EVALUATION OF BOERHAAVIA DIFFUSA FOR HEPATOPROTECTIVE ACTIVITY IN EXPERIMENTAL WISTAR RATS

Shameela S*, Shamshad S2, Indira Priyadarshini A3 and Lakshmi Devi K1

1 Department of Biochemistry, S.K.University, Ananthapur, Andhrapradesh, India.
2 Department of Zoology, K.V.R.Government College for Women, Kurnool, Andhrapradesh, India.
3 Department of Botany, K.V.R. Government College for Women, Kurnool, Andhrapradesh, India.

*Corresponding Author Email: shameela325@gmail.com

ABSTRACT

Boerhaavia diffusa (Nyctaginaceae) is conventionally used in Indian Ayurvedic system to treat liver diseases. The present study was undertaken to investigate the protective role of ethanol extract of B. diffusa on hepatic antioxidant status and the levels of diagnostic markers in isoproterenol - induced hepatitis in rats. The Prior oral administration of B. diffusa extract [150 mg/kg bodyweight/day for 45 days] significantly (P<0.05) restored the levels of diagnostic marker enzymes [alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH) and creatinine kinase (CK)] and gamma glutamyl transferase (GGT)] and Lipid peroxidation levels was decreased in liver tissues of experimental rats. Also, a significant (P<0.05) increase in reduced glutathione (GSH) and the activities of glutathione dependent antioxidant enzymes [glutathione peroxidase (GPx), glutathione-S-transferase (GST) and glutathione reductase (GR)] and antiperoxidative enzymes [catalase (CAT) and superoxide dismutase (SOD)] were observed in the liver tissue. Anti-hepatotoxic potential of B. diffusa might be due to its antioxidant property and membrane stabilizing action.

KEY WORDS

Boerhaavia diffusa, isoproterenol, Hepatitis, antioxidants, lipid peroxidation

INTRODUCTION

Herbal drugs have gained importance and popularity in recent years because of their safety, efficacy and cost effectiveness. Almost 80% of the world population depends on traditional medicine which predominantly based on plant material 1. Demand for medicinal plants is increasing in both developing and developed countries due to growing recognition of natural products. In India, of the 17,000 species of higher plants, 7,500 are known for medicinal uses. Recently, several plants of Indian origin have been found to possess medicinal properties with their beneficial effects in ailments like atherosclerosis, ischemia, cancer, diabetes and liver dysfunction. More than 87 medicinal plants were used in different combinations in the preparation of 33 patented herbal formulations 2. Only a small portion of the hepatoprotective plants as well as formulations used in traditional medicine are pharmacologically evaluated for their efficacy. Secondary metabolites, the complex substances that synthesizing in Plants are commonly referred to as phytochemicals. These Phytochemicals are biologically active compounds that have potential disease inhibiting capabilities. There are several antioxidants or plant phytochemicals which are known to improve the various damages caused by oxidative stress 3, 4. A large number of plants and purified
natural products have been screened for a wide variety of clinical diseases including liver disease. Hence, the medicinal values of these plants lie on their component phytochemicals, which produce definite physiological actions on the human body.

*Boerhaavia diffusa*, belonging to the family, Nyctaginaceae, is mainly a perennial herbaceous creeping weed of India (its traditional name is *Punarnava*) and of Brazil (known as *Erva tostão*) is widely used in traditional medicine. The word punarnava literally means, one which renews the body, that is, which brings back the youth. Punarnava enjoys an important place among medicinal herbs in India since ancient times. Pharmacological studies have demonstrated that *B. diffusa* possesses anti-inflammatory, diuretic, antifibrinolytic, anticonvulsant and antibacterial properties, which makes it a very useful medicinal plant. Various parts of *Boerhaavia diffusa* are used for the treatment of numerous disorders in different parts of India. The roots are reputed to be diuretic and laxative and are given for the treatment of anasarca, ascites and jaundice. The same hepatoprotective activity was also reported in the aerial parts of *B. diffusa*. Further experimental studies also evidenced a beneficial activity of the Punarnava root for the treatment of the jaundice.

Hepatitis is a major public health problem worldwide, responsible for considerable morbidity and mortality from chronic liver disease. Developing countries like India are also struggling to manage the impact of hepatitis along with the growing burden of obesity, Type II diabetes, hypertension and coronary heart disease. The major abnormalities noticed in hepatitis are lipidemia, peroxidation and loss of plasma membrane integrity. There is an urgent need for the clinical development of safe and non-toxic cytoprotective agents for the adequate management of hepatitis. A better understanding of the processes involved in hepatitis has stimulated the search for new drugs, which could limit the drug-induced hepatic injury.

