

Research Article



Antioxidant and Hepatoprotective Effects of *Bacopa monnieri* and *Vinca rosea* against Carbon Tetrachloride (CCl₄) Induced Liver Damage in Rats

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ABSTRACT

Liver disease is still a worldwide health problem. The liver plays an essential role in drug and xenobiotic metabolism and in maintaining the biological equilibrium of the organism. 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 that are claimed to possess hepatoprotective activity. The present study is carried out to investigate the hepatoprotective effect of ethanolic extracts of *Bacopa monnieri* (BM) and *Vinca rosea* (VR) with particular reference to antioxidant enzymes activities of Glutathione S-transferase (GST), Glutathione reductase (GR), Glutathione peroxidase (GPx), Lipidperoxidase (LP), Superoxide dismutase (SOD), Catalase (CAT) and Glutathione (GSH) content. The animals were divided into five groups with six rats in each. Group 1 received Saline water, group 2 received Carbon tetrachloride (CCl₄) through i.p. route for 14 days to induce hepatic damage. The BM and VR extracts were given (180 mg/kg/day) for 21 days orally to group 3 and group 4 respectively. Group 5 received Silymarin orally which is referred as drug control. CCl₄ treated rats exhibited significant decrease in GST, GR, GPx, LP, SOD and CAT enzyme activities and GSH levels in the liver compared to controls. Pretreatment with ethanolic extracts of BM and VR exert antioxidative effects by ameliorating the status of antioxidant metabolism, thus showing hepatoprotective activity. Our results suggest the ability of BM and VR extracts to modulate antioxidant metabolism in liver of CCl₄ induced liver damage.

Keywords: Hepatoprotective activity, antioxidant enzymes, BM and VR, CCl₄, ROS.

INTRODUCTION

It is well documented that the liver plays an essential role in the metabolism of drugs and xenobiotics and in maintaining the biological equilibrium of the organism. Major causes of hepatic disorders are due to exposure to different environmental pollutants and xenobiotics which mainly damage liver by producing Reactive Oxygen Species (ROS). Steroids, vaccines, and hepatoprotective drugs, which have been employed as therapies for liver diseases, have potential adverse effects, especially when administered for long term. Therefore, herbal products and traditional medicines with improved effectiveness and safety profiles are needed as a substitute for chemical therapeutics. It is reported that a number of herbal products have been shown to protect against liver injury, and many possess one or a combination of antioxidant, antifibrotic, immune modulatory, or antiviral activities¹⁻³. In recent years, there has been a substantial increase in the use of complementary and alternative therapies that utilize herbal medicines for the liver disease⁴⁻⁶.

Keeping in view of the relative importance of medicinal plants, the present project was taken up to study the hepatoprotective effect of two medicinal plants viz. *Bacopa monnieri* (BM) and *Vinca rosea* (VR). *Bacopa monnieri* belongs to the family of Scrophulariaceae which has been used since time immemorial by ayurvedic medical practitioners as brain tonic⁷. *Vinca rosea* belongs to the family of Apocynaceae which is traditionally being used as anticarcinogenic, antidiabetic, hypotensive and

transquilizer. *Vinca rosea* has a variety of medicinal properties such as antibacterial⁸, antifungal⁹, antiviral¹⁰ and anticancer¹¹.

It is well established that liver damage induced by several xenobiotic agents including carbon tetrachloride (CCl₄) is associated with oxidative stress leading to the production of Reactive Oxygen Species (ROS) and dysregulations of antioxidant defence mechanism. Several lines of evidence indicate that dietary phytochemicals derived from natural medicinal plants has been reported to inhibit lipid peroxidation and associated oxidative stress. Hence the present study is mainly focused to evaluate the hepatoprotective effect of ethanolic extracts of BM and VR on selected biochemical parameters and marker enzymes of free radical metabolism during oxidative damage induced by Carbon tetrachloride (CCl₄).

MATERIALS AND METHODS

Collection of plant material

Bacopa monnieri (BM) and *Vinca rosea* (VR) plants used in this work were collected in bulk from Tirumala Hills, Andhra Pradesh in India and authenticated by qualified botanist at Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh, India.

