats.

Conclusion: The results clearly demonstrate that Withania somnifera possesses promising hepatoprotective effects through its antioxidant activity and hence suggests its use as a potential therapeutic agent for protection from paracetamol overdose.

Keywords: Hepatotoxicity, Paracetamol, Withania somnifera.

INTRODUCTION

Acetaminophen (Paracetamol or AAP) is a commonly available drug well known for its analgesic and antipyretic effects. At therapeutic doses, AAP is considered a safe drug and is safely bio transformed and eliminated as non-toxic conjugates of sul fate and glucuronic acid, and a small portion is converted to NAPQI (N-acetyl-p-benzoquinone imine) which is detoxified by glutathione (GSH) and eventually eliminated in the urine or bile [1]. However, during overdose of AAP, the glucuronidation and sulfation routes become saturated and rapid depletion of hepatic GSH levels occurs which causes oxidative stress and the NAPQI thus formed binds covalently to liver proteins [2]. Hepatotoxicity induced by acetaminophen results in prominent elevations of liver marker enzymes and reactive oxygen species (ROS) which further aggravates oxidative stress and are involved in a number disease processes, including heart disease, diabetes, liver injury, cancer, cardiovascular dysfunctions and aging [3-7]. Therefore, new potential therapeutics for AAP overdose is being routinely investigated in preclinical studies.

Liver is an important organ in the body as it provides protection from potentially injurious exogenous and endogenous compounds and in this process it gets affected [8]. Thus protective mechanisms for liver are of special concern. Conventional medicines used for treatment of liver diseases have adverse side effects and are costlier. So, there is a need to evaluate natural compounds as an effective alternative which are safer and cost effective.

Withania somnifera also known as Ashwagandha or Indian Ginseng [9], dunal Solanaceae, is cultivated in drier parts of India and in Nepal. It is a well known medicine in Ayurveda and has proved to exhibit anti-inflammatory [10], immunomodulatory [11], anti-arthritis [12] and antiaging properties [13]. W. somnifera is known to alter the oxidative stress markers of the body. The root extract has found to significantly reduce the lipid peroxidation [14] and increase the superoxide dismutase (SOD) and catalase activities, thus carrying free radical scavenging property [15]. It is been proved to have hepatoprotective effect against radiation induced [16] and iron induced toxicity [17]. However, protective activity of Withania somnifera has not been scientifically investigated against paracetamol induced hepatotoxicity. Hence, an attempt was made to investigate the effects of aqueous extract of Withania somnifera against hepatic injury induced by acetaminophen hepatotoxicity.

MATERIALS AND METHODS

Drugs and chemicals

Commercially available Ashwagandha (W.somnifera) powder was obtained from Indian Medical Practitioners Co-operative Stores and Society, Mylapore, Chennai, Tamilnadu, India. Its aqueous suspension at dose 500mg/kg body weight and 1000mg/ kg body weight was injected intraperitoneally. Silymarin and Paracetamol were obtained from Natural remedies private limited, Bangalore. Paracetamol was dissolved in double distilled water and injected intravenously. All other reagents used were standard laboratory reagents of analytical grade.

Experimental Animals

The study was performed using rats of either sex, having a mean weight of 190 grams, procured from VIT Animal house, VIT University, Vellore, Tamilnadu, India. The rats were fed commercial pellet fed from Hindustan Lever Ltd. (Mumbai, India) and water ad libitum. The animals were well treated and cared for in accordance of the guidelines recommended by the Committee for the Purpose of Control and Supervision of Experiments on Animals, Ministry of Culture, Govt. of India, Chennai, India.

Evaluation of hepatoprotective activity

Animals were divided into 5 groups. All animals were made to fast 24 hours before the commencement of the study. Group I or the control group, received saline (0.89 % NaCl). In Group II, Paracetamol induced test group, hepatotoxicity was induced by single dose of paracetamol (900 mg/kg body weight i.p. dissolved in distilled water); Group III i.e. drug treated group, were given Ashwagandha suspended in distilled water (500 mg/kg/ body weight /day, orally) administered 30 minutes after the single injection of Paracetamol (900 mg/kg body weight, i.p.); Group IV...
received Ashwagandha (1000mg/ kg body weight, orally) administered 30 minutes after the single injection of Paracetamol (900 mg/kg body weight, i.p.). Group V was administered Silymarin (25 mg/kg body weight, i.p.) 30 minutes after the single injection of Paracetamol (900 mg/kg body weight, i.p.). Rats were decapitated after 4 hours of Paracetamol injection; blood was collected from the trunk, serum was separated and stored at -70°C. Tissue samples from the liver were processed for biochemical and histological analysis.

