Protective Effect of a Herbal Formula Against Carbontetrachloride Induced Hepatotoxicity

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Abstract: This study investigated the protective effects of a polyherbal formula BCEZ, containing extracts of *Bacopa monnieri* Linn. Penn., *Curcuma longa* Linn., *Emblcia officinalis* Gaertn. and *Zingiber officinale* Rosc., on the carbon tetrachloride (CCL) induced hepatotoxicity in rats. Hepatic injury was achieved by injecting 0.5 mL kg⁻¹, i.p. of CCL. The BCEZ at the doses 50, 100 and 250 mg kg⁻¹, p.o. offered significant hepatoprotective action by reducing the serum marker enzymes like Serum Glutamate Oxaloacetate Transaminase (SGOT) and Serum Glutamate Pyruvate Transaminase (SGPT). They also reduced the elevated levels of alkaline phosphatase (ALP). Histopathological studies further confirmed the hepatoprotective activity of BCEZ when compared with the CCL treated control groups. The results obtained were compared with silymarin (100 mg kg⁻¹, p.o.), the standard drug. Thus it can be concluded, BCEZ might be a potential herbal agent for its hepatoprotective activity.

Keywords: Hepatoprotective, serum glutamate oxaloacetate transaminase, serum glutamate pyruvate transaminase, herbal formula, carbontetrachloride

INTRODUCTION

Liver diseases are world wide problem. Liver is the most important organ, which plays a pivotal role in regulating various physiological processes in the body. It has great capacity to detoxicate toxic substances and synthesize useful principles. The spectrum of its functions include metabolism and disposition of chemicals (xenobiotics) to which the organ is exposed directly or indirectly, metabolism of lipids, carbohydrates and proteins, blood coagulation and immunomodulation. Accordingly, the disorders associated with this vital organ are numerous and varied. Conventional synthetic drugs used in the treatment of liver diseases are sometimes inadequate and can have serious adverse effects. So there is a worldwide trend to go back to traditional medicinal plants (Venkateswaran et al., 1997; Mitra et al., 2000; Dhale and Naik, 1997). Therefore, damage to the liver inflicted by hepatotoxic agents is of grave consequences. There is an ever increasing need of an agent which could protect it from such damage. In view of severe undesirable side effects of synthetic agents, there is growing focus to follow systematic research methodology and to evaluate scientific basis for the traditional herbal medicines which are claimed to possess hepatoprotective activity (Subramoniam et al., 1998). The development of herbal formulation as naturally occurring inhibitors of peroxidation and resulting to reduce cell damage without any side effects. Therefore, lead to important new strategies for disease prevention in human beings.

BCEZ is a herbal formula containing whole plant of *Bacopa monnieri* Linn. Penn., fruits of *Emblica officinalis* Gaertn, rhizome of *Curcuma longa* Linn. and *Zingiber officinale* Rosc. All the 4 Indian medicinal plants have different biological properties. *Curcuma longa* Linn. (Zingiberaceae), commonly known as haridra in Hindi and turmeric in English is commonly used as an antacid, carminative, stomachic, blood purifier, wound healing, anti-inflammatory, antibacterial, antiviral and antioxidant activities (Ammon and Whal, 1991). It is widely used in the coloring agent in food items and it is a major component of curry powder. Its medicinal properties have been attributed mainly due to the presence of
curcuminoids. The main component present in the rhizome includes curcumin, demethoxy curcumin and bisdemethoxy curcumin. *Zingiber officinale* Rosc. (Zingiberaceae), is commonly known as suniti in Hindi and ginger in English. Ginger taste is sweet, pungent, act as appetizer, an aphrodisiac and carminative in nature. In Ayurveda, it is considered a valuable medicine because of its action as rubificant, antiasthmatic and stimulant to the gastrointestinal tract. Ginger has been showed the hypolipidaemic, anti-inflammatory and antihepatotoxic activity (Bhandari et al., 1998; Penna et al., 2003). The main active ingredients present in the ginger are gingerols. *Emblia officinalis* Gaertn., (Euphorbiaceae), commonly known as Amla in Hindi. The fruits of the plant are fleshy with sour, astringent taste and are consumed as raw, cooked, or even pickled locally. The fruits have been reported to possess antioxidant, adaptogenic, hepatoprotective, antifungal, antipyretic, analgesic, gastroprotective, hypolipidaemic, antiluorogenic activities (Bhattacharya et al., 1999; Rege et al., 1999; Jose and Kuttan, 2000; Dutta et al., 1998; Periamyagam et al., 2004; Al-Rehailiya et al., 2002; Mathur et al., 1996; Saram et al., 2002a, b). *Bacopa monnieri* Linn. Penn. (Scrophulariaceae), commonly known as Brahmi, is reported to be useful in the treatment of insanity, epilepsy and as an effective drug for nerve tonic and its bacosides are used as a memory enhancer. Brahmi has been reported to possess antioxidant activity (Tripathi et al., 1996).