Liver is the largest organ in human body and the principal site for intense metabolism and excretion. It has a surprising role in the maintenance, performance and regulating the homeostasis of the body. The major functions of the liver are carbohydrate, protein and fat metabolism, detoxification, blood coagulation, immunomodulation, secretion of bile and storage of vitamins. It is involved with almost all the biochemical pathways responsible for growth, fight against disease, nutrient supply, energy provision and reproduction. Liver diseases are among the most serious ailments. They may be classified as acute or chronic hepatitis (inflammatory liver diseases), hepatosis (non-inflammatory diseases) and cirrhosis (degenerative disorder resulting in fibrosis of the liver). Liver diseases are mainly caused by toxic chemicals (certain antibiotics, chemotherapeutics, peroxidised oil, aflatoxin, carbon-tetrachloride, chlorinated hydrocarbons, etc.). Most of the hepatotoxic chemicals damage liver cells mainly by inducing lipid peroxidation and other oxidative damages in liver. Malondialdehyde (MDA), the end product of lipid peroxidation, is an important marker for assessment of oxidative status. The body has a protective mechanism against these free radicals in the form of antioxidant scavenging system which comprises of enzymatic and non enzymatic defense mechanisms. These include superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione-S-transferase (GST) and reduced glutathione (GSH). The imbalance between oxidative stress and antioxidant defense mechanism underlies the etiopathogenesis of various diseases. The ALT, AST and ALP activity levels were largely used as most common
biochemical markers to evaluate liver injury \cite{22,23}. The rise in serum levels of SGPT, SGOT, ALP and CK has been attributed to the damaged structural integrity of the liver, because they are cytoplasmic in location and released into circulation during cellular damages.

Isoproterenol hydrochloride (ISPH), a synthetic catecholamine, was administered in the present study, is generally serves as a standard model to study the beneficial effect of many drugs on cardiac function. ISPH causes severe stress in the myocardium and cause necrosis in the heart muscle. ISPH-induced myocardial necrosis showed membrane permeability alterations, which bring about the loss of function and integrity of myocardial membranes \cite{24,25}. There is no study reported on isoproterenol induced toxicity in liver tissue. Hence, the present study was carried out to evaluate the hepatoprotective activity of ethanolic extract of \textit{B. diffusa} in isoproterenol induced hepatotoxicity.

**Materials and Method**

**Plant Material:**
The whole plant of fresh \textit{Boerhaavia diffusa} was collected from the Braghadevum village of Anantapur district, Andhrapradesh, was used for extraction with ethanol which was already described in our earlier paper \cite{26}.

**Experimental Animals:**
In this experiment twenty four healthy male albino Wistar strains rats, 3 months of age, weighing 150 – 190g were procured from sri venkateswara enterprises, Bangalore, India. Selected rats were acclimatized for a period of two weeks in laboratory animal house and maintained under standard conditions of temperature 27 ± 2°C, relative humidity of 60 ± 5% and 12: 12 hour light: dark cycle prior to experimentation. The animals were fed with standard pellet diet and water ad libitum. The study was approved by Animal Ethics Committee of S.K. University, Anantapur (Reg. No 470/01/a/CPCSEA, dt.24\textsuperscript{th} Aug 2001).

**Drug treatment protocol:**
After acclimatization, the rats were divided into four groups of 6 rats each. Group I rats served as the control and were injected with 20% Dimethyl sulfoxide for 45 days through oral intubation. Group II animals were injected with 20% Dimethyl sulfoxide for 45 days through oral intubation and administrated of 85mg lKg\textsuperscript{-1} body wt day\textsuperscript{-1}, i. p for 2 days for the induction of hepatotoxicity for last 2 days of experimental period. Group III rats injected with 150 mg of Ethanol extract from whole plant of \textit{B.diffusa} dissolved in 20 % DMSO administered /Kg body weight/day for 45 days through oral intubation. Group IV animals were injected with ethanolic extract of \textit{B.diffusa} at the above dosage for 45 days and then injected with isoproterenol [85mg lKg\textsuperscript{-1} body wt day\textsuperscript{-1}, i. p] for 2 days in the end of the experiment period.

At the end of the experimental period, i.e., 24 h after last injection of isoproterenol, the experimental animals were sacrificed, and blood was collected for the separation of serum. The liver tissue was excised immediately and washed with chilled isotonic saline. Then the tissue was homogenized in ice-cold 0.1 M Tris-HCl buffer, pH 7.2 and centrifuged and tissue homogenates were used for various biochemical analysis.

