

AQUEOUS EXTRACT OF *BRASSICA RAPA CHINENSIS* AMELIORATES TERT- BUTYL HYDROPEROXIDE INDUCED OXITATIVE STRESS IN RATS

SHWETHA KALAVA, DHARANI MAYILSAM Y *

Department of Biochemistry, Kongunadu Arts and Science College, Coimbatore - 641029, Tamilnadu, India. Email: dr.sdbio@gmail.com

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ABSTRACT

Objectives: Tert-butyl hydroperoxide (t-BHP) is a membrane-permeant oxidant compound that can induce cell death via apoptosis or necrosis. The present study aims to study the effect of aqueous extract of *Brassica rapa chinensis* (250, 500 mg/kg, p.o) against the oxidative stress induced by t-BHP in rats.

Methods: The experimental rats were divided into 5 groups. Group I served as normal control, group II served as t-BHP control, group V rats were treated with silymarin and t-BHP induced, group III and IV rats were treated with the extract of *Brassica rapa chinensis* at 250 and 500 mg/kg respectively and induced with t-BHP.

Results: Prior treatment of the extract to groups III and IV rats, significantly reduced lipid peroxidation induced by t-BHP (observed as levels of hepatic malondialdehyde and hydroperoxides). The extract improved the hepatic tissue antioxidant status by improving the activity of the antioxidant enzymes - Catalase (CAT), Superoxide dismutase (SOD), Glutathione reductase (GR), Glutathione peroxidase (GPx) and also markedly normalizing the levels of non-enzymic antioxidants - vitamin E (vit E), vitamin C (vit C) and hepatic glutathione (GSH) in line with the standard drug, silymarin.

Conclusion: The treatment with aqueous extract of *Brassica rapa chinensis* significantly combats the oxidative stress imposed by t-BHP in the hepatic tissues as evidenced by marked improvement in the antioxidant status and suppressing lipid peroxide levels. The results obtained were dose dependent with 500 mg/kg b.wt, dosage of *Brassica rapa chinensis* aqueous extract revealing more potential in curbing toxic insult of t-BHP.

Keywords: Antioxidants; *Brassica rapa chinensis*; oxidative stress; Tert-butyl hydroperoxide

INTRODUCTION

Liver is the most important organ, which plays a pivotal role in regulating various physiological processes in the body. Liver injury induced by chemicals and drugs is a well-recognized toxicological problem. Many risk factors, including hepatic viruses, alcohol consumption, chemical agents, environmental pollutants and improper dietary habits, are considered to be the main causative agents of hepatic injury.

In the process of detoxification of the xenobiotics, the liver often produces free radicals. The oxidative stress imposed by the reactive oxygen species (ROS) has been shown to be associated to liver ailments, such as hepatotoxicity[1].

A number of xenobiotics have been implicated in producing oxidative stress and cell injury resulting from the intracellular production of ROS[2]. Tert-butyl hydroperoxide (t-BHP) is a short-chain analog of lipid hydroperoxide and has been often employed as a model to investigate the mechanism of cell injury initiated by acute oxidative stress [3-5]. t-BHP has been reported to be metabolized to free radical intermediates by cytochrome P450 in hepatocytes or by hemoglobin in erythrocytes, which in turn can initiate lipid peroxidation and glutathione (GSH) depletion, affect cell integrity, and result in cell injury in hepatocyte cultures and liver tissues [5]. It also causes a mitochondrial depolarization within intact hepatocytes and mediates DNA base damage in cultured mammalian cells [6].

In spite of remarkable advances in modern medicine, there are no effectual drugs available that promote liver function and offer effective protection to the liver from damage or aid in the regeneration of hepatocytes[7]. A number of plants have been reported to harbor hepatoprotective property by improving the antioxidant status of the cells[8].

Phytochemicals derived from plants are excellent antioxidants. Antioxidants appear to act against diseases by raising the levels of endogenous defense and /or by up-regulating gene expressions of the antioxidant enzymes [9,10].