Recently, several traditional formula comprising 4 or more herbs have been successfully used to treat liver disorders (Acharya et al., 2004; Mitra et al., 1998). In view of severe undesirable side effects of synthetic drugs, there is growing focus to follow systematic research methodology develop and evaluate the traditional herbal formulations which can be claimed to possess hepatoprotective activity. The aim of present experimental study on rats, a systematic research was undertaken to evaluate the possible effect of the formulation on the hepatotoxicity induced by CCl4 agents and this communication substantiates the therapeutic utility of the formulation as a hepatoprotective agent.

**MATERIALS AND METHODS**

**Plant material:** The plant material used in this study were authenticated by Dr. H.B. Singh of National Institute of Science Communication and Information Resources, New Delhi, India. The authenticated sample of plant material of *Bacopa monnieri* Linn. Penn. (whole plant, voucher specimen no. NISCAIR/RHMD/Consult/07-08/882/66/4), *Curcuma longa* Linn. (rhizome, voucher specimen no. NISCAIR/RHM/F-3/2006/Consult/723/40), *Emblia officinalis* Gaertn. (fruits, voucher specimen no. NISCAIR/RHMD/Consult/06-07/790/107) and *Zingiber officinale* Rosc. (rhizome, voucher specimen no. NISCAIR/RHM/F-3/2004/Consult/495/71) were collected from Bangalore in the month of June and provided by M/s Natural Remedies Pvt. Ltd. Bagalore, India.

**Preparation and standardization of plant extracts:** Coarse powder of the dried material of *Bacopa monnieri*, *Curcuma longa*, *Emblia officinalis* and *Zingiber officinale* was separately extracted with methanol using a soxhlet apparatus. The methanolic extract thus obtained was dried separately under reduced pressure at a room temperature not exceeding 40°C.

**Herbal formulation BCEZ:** The polyherbal formulation consists of methanolic extract of *Bacopa monnieri*, *Curcuma longa*, *Emblia officinalis* and *Zingiber officinale* in the ratio of 1:1:2:1. The extracts were mixed properly.

**Experimental animals:** The study was carried out on mixed sex of Wistar albino rats (175-210 g) inbred at our animal house. They were fed with a standard pellet (Goldfin Feed, New Delhi, India) and water *ad libitum*. The rats were kept in standard environmental conditions (temperature 25-28°C and 12 h light/12 h dark cycle) at 37°C and was used for the estimation of various biochemical parameters.

**Treatment:** All plant extracts were individually weighed and mixed properly. The drugs were administered as oral 2% gum acacia suspension. The group 1 animals of the control group received Vehicle (2% gum acacia suspension) for seven days. The animal of group 2 also received vehicle for seven days. The animals of group 3 received the silymarin (100 mg kg⁻¹ p.o.) for seven days. Animals of group 4, 5 and 6 received BCEZ at a dose of 50, 100 and 250 mg kg⁻¹ p.o., respectively for seven days. On 8 day groups 2-6 received carbon-tetra chloride (CCl₄, 0.5 mL kg⁻¹, i.p.). The liver was excised out after perfusion, washed with chilled normal saline solution and 10% w/v liver homogenates were prepared in ice-cold 0.15 M KCl solution.

**Assessment of liver functions:** Twenty-four hours after the toxin administration, the rats of each group were anaesthetized and blood was collected directly from the heart. The blood samples were allowed to clot for
20-30 min. Serum was separated by centrifugation at 37°C and used for estimation of various biochemical parameters.

**Assay of serum transaminases:** The activities of Serum Glutamate Oxaloacetate Transaminase (SGOT) and of Serum Glutamate Pyruvate Transaminase (SGPT) were estimated by using Ecolline kit (E-merck). The enzyme activity was expressed as mL^{-1}.

**Assay of alkaline phosphatase:** The activity of serum alkaline phosphatase (ALP) was estimated by using the Ecolline kit (E-merck). The enzyme activity was expressed as KA unit.

**Estimation of total protein:** Total protein (TP) were estimated by the methods of Lowry et al. (1951). The units were expressed as g dL^{-1}.

**Statistical analysis:** Results of the biochemical estimations are reported as Mean±SD. Total variation, present in a set of data was estimated by one-way analysis of variance (ANOVA) followed by Dunnett’s test. Minimum level of significance was fixed at p<0.05.

**RESULTS AND DISCUSSION**

Rats treated with a single dose of CCl_{4} developed significant hepatic damage as observed from elevated serum levels of hepatospecific enzymes as well as severe alterations in different liver parameters (Table 1). Oral administration of BCEZ is seen to lower the levels of marker enzymes namely SGOT, SGPT and ALP compared to CCl_{4} treated group (Table 1). The level of serum proteins was significantly (p<0.01) increased in rats, which received BCEZ as compared to CCl_{4} group (Table 1).