The activities of AST [18], ALT [18], Alkaline Phosphatase [19], and Bilirubin [20] were determined in serum of control and experimental rats. Superoxide dismutase was assayed according to Marklund and Marklund [21] and the unit of enzyme activity was defined by the enzyme required to give 50% inhibition of pyrogallol autoxidation. Catalase [22], Glutathione-S-transferase [23] and Reduced Glutathione [24] were evaluated and Total Protein was estimated using Lowry’s method [25] using bovine serum albumin as standard.

**Histopathological Studies**

Immediately after sacrifice, a portion of the liver was fixed in 10% formalin, then washed, dehydrated in descending grades of isopropanol and finally rinsed with xylene. The tissues were then embedded in molten paraffin wax. Sections were cut at 5 mm thickness, stained with haematocytin and eosin was observed microscopically for histopathological changes.

**Statistical Analysis**

Results were expressed as mean± SD and statistical analysis was performed using ANOVA, to determine the significant differences between the groups, followed by Student Newman-Keul’s test. p<0.05 implied significance.

### Table 1: Effect of W.somnifera on liver marker enzymes and protein content in serum of control and Paracetamol - intoxicated rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
<th>Group 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine transaminase (U/dl)</td>
<td>86.5±2.857</td>
<td>180.9±6.21a'</td>
<td>95.5±4.98a'b'</td>
<td>88.3±4.78a'c'</td>
<td>88.4±4.62ad'</td>
</tr>
<tr>
<td>Aspartate transaminase (U/dl)</td>
<td>73.3±5.386</td>
<td>230.4±6.16a'</td>
<td>123.2±6.57a'b'</td>
<td>100.2±6.21a'c'</td>
<td>98.3±6.37a'</td>
</tr>
<tr>
<td>Alkaline phosphatase (U/dl)</td>
<td>99.1±5.08</td>
<td>279.2±7.44a'</td>
<td>143.6±5.28a'b'</td>
<td>131.5±4.9a'c'</td>
<td>134.0±5.21a'</td>
</tr>
<tr>
<td>Total bilirubin (mg/dl)</td>
<td>0.57±0.72</td>
<td>5.68±1.03a'</td>
<td>2.02±0.98a'b'</td>
<td>1.81±0.9a'c'</td>
<td>1.97±0.83a'</td>
</tr>
<tr>
<td>Total protein (mg/dl)</td>
<td>6.86±0.04</td>
<td>3.22±0.78a'</td>
<td>6.73±0.42a'b'</td>
<td>6.68±0.13a'c'</td>
<td>5.58±0.37a'</td>
</tr>
</tbody>
</table>

For each group n=6, the values are mean ± SD. Comparisons indicated by lowercase letters were made as follows: a—group I vs. groups II, III, IV and V; b—group II vs. group III; c—group II vs. group IV. Statistical analysis was calculated by one way ANOVA followed by Student’s Newman-Keul’s test. The symbols represent statistical significance at: * p<0.05

### Table 2: Effect of W. somnifera on antioxidant status in serum of control and Paracetamol - intoxicated rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
<th>Group 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superoxide dismutase (U/mg)</td>
<td>24.4±2.00</td>
<td>45.3±2.62a'</td>
<td>20.4±1.70a'c'</td>
<td>20.5±1.79a'</td>
<td>22.0±1.64a'</td>
</tr>
<tr>
<td>Catalase</td>
<td>78.31±1.70</td>
<td>69.96±8.35a'</td>
<td>63.1±7.39a'c'</td>
<td>70.3±7.41a'</td>
<td>70.3±7.41a'</td>
</tr>
<tr>
<td>Glutathione-S-transferase</td>
<td>2.7±0.53</td>
<td>2.2±0.27a'</td>
<td>2.3±0.39a'c'</td>
<td>2.80±0.21a'</td>
<td>2.80±0.21a'</td>
</tr>
<tr>
<td>Reduced glutathione</td>
<td>24.4±2.29</td>
<td>21.3±2.61a'</td>
<td>19.4±2.38a'c'</td>
<td>22.0±1.64a'</td>
<td>22.0±1.64a'</td>
</tr>
</tbody>
</table>

For each group n=6, the values are mean ± SD. Comparisons indicated by lowercase letters were made as follows: a—group I vs. groups II, III, IV and V; b—group II vs. group III; c—group II vs. group IV. Statistical analysis was calculated by one way ANOVA followed by Student’s Newman-Keul’s test. Units: lipid peroxidation—nanomoles of MDA formed/milligram protein, CAT—micromoles of H2O2 consumed/minute/ milligram protein, SOD—units/milligram protein (1 U= amount of enzyme that inhibits the autoxidation of pyrogallol by 50 %), GST—nanomoles of 1-chloro-2, 4-dinitrobenzene—GSH conjugate formed/minute/milligram protein, reduced glutathione—nanomoles/milligram/protein *p<0.05 (statistically significant)
Fig 1: Histopathological monograph of extract and standard. a : control; b: Paracetamol (900 mg/kg) alone; c: Paracetamol + Ashwagandha (900 mg/kg +500 mg/kg); d: Paracetamol + Ashwagandha (900 mg/kg +1000 mg/kg); e: Paracetamol + Silymarin(25 mg/kg+ 900 mg/kg).