Lipid peroxidative extent was measured by the formation of malondialdehyde (MDA) by using the method of Okhawa \cite{27}. The diagnostic marker enzymes such as LDH, AST, ALT, ALP was estimated by Teitz method \cite{28}, using Robonik Diagnostic Kit, CK by Roasli method \cite{29} and GGT by young method \cite{30}. Reduced Glutathione was estimated by Ellman’s method \cite{31}. Glutathione dependent antioxidant enzymes like GPX was estimated by Nakamura’s method \cite{32}. Glutathione dependent antioxidant enzymes like GPX was estimated by Nakamura’s method \cite{32}, GST by Habig method \cite{33}, GSR by pinto and Bartley method \cite{34} and the peroxidative enzymes like catalase by
Beers and Sizer method \(^{35}\) and Superoxide dismutase by Soon and Tan method \(^{36}\).

**Statistical analysis**

Statistical analysis was performed by one way analysis of variance (ANOVA) followed by Duncan’s Multiple Range Test (DMRT). The results were expressed as mean ± S.E.M for rats in each group. Values of P < 0.05 were considered as significant.

**RESULTS & DISCUSSION**

Liver diseases represent a major global health problem that still has no cure in modern medicine. Developing countries like India are also struggling to manage the impact of hepatitis along with the growing burden of obesity, Type II diabetes, hypertension and coronary heart disease \(^{37}\). Some of the traditional plants used for treatment of liver disorders provided useful therapeutic agents. The major abnormalities noticed in hepatitis are lipidemia, peroxidation and loss of plasma membrane integrity. The increase in MDA level in liver suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defence mechanisms to prevent formation of excessive free radicals. It is now generally accepted that lipid peroxidation and its product play an important role in liver, kidney, heart and brain toxicity \(^{38}\). Table I represents the effect of BDEEWP on the levels of myocardial LPO marker MDA and amount of reduced glutathione (GSH) in liver tissues of control and ISPH administered rats. The content of MDA, in the liver was elevated significantly (P<0.05) by 61.5% and the GSH is decreased significantly (P<0.05) by 51.47 % in the ISPH administered group when compared to normal control group. Animals treated with BDEEWP followed by ISPH injection showed significant (P<0.05) decrease in MDA levels by 78.36% and increase in GSH levels by 94.51%. There is no significant difference between the control and BDEEWP alone treated rats i.e. in Group I and III. Glutathione is one of the abundant tripeptide non-enzymatic biological antioxidants present in the liver \(^{39}\). Depletion of GSH results in enhanced lipid peroxidation and excessive lipid peroxidation can cause increased GSH consumption as observed in the present study, which was restored by the pretreatment with *B. diffusa*.

### Table: I levels of GSH and LPO in the liver tissue of Control and Experimental Rats

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>ISPH</th>
<th>CON+BDEEWP</th>
<th>ISPH+BDEEWP</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSH</td>
<td>38.54±0.09(^a)</td>
<td>18.71±0.10(^d)</td>
<td>41.47±0.15(^c)</td>
<td>37.46±0.17(^b)</td>
</tr>
<tr>
<td>LPO</td>
<td>18.35±0.18(^a)</td>
<td>29.72±0.15(^b)</td>
<td>18.69±0.12(^a)</td>
<td>20.81±0.24(^b)</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M for six rats in each group. Values in the same row not sharing a common superscript (a-c) differ significantly P<0.05 with each other.

Antioxidants play an important role in providing protection of humans against infection and degenerative diseases \(^{40}\). Data presented in table II indicates the activities of glutathione dependent antioxidant enzymes (GPX, GST and GR) and antiperoxidative enzymes (SOD and CAT) in liver of control and experimental rats. There is a significant (P<0.05) decrease in the activities of glutathione dependent enzymes, GPX, GST and GR by 46.19%, 35.50% and 25.20% and a significant (P<0.05) decrease is observed in the antiperoxidative enzymes, SOD, CAT by 38.61% and 52.94% in livers of ISPH administered rats compared to controls. Pretreatment with BDEEWP increased the activities of antioxidant enzymes by 65.93%, 32.87%, 15.50%, 57.99 and...
74.04% respectively for GPX, GST, GR, CAT and SOD. However, BDEEWP treatment alone does not show much significant change compared with the control groups of liver. Previous studies have shown that natural antioxidant molecules impart stabilization to cell membranes in relation to the degree of their free radical scavenging ability. Hence, it is possible that likewise *B. diffusa* may also prolong the viability of liver cell membranes from ISPH-induced necrotic damage by its membrane stabilizing action.