BCEZ demonstrated protective effect in rats against CCl_{4} induced hepatotoxicity in doses ranging from 50-250 mg kg^{-1}. The effect of BCEZ seems to be dose dependent. However, the protection offered by silymarin seemed relatively greater. Figure 1 exhibits the histological section of liver of rats treated with BCEZ. The normalcy of hepatic cells, central vein and portal triad can be easily observed. The degree of protection was observed maximally with the highest dose of the extract.

The present study brings about the potential hepatoprotective activity of BCEZ and gives insight into its mechanism of action. Liver injury induced by CCl_{4} is the best-characterized system of the xenobiotic-induced hepatotoxicity and is a commonly used model for the screening the anti-hepatotoxic/hepatoprotective activity of drugs (Brautbar and Williams, 2002; Brent and Rumack, 1993). In this study, rat treated with single dose of CCl_{4} developed a significant hepatic damage, which was observed from a substantial increase in the activities of serum, SGOT and SGPT. This is indicative of cellular leakage and loss of functional integrity of cell membrane in liver (Sallie et al., 1991). Reduction in the levels of SGOT and SGPT towards the respective normal values by herbal formula of three different doses (50, 100 and 500 mg kg^{-1}) is an indication of the stabilization of plasma membranes as well as repair of hepatic tissue damage caused by CCl_{4}. This effect is in agreement with the commonly accepted view that serum levels of transaminases return to normal with healing of hepatic parenchyma and the regeneration of hepatocytes (Mahi et al., 2005). In the present study, also it was seen that administration of CCl_{4} elevates the levels of serum marker enzymes SGPT, SGOT and ALP and level of total protein is lowered. BCEZ and silymarin treated groups exhibited lower levels of SGPT, SGOT and ALP as compared to CCl_{4} treated group. The treatment with BCEZ also significantly elevated total protein levels. The stabilization of serum SGPT, SGOT and ALP levels by BCEZ is a clear indication of the improvement of the functional status of the liver cells. The characteristics feature of experimental hepatic damage observed is significant decrease in protein level. The rats in a group which received BCEZ showed rectification of lowered protein levels. These findings can be further corroborated with histopathological studies. The histopathological examination clearly reveals that the hepatic cells, central vein and portal triad are almost normal in BCEZ (250 mg kg^{-1}, p.o.) group in contrast to group which received CCl_{4}.

<table>
<thead>
<tr>
<th>Treatment/group</th>
<th>SGOT (U mL^{-1})</th>
<th>SGPT (U mL^{-1})</th>
<th>ALP (KA unit)</th>
<th>Total protein (g dL^{-1})</th>
</tr>
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<tbody>
<tr>
<td>Normal (I)</td>
<td>41.15±5.23**</td>
<td>29.45±6.21**</td>
<td>10.21±4.23**</td>
<td>7.54±0.65**</td>
</tr>
<tr>
<td>CCl_{4} Control (II)</td>
<td>118.67±10.46</td>
<td>121.83±7.42</td>
<td>34.01±4.12</td>
<td>3.48±0.59</td>
</tr>
<tr>
<td>Silymarin (100 mg kg^{-1})+CCl_{4} (III)</td>
<td>49.00±8.77**</td>
<td>40.55±8.43**</td>
<td>14.67±3.09**</td>
<td>6.89±0.81**</td>
</tr>
<tr>
<td>BCEZ (50 mg kg^{-1})+CCl_{4} (IV)</td>
<td>116.67±8.98</td>
<td>115.25±12.34</td>
<td>24.78±2.98**</td>
<td>5.68±0.89**</td>
</tr>
<tr>
<td>BCEZ (100 mg kg^{-1})+CCl_{4} (V)</td>
<td>99.78±11.24*</td>
<td>81.45±10.25**</td>
<td>18.43±0.96**</td>
<td>6.02±0.23**</td>
</tr>
<tr>
<td>BCEZ (250 mg kg^{-1})+CCl_{4} (VI)</td>
<td>60.05±10.25**</td>
<td>49.29±6.25**</td>
<td>15.45±1.34**</td>
<td>5.98±0.59**</td>
</tr>
</tbody>
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*p<0.05 and **p<0.01 as compared to control group by Dunnett’s test.
CONCLUSION

Thus, BCEZ can be considered to be an effective hepatoprotective herbal formula as it ameliorates almost to normalcy the damage caused by CCL\textsubscript{4} to hepatic function. It is difficult at this stage to comment on the rational of inclusion of such herbs together in single formulation for hepatic protection but the results of this study demonstrated that pretreating the rats with BCEZ effectively protected the rats against CCL\textsubscript{4} induced hepatotoxicity, as evidenced by a significant reduction in the CCL\textsubscript{4} induced rise in SGOT and SGPT levels in rats in a dose-dependent manner. This phenomenon was also confirmed by histological observation. The BCEZ formulation used in this study seems to preserve the structural integrity of the hepatocellular membrane.

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REFERENCES