Extract Preparation

The whole plant of BM and leaves of VR was shade-dried and powdered. The plant material was percolated with circulating 95% ethanol (200 ml) for three rounds. The residue was extracted twice using the same procedure.



The extract was filtered and concentrated under reduced pressure in the Buchi rotavapour. Finally the extract was freeze-dried and was used for further studies.

Experimental design

The present work was conducted on male Wistar rats weighing 150 ± 25 g. They were maintained at a temperature of $25 \pm 2^\circ\text{C}$ and relative humidity of 45-55% with 12:12 h dark: light cycle. The rats were divided into 5 groups each consisted of 6 rats as mentioned below. The rats were maintained according to the ethical guidelines for animal protection and welfare bearing no.04a/a/CPCSEA/IAEC/08-09/SVU/Zool/WR-GS/dt.1.9.2009.

Group I: Served as normal control group, received vehicle at a dose of (olive oil) 1.0 ml/kg/day i.p. for 14 days.

Group II: Hepatic damage was induced by Carbon tetrachloride (CCl_4) at a dose of 2.5 mg/kg once daily for 14 days.

Group III: Hepatic damaged rats were treated with BM extract with a dose of 180 mg/kg/day orally for 21 days, started before 7 days from induction of CCl_4 .

Group IV: Hepatic damaged rats were treated with VR extract with a dose of 180 mg/kg/day orally for 21 days, started before 7 days from induction of CCl_4 .

Group V: Hepatic damaged rats were treated with Silymarin drug (reference drug) (25mg/kg) orally for 14 days from induction of CCl_4 .

After completion of 21 days treatment the animals were sacrificed by cervical dislocation and the liver tissue was excised. The tissues were washed with ice-cold saline, immersed in liquid nitrogen and immediately stored in deep freezer at -80°C for further biochemical analysis.

Biochemical Analysis

The levels of Lipid peroxidation was measured by determining malondialdehyde (MDA) content using Thiobarbituric acid (TBA)¹². Serum levels of hepatic enzyme markers such as Aspartate aminotransferase (AST) and Alanine aminotransferase (ALAT) were measured¹³. Glutathione (GSH) content was measured¹⁴. The activity levels of antioxidants enzymes such as Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (Gpx), Glutathione-S- transferase (GST) and Glutathione reductase (GR) were determined by the methods¹⁵⁻¹⁹ respectively.

Statistical Analysis

Values of the measured parameters were expressed as Mean \pm SEM of six individual observations. One way-ANOVA (F value) was used to test the significance of difference among more than two arithmetic means, followed by Post – hoc test (Scheffe multiple comparison) to test the difference between each two means. The significance was considered at p values < 0.05 . All the statistical analyses were processed using Statistical

Program of Social Sciences (SPSS) for Windows, version 11.5.

RESULTS

Lipid peroxidation

Malondialdehyde (MDA) is the product of lipid peroxidation and is a common marker of lipid peroxidation. The content of MDA was significantly increased in the liver of CCl_4 -treated rats as compared with the control group (Table 1). BM, VR and Silymarin treated animals significantly suppressed the MDA content of liver. These results suggest that oxidative stress induced by CCl_4 was blocked by the supplementation of BM and VR.

Hepatic antioxidant enzyme activities

The hepatic antioxidant enzymes activities were decreased with the treatment of CCl_4 . However, GPx, GST, GR, SOD and CAT activities were observed to be significantly elevated during treatment with BM and VR extracts when compared to CCl_4 treated group. The GSH content was decreased in CCl_4 treated group as compared to control (Table 1). The groups treated with BM, VR and Silymarin showed significant increase in the level of GSH content as compared with CCl_4 treated group.

Serum marker enzymes

The serum activities of AST and ALT were used as biochemical markers for the early acute hepatic damages. As shown in Table 2, the groups pre-treated with both doses of the BM and VR extract showed significantly lower activities of AST and ALT than did the CCl_4 treated group. The Silymarin group also demonstrated significantly lower serum activities of both the enzymes in comparison to the CCl_4 treated group.

