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Challenges in Biochemical Engineering and Biotechnology for Sustainable Environment

## Hepatic Antioxidant and Lipid Peroxidative Efficacy of Aqueous Extract of *Boerhaavia Diffusa* (Linn.) against Antituberculosis Drug Rifampicin Induced Hepatotoxicity in Male Albino Wistar Rats

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**Abstract :** Numbers of plant remedies have been used by traditional practitioners for the treatment of liver disorders for centuries. The scientific evaluation of medicinal plants used in the preparation of folk remedies has provided modern medicine with effective pharmaceuticals for the treatment of diseases. The aqueous extract of *Boerhaavia diffusa* leaves was investigated for its antioxidant and lipid peroxidative efficacy on antituberculosis drug rifampicin (1g/kg) induced liver damage in male albino wistar rats. Antioxidant and lipid peroxidative activity was measured by using biochemical parameters such as superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH) and TBARS in liver. Oral administration of the aqueous leaf extract of *Boerhaavia diffusa* at the doses of (125, 250 and 500 mg/kg) to rifampicin treated rats produced significant antihepatotoxic effect by decreasing the level of TBARS and enhance the levels of antioxidant activity in liver. The effects aqueous leaf extract of *Boerhaavia diffusa* were comparable to standard drug silymarin. These results suggest that aqueous leaf extract of *Boerhaavia diffusa* have potential therapeutic value in the treatment of liver diseases, probably by its antioxidative efficacy in liver.

**Keywords:** Antioxidant enzymes, *Boerhaavia diffusa*, Lipid peroxidation, Rifampicin.

### Introduction

Liver is one of the most important organ that functions as a centre of metabolism of nutrients such as carbohydrates, proteins and lipids. It is also handling the metabolism and excretion of drugs and other xenobiotics from the body thereby providing protection against foreign substances by detoxifying and eliminating them. The bile secreted by the liver has, among other things, plays an important role in digestion. Liver cell injury caused by various toxicants such as certain chemotherapeutic agents, carbon tetrachloride, thioacetamide etc., Drugs are curing agents of particular diseases however side effects of drugs induced toxicity is inevitable. *Mycobacterium tuberculosis* causes infection throughout the universe and results in more deaths than any other microbial agent. Approximately one third of World's population is infected with *M.tuberculosis*. It is estimated that each year three million people die of tuberculosis and eight million new cases occur<sup>1,2</sup>.

Rifampicin is an effective drug can able to cure tuberculosis but ultimately it will damage the liver. Since time immemorial, mankind has made the use of plants in the treatment of various ailments. The Indian Traditional Medicine like Ayurveda, Siddha and Unani are predominantly based on the use of plant materials.

Herbal drugs have gained importance and popularity in recent years because of their safety, efficacy, cost effectiveness and minimum or less side effects.<sup>3</sup>

Hepatotoxicity is one of the most important adverse drug reactions associated with antituberculosis chemotherapy.<sup>4</sup> Hepatitis has been reported to occur in 0.46% of patients receiving antitubercular drug<sup>5</sup> and the rate of hepatotoxic reaction was reported much higher in Indian patients.<sup>6</sup> Hepatitis is a common disease in the world especially in the developing countries. Despite considerable progress in the treatment of liver diseases by oral hepatoprotective agents, search for newer drugs continues because the existing synthetic drugs have several limitations. Hence there are many researchers of traditional medicines attempting to develop new drugs for hepatitis.<sup>7</sup>

*Boerhaavia diffusa* (Nyctaginaceae) distributed in tropical, subtropical and temperate regions of the world. The plant is consumed as vegetable as it is believed to be a rich source of vitamins, minerals, protein and carbohydrate.<sup>8</sup> It has been shown to contain a large number of compounds such as flavonoids saponins, steroids and alkaloids.<sup>9</sup> *Boerhaavia diffusa* possess a rotenoid named boeravinone G which has been shown to be a very powerful antioxidant and genoprotective agent.<sup>10</sup> These effect of rotenoids in the prevention against the diseases causes by free radical, cancer and aging which cause damage to DNA macromole. The extraordinary antioxidant property of boeravinone G could possibly be use in the near future in order to develop drugs against different pathological conditions and theses related to reactivate oxygen species (ROS) mediated injuries.<sup>11</sup>