Bokchoy, Chinese cabbage or Chinese chard as commonly called is a widely consumed vegetable in china and in other parts of Asia. The present study was undertaken to evaluate the potential of the

aqueous extract of bok choy, *Brassica rapa chinensis* Linn against t-BHP-induced oxidative stress.

MATERIALS AND METHODS

Preparation of the extract

Bok choy, *Brassica rapa chinensis* was obtained from local department store, Coimbatore, Tamilnadu, India. The leaves were cleaned, shade dried and powdered. 10g of the powder was extracted with 100 ml of water at 100°C for 4 hours, centrifuged at 5000 rpm for 15 minutes and filtered through Whatman No.1 filter paper. The residue was extracted twice with 100 ml portions of water, as described above. The extracts were combined and vacuum evaporated. The extract obtained after vacuum evaporation was freeze dried and stored at 4°C until further use.

Drugs and chemicals

Silymarin was obtained from Himedia, Bangalore, India. Tert-butyl hydroperoxide (t-BHP) was purchased by Sigma Aldrich, Bangalore, India. All other chemicals used in this study were obtained commercially and were of analytical grade.

Experimental Animals

Female Sprague Dawley rats, weighing, 160g-180g were purchased from, Small Animal Breeding Centre, College of Veterinary and Animal Science, Mannuthy, Kerala, India. The animals were maintained under standard conditions of humidity, temperature (25 ± 2°C) and light (12 h light/dark). They were acclimatized to animal house conditions and were fed on a commercial pelleted rat chow (AVM Cattle Feeds, Coimbatore, Tamilnadu) and water *ad libitum*. Experimental animals were handled according to the University and Institutional Legislation, regulated by the Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

Experimental Design

The animals were divided into 5 groups of six animals each. Hepatotoxicity was induced by single i.p dose of t-BHP[11]. The animals were grouped as follows- Group I - Rats in this group served as control, Group II - hepatotoxicity was induced with a

single dose of t-BHP (i.p.; 0.2 mmol/kg b.wt), Group III - rats were treated with 250 mg/kg body weight/ day of aqueous extract of *Brassica rapa chinensis* (BRCAE) orally, for a period of 7 days and on the 8th day hepatotoxicity was induced with a single dose of t-BHP (i.p.; 0.2 mmol/kg b.wt), Group IV -rats were treated with 500 mg/kg body weight/ day of aqueous extract of *Brassica rapa chinensis* (BRCAE) orally, for a period of 7 days and on the 8th day hepatotoxicity was induced with a single dose of t-BHP (i.p.; 0.2 mmol/kg b.wt) and Group V - rats were treated with 25 mg/kg body weight of silymarin orally, to the animals for 7 days and hepatotoxicity was induced on the 8th day with t-BHP (i.p.; 0.2 mmol/kg b.wt.).

Biochemical Analysis

At the end of the last injection, the animals were subjected to fasting for a period of 12 hours. At the end of 12 hours fasting the animals were sacrificed, blood was collected and the liver, were excised and washed in saline. 10% homogenate of the liver tissues was prepared with 0.1 M Tri-HCl buffer, pH 7.4. The homogenates were centrifuged at 3000 rpm for 15 min at 4°C for cytosolic separation and were used up for analysis. The levels of hepatic lipid peroxides (LPO) were determined by measuring malondialdehyde (MDA) content according to the method of Niehus and Samuelsson [12] and hydroperoxides by the method of Jiang et al [13].

The enzymatic activity of hepatic SOD was assessed according to the method of Das et al [14] and CAT by the method of Sinha [15]. The activity of glutathione metabolizing enzymes - GR and GPx were assessed by the method of Beutler et al [16] and Ellman [17] respectively. Glutathione (GSH) content of hepatic tissues were assessed according to the method described by Moron et al [18]. Protein levels were determined as described by Lowry et al [19]. Vitamin C content was evaluated as described by Omaye et al [20] and vitamin E by the method described by Deasai [21].