**Table: II Effect of BDEEWP on Hepatic Tissue Antioxidant Enzyme Levels in Control and Experimental Rats**

<table>
<thead>
<tr>
<th>ANTIOXIDANT ENZYMES</th>
<th>CONTROL</th>
<th>ISPH</th>
<th>CON+BDEEWP</th>
<th>ISPH+BDEEWP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutathione Peroxidase</td>
<td>76.84±0.15a</td>
<td>41.35±0.13c</td>
<td>76.51±0.22a</td>
<td>68.61±0.17b</td>
</tr>
<tr>
<td>Glutathione – S- Transferase</td>
<td>26.60±0.34a</td>
<td>17.16±0.07c</td>
<td>26.55±0.22a</td>
<td>22.80±0.22b</td>
</tr>
<tr>
<td>Glutathione Reductase</td>
<td>35.57±0.21a</td>
<td>26.60±0.34c</td>
<td>34.37±0.20a</td>
<td>30.73±0.17b</td>
</tr>
<tr>
<td>Sodium dismutase</td>
<td>38.07±0.20a</td>
<td>23.37±0.15b</td>
<td>37.13±0.21a</td>
<td>36.93±0.18c</td>
</tr>
<tr>
<td>Catalase</td>
<td>49.56±0.08a</td>
<td>23.32±0.15d</td>
<td>47.35±0.27b</td>
<td>40.59±0.21c</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M for six rats in each group. Values in the same row not sharing a common superscript (a-c) differ significantly P<0.05 with each other.

Data in the table III represents the levels of the biochemical marker enzymes such as AST, ALT, ALP, LDL, CK and GGT in liver tissues of the control and experimental rats. A significant decrease (P < 0.50) 31.05%, 40.56%, 42.96%, 35.32%, 59.16% and 12.46% was observed in the liver tissues of ISPH induced rats compared to the normal rats. The present observation is in agreement with earlier reported studies, which have shown that the amount of diagnostic marker enzymes present in plasma is directly proportional to the number of necrotic cells present in the liver tissue. Oral pre-treatment of BDEEWP restores the activities of marker enzymes in liver tissues (33.55%, 64.61%, 70.46%, 41.27%, 79.80% and 78.42%) of ISPH administered rats. There is no significant difference between the control and BDEEWP alone treated rats i.e. in Group I and III. Decrease in liver AST, ALT, ALP, LDL, CK, and GGT has been attributed to the damaged structural integrity of the liver, because they are cytoplasmic in location and released into serum after cellular damage. Thereby increased in the concentrations of serum diagnostic markers were reported earlier in our previous paper.

**Table: III Effect of BDEEWP treatment and pre-treatment on liver tissue marker enzymes in Control and Experimental groups**

<table>
<thead>
<tr>
<th>MARKER ENZYME</th>
<th>CONTROL</th>
<th>ISPH</th>
<th>CON+BDEEWP</th>
<th>ISPH+BDEEWP</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST</td>
<td>72.43±0.24a</td>
<td>49.94±0.29c</td>
<td>71.75±0.19a</td>
<td>68.73±0.34b</td>
</tr>
<tr>
<td>ALT</td>
<td>120.65±1.37a</td>
<td>71.72±0.41c</td>
<td>120.41±0.16a</td>
<td>103.34±0.47b</td>
</tr>
<tr>
<td>ALP</td>
<td>362.04±1.80a</td>
<td>206.50±0.25c</td>
<td>364.49±0.44a</td>
<td>316.10±0.65b</td>
</tr>
<tr>
<td>LDH</td>
<td>1269.07±7.06a</td>
<td>820.80±0.77d</td>
<td>1233.59±2.92b</td>
<td>1005.81±0.52c</td>
</tr>
<tr>
<td>CK</td>
<td>228.31±0.34a</td>
<td>93.23±0.15d</td>
<td>233.65±0.53b</td>
<td>201.03±0.27c</td>
</tr>
<tr>
<td>GGT</td>
<td>6.63±0.01a</td>
<td>6.68±0.01c</td>
<td>7.64±0.01c</td>
<td>7.42±0.01b</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M for six rats in each group. Values in the same row not sharing a common superscript (a-c) differ significantly P<0.05 with each other. All the Values are expressed as U/mg protein.
CONCLUSION
The results of the present study indicate that the prior administration of B. diffusa at 150 mg/kg body weight/day for 45 days prevents the isoproterenol induced hepatitis in rats. In the present study, the prior treatment with B. diffusa significantly (P<0.05) decreased all these ISPH-induced alterations in the activities of antioxidant enzymes, marker enzymes and maintained the rats at a near normal status. The overall hepatoprotective effect of B. diffusa is probably related to a counteraction of free radicals by its antioxidant property, or by its membrane stabilizing action, or to its ability to maintain near to normal status the activities of free radical enzymes and the level of GSH, which protect hepatocellular membrane against oxidative damage by decreasing lipid peroxidation.

REFERENCES
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*Corresponding Author: shameela325@gmail.com*