Silymarin is a standardized mixture of antioxidant flavonolignans (silybin and silibinin) extracted from the medicinal plant *Silybum marianum*.<sup>12</sup> It is a free radical scavenger and a membrane stabilizer that prevents lipid peroxidation and its associated cell damage in some experimental models.<sup>13</sup> Silymarin was proved to have a protective effect against experimental hepatotoxicity by regulating the actions of the ultrastructures of liver cells, and improving the performance of hepatic enzymes and bile production.<sup>14,15</sup> There is no available report on the effect of *Boerhaavia diffusa* on rifampicin induced liver damage. Therefore, the present investigation to evaluate the antioxidant and lipid peroxidative efficacy of aqueous extract of *Boerhaavia diffusa* on rifampicin induced liver injury in rats.

## Materials and Methods

### Procurement and rearing of experimental animals

Adult male albino rats (Wistar strain) were collected from Central Animal House, Rajah Muthiah Medical College, Annamalai University and were used for the present study. The rats were housed in polypropylene cages at room temperature ( $27 \pm 2^\circ \text{C}$ ). The animals were randomized and separated into normal and experimental groups of body weight ranging from 150-180 g. The animals received a diet of standard pellets (Hindustan Lever Ltd., Bombay). Rats were provided free access to water *ad libitum* and food through the tenure of acclimatization to the environment for a minimum period of two weeks prior to commencement of experiment. The study was approved by the Institutional Animal Ethical Committee of Rajah Muthiah Medical College and Hospital (160/1999/CPCSEA, Proposal No. 962), Annamalai University, Annamalainagar, Chidambaram.

### Preparation of aqueous extract

The collected leaves of *Boerhaavia diffusa* were air dried and powdered. The powdered *Boerhaavia diffusa* were kept in airtight containers in a deep freeze until the time of use. A sample containing 250 g of *Boerhaavia diffusa* was mixed with 1000 mL of distilled water and stirred magnetically overnight (12 h) at  $37^\circ \text{C}$ . This was repeated three consecutive times. The residue was removed by filtration and the extract evaporated to dryness at a lower temperature ( $<40^\circ \text{C}$ ) under reduced pressure in a rotary evaporator. The residual extract was dissolved in normal physiological saline and used in the study. The yield of the extracts was approximately 13.5 g. The suitable optimum dosage schedule were identified by administering the aqueous extract of *Boerhaavia diffusa* extracts at different dosages (125, 250, 500 and 1000 mg/kg body weight) in a day daily for twenty eight days. The optimum doses were selected as 250 and 500 mg/kg body weight of the animals for twenty eight days respectively.

### Experimental design

The animals were divided into 7 groups of 6 rats each.

Group 1: Control rats given physiological saline solution 10 mL/kg body wt.

Group 2: Rats given rifampicin (1 g/kg body wt./p o) for one day only.

Group 3: Rats given rifampicin + *Boerhaavia diffusa* (125 mg/kg body wt / p o)

Group 4: Rats given rifampicin + *Boerhaavia diffusa* (250 mg/kg body wt / p o)

Group 5: Rats given rifampicin + *Boerhaavia diffusa* (500 mg/kg body wt / p o)

Group 6: Rats given rifampicin + silymarin (25 mg/kg bodywt / p o)

Group 7: Rats given *Boerhaavia diffusa* (500 mg/kg body wt / p o) alone

At the end of the experimental period in 24 h after last treatment the animals were killed by cervical decapitation. The liver tissues were excised immediately and washed with chilled physiological saline.