DISCUSSION

The hepatotoxicity of CCl_4 in experimental animals has been studied extensively and used widely as a model agent for inducing free radical damage. The toxicity of CCl_4 probably depends on conversion of CCl_4 to highly toxic trichloromethyl free radical ($\text{CCl}_3\cdot$) by cytochrome P450 leading to hepatocellular injury. Lipid peroxidation is thought to be one of the major pathways of disease initiation and proliferation. It is initiated by the interaction of the trichloromethyl radical with unsaturated fatty acids of membrane lipids²⁰. Increased lipid peroxidation and impaired antioxidant defense mechanism in the liver tissue are characteristic observations in CCl_4 -treated rats^{20,21}. Hence hepatoprotective effect of BM, VR was evaluated in the present study against acute oxidative stress induced by Carbon tetrachloride (CCl_4).

It is well known that xenobiotic agents induce hepatic injury causing marked elevation in the activity of serum AST and ALAT activities²¹, which are considered as serum marker enzymes of liver function.



Table 1: Alterations in the levels of MDA, GSH, SOD, CAT, GPx, GST, and GR in rat liver during CCl₄ induced acute liver damage and on pre-treatment with ethanolic extracts of BM, VR and Silymarin groups

Parameters	SC	CCl ₄	BM+CCl ₄	VR+CCl ₄	REF+CCl ₄
GSH ^a	6.29±0.04	2.37±0.27*	5.71±0.13 [#]	5.38±0.13 [#]	5.80±0.08 [#]
GR ^b	8.47±0.12	4.32±0.16*	5.96±0.21 [#]	6.27±0.32 [#]	6.33±0.15 [#]
GPx ^c	2.1±0.08	0.95±0.54*	1.86±0.24 [#]	1.68±0.42 [#]	1.69±0.32 [#]
GST ^d	6.33±0.14	2.95±0.07*	3.77±0.23 [#]	3.29±0.19 [#]	3.95±0.08 [#]
CAT ^e	5.28±0.15	2.60±0.16*	4.09±0.24 [#]	3.90±0.33 [#]	3.84±0.20 [#]
SOD ^f	42.14±0.53	21.78±0.78*	31.23±0.64 [#]	30.12±0.56 [#]	31.97±0.62 [#]
MDA ^g	93.9±1.34	151.41±3.05*	105.03±3.89 [#]	98.13±3.53 [#]	94.83±4.24 [#]

The values are expressed as Mean ± SEM of six individual observations.; *Significant at P<0.05 when compared with Saline control, # Significant at P<0.05 when compared with CCl₄ induced liver damage.;

a. Values were expressed in nano moles / gm wet weight of the tissue.; b. Values were expressed in μ moles of NADPH oxidized / mg protein / min.; c. Values were expressed in μm of NADPH oxidized / mg protein / min.; d. Values were expressed in μ moles of thioether formed / mg protein / min.; e. Values were expressed in moles of H₂O₂ degraded / mg protein / min.; f. Values were expressed in μ moles of epinephrine oxidized/ mg of protein/min.; g. Values were expressed in μ moles of malondialdehyde formed / gram wet weight of the tissue.

Table 2: Alterations in the levels of AST and ALT in rat liver during CCl₄ induced acute liver damage and on pre-treatment with ethanolic extracts of BM, VR and Silymarin groups.

Parameter	SC	CCl ₄	BM+CCl ₄	VR+CCl ₄	REF+CCl ₄
AST	69.5	123.78*	82.876 [#]	87.365 [#]	72.723 [#]
ALT	13.743	35.963*	21.426 [#]	26.396 [#]	17.594 [#]

The values are expressed as Mean ± SEM of six individual observations. *Significant at P<0.05 when compared with Saline control, # Significant at P<0.05 when compared with CCl₄ induced liver damage. The activity levels were expressed in μ moles of pyruvate formed or ketoacid formed. mg protein⁻¹.hr⁻¹.