RESULTS

Hepatoprotective activity

The activities of Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Alkaline Phosphatase (ALP) in serum were significantly increased (p < 0.05) in the acetaminophen-treated group as compared with the normal control group [Table 1]. The increase in the levels of these liver marker enzymes clearly indicated the damage of the hepatic cells. However, treatment with Withania somnifera prevented the alteration in the above to levels similar to those in control rats. The levels of serum bilirubin was also increased significantly (p < 0.05) as compared to the normal rats which was brought to normal after treatment with Withania somnifera. In acetaminophen treated rats, the total protein content was also decreased significantly (p < 0.05) as compared to the normal rats [Table 1]. On the other hand, Withania somnifera reversed the effect of Acetaminophen toxicity and thus protein level was brought to normal as that of control rats.

Antioxidant activity

In rats treated with acetaminophen, a significant decrease in antioxidant enzymes (superoxide dismutase, catalase and glutathione-S-transferase) and total reduced glutathione was observed [Table 2]. However, treatment of W.somnifera significantly increased the antioxidant status as compared with the control group.

Histopathological results

The hepatoprotective effect of Ashwagandha was confirmed by histopathological examination of the liver tissue of control and treated animals [Figure 1]. The histological architecture of control rats was found to be normal with distinct hepatic cells and sinusoidal space. In group II i.e. the paracetamol treated liver sections showed congestion, mild centrilobular degeneration of hepatocytes, mild bile duct hyperplasia and multifocal cell infiltration. The histopathological profile of the Group III rats showed mild degeneration of hepatocytes and in group IV rats no visible changes were observed confirming the safety of the extract at selected dose. In group V, rats treated with silymarin, intoxicated with paracetamol showed less disarrangement and degeneration of hepatocytes.

DISCUSSION

Paracetamol-induced hepatic failure is the second leading cause of liver transplantation and accounts for considerable levels of morbidity and mortality. The advantage of this model is that being a dose-dependent toxicant, the experiments are technically easy to perform and, most importantly, it is a clinically relevant. The estimation of enzymes in the serum is a useful quantitative marker of the extent and type of hepatocellular damage. The rise in serum AST and ALT levels has been attributed to the damaged structural integrity of the liver because these are cytoplasmic in location and
are released into circulation after cellular damage. In this study, significant elevation in the liver marker enzymes namely aspartate transaminase, alanine transaminase, alkaline phosphatase, and total bilirubin in serum was caused by a single dose of paracetamol (900mg/kg bodyweight, ip.) treatment as compared to the control group. The increased serum transaminases, alkaline phosphatase, and total bilirubin levels in paracetamol-intoxicated rats indicate a deterioration of the hepatic functions due to liver membrane damage resulting from acetaminophen toxicity which leads to the escape of these enzymes in serum (circulation) [26]. However, we observed that administration of Ashwagandha (Withania somnifera) after paracetamol intoxication resulted in subsidence of the increased activities of liver markers indicating that Ashwagandha treatment may enhance recuperation of liver from paracetamol induced damage in paracetamol intoxicated rats [Table 1]. The prevention of the leakage of intracellular enzymes in serum of acetaminophen-intoxicated mice might be due to the membrane stabilizing activity of Withania somnifera.

Bilirubin is a yellow pigment produced when heme is catabolized. Hepatocytes render bilirubin water-soluble and therefore easily excretable by conjugating it with glucuronic acid prior to secreting it into bile by active transport. Hyperbilirubinemia may result from the production of more bilirubin than the liver can process, damage to the liver impairing its ability to excrete normal amounts of bilirubin or obstruction of excretory ducts of the liver. Serum bilirubin is considered as one of the true test of liver functions since it reflects the ability of the liver to take up and process bilirubin into bile [6]. Withania somnifera helped in decreasing the significantly altered levels of Bilirubin thus bringing liver to function normally [Table 1].

Production of ROS and glutathione depletion are key players in AAP induced toxicity [27]. This is evident from the reduction of antioxidant status (Superoxide dismutase, Catalase). Reduced glutathione and Glutathione-S-Transferase activities of paracetamol intoxicated rats. Wsomnifera was able to restore the levels of Antioxidants suggesting its protective role in AAP mediated liver injury. This was confirmed by the histopathological observations [Figure 1]. In conclusion, the results obtained in the present study suggest that Ashwagandha possesses a promising hepatoprotective and antioxidant effect in acetaminophen-intoxicated rats probably due to its antioxidant effects. However, further pharmacological evidence at the molecular level is required to establish the actual mechanism of the action of the drug and research into this area is underway.

REFERENCES