### Biochemical analysis

Liver tissues were taken into centrifuge tube with rupper caps labeled and centrifuged at 3000 rpm for 15 minutes. Biochemical parameter such as lipid peroxidation (TBARS), reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) activities were estimated according to standard methods<sup>16,17,18,19</sup> respectively.

### Statistical analysis

Statistical analysis was done by analysis of variance (ANOVA) and the groups were compared by Duncan's multiple range test (DMRT). The level of statistical significance was set at  $p < 0.05$ .<sup>20</sup>

### Result and Discussion

Liver is the key organ in the metabolism, detoxification and secretory functions in the body and its disorders are numerous with no effective remedies and however, the search for new medicines is still ongoing.<sup>21</sup> Many folk remedies from plant origin have been long used for treatment of liver diseases.<sup>22</sup> Liver injury in a patient on antituberculosis treatment often presents the clinician with a difficult problem of management.<sup>23</sup> Management of liver diseases is still a challenge to the modern medicine. In Ayurveda, various herbal and herbomineral preparations are extensively used for the treatment of various liver disorders.<sup>24</sup>

**Table 1. Activities of lipid peroxidation and antioxidants in liver of control and experimental groups**

Groups	TBARS (nmoles/mL)	GSH (mg/dL)	SOD (Units <sup>A</sup> )	CAT (Units <sup>B</sup> )
Control	1.17±0.04 <sup>a</sup>	9.21±0.43 <sup>c</sup>	6.18±0.30 <sup>d</sup>	60.31±3.48 <sup>c</sup>
Rifampicin (1g /kg)	3.47±0.17 <sup>d</sup>	4.55±0.17 <sup>a</sup>	4.31±0.22 <sup>a</sup>	26.18±1.31 <sup>a</sup>
Rifampicin (1g /kg) + <i>Boerhaavia diffusa</i> (125 mg/kg)	1.67±0.07 <sup>c</sup>	6.40±0.35 <sup>b</sup>	4.78±0.20 <sup>b</sup>	39.15±2.21 <sup>b</sup>
Rifampicin (1g /kg) + <i>Boerhaavia diffusa</i> (250 mg/kg)	1.34±0.04 <sup>b</sup>	7.67±0.45 <sup>c</sup>	5.64±0.25 <sup>c</sup>	48.48±3.41 <sup>c</sup>
Rifampicin (1g /kg) + <i>Boerhaavia diffusa</i> (500 mg/kg)	1.20±0.05 <sup>a</sup>	8.55±0.51 <sup>d</sup>	6.09±0.29 <sup>cd</sup>	56.65±2.40 <sup>d</sup>
Rifampicin + Silymarin (25 mg/kg)	1.24±0.04 <sup>ab</sup>	7.87±0.55 <sup>c</sup>	6.02±0.25 <sup>cd</sup>	51.23±2.20 <sup>d</sup>
<i>Boerhaavia diffusa</i> (500 mg/kg) alone	1.16±0.04 <sup>a</sup>	9.24±0.45 <sup>e</sup>	6.19±0.32 <sup>d</sup>	61.38±3.70 <sup>e</sup>

All the values are mean ± SD of six observations

Values which are not sharing common superscript differ significantly at 5% level ( $P < 0.05$ )

Duncan Multiple Range Test (DMRT)

Units<sup>A</sup> = one unit is as 50% inhibition of NBT/mg protein

Units<sup>B</sup> =  $\mu$ moles of H<sub>2</sub>O<sub>2</sub> utilised/min/mg protein.