Pre-treatment with BM and VR to the CCl₄ treated rats suppressed the hepatic damage as evidenced by significant depletion of above serum marker enzymes which suggest that BM and VR could play a significant role in protecting the liver from oxidative injury caused by CCl₄ similar to the reference drug silymarin (Table 1). Reactive oxygen species (ROS), such as superoxide anions and H₂O₂, are produced throughout cells during normal aerobic metabolism. The intracellular concentration of ROS is a consequence of both their production and their removal by various antioxidants. The major components of the antioxidant system consists of two enzymes, namely, superoxide dismutase (SOD) and catalase (CAT) (Table 2) which work in concert to detoxify superoxide anion and H₂O₂ in cells. In the present study SOD and Catalase activity levels were significantly reduced in the liver of CCl₄-intoxicated rats which were restored to normal on pretreatment with the extracts of BM and VR on par with silymarin.

GSH-related enzymes play pivotal role in detoxification of xenobiotics through the conjugation with glutathione or reduction of free radicals. Acute CCl₄ damage significantly decreased the expression of antioxidant enzymes in liver such as GPx, GR and GST. GR is responsible for the regeneration of GSH, and GPx working in conjugation with GSH helps in disintegrating hydrogen peroxide or other organic hydroperoxides. Since maintenance of intracellular levels of GSH is critical for the proper functioning of important antioxidant defence mechanism of CCl₄, the failure of GSH replenishment by reduced GR activity might have caused an inefficient operation of antioxidant mechanism during CCl₄ toxicity causing

oxidative stress. Similar to silymarin, BM and VR pretreatment reversed the activities of Gpx, GR, and GST and showed an improved effect on the expression of antioxidant enzymes as compared to CCl₄ treated group. The reversal of the oxidative damage by the BM and VR may be attributed to a direct action of the extract on the activities of hepatic antioxidant mechanism, the mechanism of which needs further investigation. Sulfhydryl compounds such as glutathione (GSH) are well known to be an antioxidant substance in organisms, playing a critical role against CCl₄-induced injury by covalently binding to CCl₃. This is considered as the initial reactant in the chain reaction of oxidation, and then result in the lipid peroxidation and the cell membrane disruption²². Similar to Silymarin treatment, pre-treatment with BM and VR resulted in elevation of GSH content in the liver of CCl₄ intoxicated rats as compared with the control group. Several diseases have been associated with the changes in GSH levels, and reduce the resistance to oxidative stress. The levels of GSH and the activities of the GPx, GR and GST were used to monitor the balance of oxidative stress and chemopreventive ability²³. In our study, the BM and VR exhibited protective effects against liver damage from CCl₃.

Lipid peroxidation, a reactive oxygen species-mediated mechanism, has been implicated in the pathogenesis of various liver injuries and subsequent liver fibrosis in experimental animals and humans^{24,25}. MDA is a major reactive aldehyde that appears during the peroxidation of polyunsaturated fatty acids present in biological membrane²⁶. Therefore, the hepatic content of MDA is used as an indicator of liver tissue damage and as a

marker for lipid peroxidation²⁷ involving a series of chain reactions¹². It has been documented that lipid peroxidation of hepatocyte membranes is one of the principal causes of CCl₄-induced hepatotoxicity, and is mediated by the production of free radical derivatives of CCl₄^{20,21,28}. Rats treated with CCl₄ showed significant increase in the levels of MDA compared with the control group. Pretreatment with BM and VR caused significant reduction in lipid peroxidation when compared to CCl₄ treated group. Earlier research findings have reported oxidative stress in rats exposed to CCl₄ and other xenobiotic compounds which were countered by dietary antioxidants²⁹. Since dietary phytochemicals such as polyphenols and flavonoids could provide potent antioxidant effects³⁰. It is possible that the BM and VR extracts exerted antioxidant effects against free-radical induced damage which are rich in antioxidant bioactive compounds.

CONCLUSION

In conclusion, pre-treatment with BM and VR could reduce the damage induced by CCl₄. The mechanisms of protection include the inhibition of lipid peroxidation, increasing the content of GSH, elevating the expression of antioxidant enzymes (GPx, GST, GR, SOD and CAT), all of which result in the recuperation of antioxidant defense mechanism and attenuate the deleterious effects of CCl₄ induced liver damage.

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