Table 1 shows the results of lipid peroxidation and antioxidant activities in control and experimental rats. Oral administration of antituberculosis drug, rifampicin to rats induced an increase in lipid peroxidation with a concomitant decline in SOD, CAT and GSH activities in liver. This confirms that rifampicin accentuates lipid peroxidation an indicator of tissue damage and antioxidant enzymes are presumed to be an important

endogenous defense against peroxidative destruction of cellular membranes. Lipid peroxidation has been identified as one of the basic reactions involved in oxygen free radical induced cellular damages.<sup>25</sup> Peroxidation reactions in biological systems are the underlying causes for a variety of pathological condition.<sup>26</sup> Lipid peroxidation is a measurement of function of cellular membranes. The levels of TBARS are an indirect measurement of the lipid peroxidation.<sup>27</sup> The reactive free radicals initiate cell damage through two major mechanisms of covalent binding to cellular macromolecules and lipid peroxidation.<sup>28</sup> The free radicals initiate lipid peroxidation and could produce a range of enzymatically damaging consequences and could result in membrane disorganization by peroxidizing mainly the highly unsaturated and polyunsaturated fatty acids by attacking the methylene bridge hydrogen.<sup>29</sup>

Reduced glutathione (GSH) plays a key role in protecting cells against electrophiles and free radicals. This is due to the nucleophilicity of the SH group and to the high reaction rate of thiols with free radicals.<sup>30</sup> The role of extracellular GSH in detoxification of reactive oxygen intermediate has been well established. GSH as a co-substrate for glutathione peroxidase (GPx) plays an essential protective role against reactive oxygen species that may be generated under several stress conditions. It has been shown that glutathione 'redox cycle' dynamic balance between reduced glutathione represents one of the most effective endothelial cell antioxidant mechanisms.<sup>31, 32</sup> Reduced glutathione is a cofactor for enzymes involved in protecting membrane against oxidative damage. GSH scavenges hydrogen peroxide in the reaction catalysed by glutathione peroxidase. A deficiency of glutathione and its antioxidant partners in the liver and an increase in toxic free radicals may contribute the progression of liver disease. Catalase catalyses the decomposition of H<sub>2</sub>O<sub>2</sub> to water and oxygen and thus protecting the cell from oxidative damage by H<sub>2</sub>O<sub>2</sub> and OH.<sup>33</sup>

In the present study administration of rifampicin treated rats showed decrease in the activities of superoxide dismutase, catalase and reduced glutathione whereas lipid peroxidation level was increased when compared with control rats. Oral administration of aqueous extract of *Boerhaavia diffusa* (125, 250 and 500 mg/kg body wt.) and silymarin to rifampicin treated rats showed an elevated levels of superoxide dismutase, catalase and reduced glutathione whereas lipid peroxidation level was decreased than rifampicin alone treated rats. Similarly administration of *Astercantha longifolia* extract and silymarin to CCl<sub>4</sub> treated rats showed increased the activities of SOD, CAT and GSH whereas lipid peroxidation level was decreased when compared with CCl<sub>4</sub> alone treated rats.<sup>34</sup> Oral administration of *Cajanus indicus* to thioacetamide treated rats showed an increase in SOD and CAT activity where as lipid peroxidation level was decreased.<sup>35</sup> Administration of HD-03, a herbal formulation to paracetamol treated rats showed lipid peroxidation level was decreased and GSH activities was increased.<sup>36</sup> Oral administration of *Agaricus blazei* to CCl<sub>4</sub> treated rats showed that SOD, CAT and GSH activities were enhanced where as lipid peroxidation level was minimized.<sup>37</sup> SOD, CAT and GSH activities were significantly enhanced whereas lipid peroxidation level was decreased with administration of *Indigofera tinctoria* to paracetamol induced hepatotoxicity rats<sup>38</sup>.

It is concluded that treatment with aqueous extract of *Boerhaavia diffusa* decreases the rifampicin induced toxicity in biochemical parameters. These findings suggest that the aqueous extract of *Boerhaavia diffusa* was effective in bringing about functional improvement of hepatocytes. The enhancement of the antioxidant effect of this extract was also confirmed by minimize the lipid peroxidative activities were observed. The study demonstrates that, aqueous extract of *Boerhaavia diffusa* have a potential therapeutic approach to antihepatotoxic properties.

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