Statistical analysis

The data are expressed as mean \pm S.D. Statistical comparison was done at significance level, $p < 0.05$ using SPSS package version 10.0. One way ANOVA followed by post hoc analysis of LSD was performed.

RESULTS

Table 1 represents the levels of peroxidation in the hepatic tissues of experimental animals. There was observed a significant ($p < 0.05$) increase in the levels of MDA and hydroperoxides in t-BHP intoxicated rats (group II). The increase in the peroxides was nearly 2- fold as compared to normal control rats. The treatment with BRCAE to the animals of group III and IV at a dose of 250 mg/kg and 500 mg/kg body weight (b.wt), respectively, resulted in a significant ($p < 0.05$) decrease in the levels of MDA and hydroperoxides in a dose dependent manner. Silymarin administration to the group V animals, also resulted in a marked reduction ($p < 0.05$) in the hepatic peroxidation status.

The effect of BRCAE on the activity of the antioxidant enzymes are presented in **Table 2**. A significant ($p < 0.05$) reduction in the activity of SOD, CAT, GPx and GR were observed in the t-BHP control animals. Treatment with BRCAE at (250 mg/kg b. wt and 500 mg/kg b.wt) resulted in a marked improvement in the activity of the antioxidant enzymes Group V animals that were supplemented with the standard, silymarin effectively normalized ($p < 0.05$) the activity of the enzymes. The 500mg dose of BRCAE was found to be more effective in normalizing the activity of the enzymes than the 250mg dose of BRCAE.

The induction of t-BHP resulted in a marked ($p < 0.05$) decrease in the levels of GSH and vitamins C and E in the hepatic tissue as compared to normal control rats (Table 3). Prior treatment with BRCAE and silymarin was found to significantly improve and stabilize the levels of GSH, vitamin C and vitamin E compared to the t-BHP alone induced group II animals. The treatment with both the doses of BRCAE and silymarin increased the levels of non-enzymic antioxidants.

Table 1: Effects of aqueous extract of *Brassica rapa chinensis* on hepatic peroxidation status

Groups	Lipid peroxides (n moles of MDA formed /min/mg protein)	Hydroperoxides (m moles/g tissue)
Control	1.02 \pm 0.07 ^b	1.09 \pm 0.02 ^b
t-BHP (0.2 mmol/kg b.wt)	3.22 \pm 0.16 ^a	2.85 \pm 0.10 ^a
BRCAE (250 mg/kg b.wt) + t-BHP	1.51 \pm 0.06 ^b	1.59 \pm 0.05 ^{ab}
BRCAE (500 mg/kg b.wt) + t-BHP	0.98 \pm 0.05 ^b	1.23 \pm 0.06 ^b
Silymarin (25 mg/kg b.wt) + t-BHP	1.07 \pm 0.06 ^b	1.17 \pm 0.04 ^b

Values are expressed as mean \pm SD for six animals. , Group comparison and statistical significance at $p < 0.05$: ^a: Group I vs. II, III, IV, V ^b: Group II vs. I, III, IV, V

Table 2: Effects of aqueous extract of *Brassica rapa chinensis* on the activity of hepatic enzymic antioxidants

Groups	SOD (U/mg protein)	CAT (U/mg protein)	GPx (U/mg protein)	GR (U/mg protein)
Control	6.19 \pm 0.19 ^b	11.25 \pm 0.67 ^b	5.77 \pm 0.26 ^b	18.80 \pm 0.82 ^b
t-BHP (0.2 mmol/kg b.wt)	1.94 \pm 0.02 ^a	4.96 \pm 0.24 ^a	3.71 \pm 0.14 ^a	6.45 \pm 0.33 ^a
BRCAE (250 mg/kg b.wt) + t-BHP	4.21 \pm 0.26 ^b	8.36 \pm 0.47 ^{ab}	4.81 \pm 0.13 ^b	12.32 \pm 0.57 ^b
BRCAE (500 mg/kg b.wt) + t-BHP	5.89 \pm 0.31 ^b	10.78 \pm 0.39 ^b	5.12 \pm 0.30 ^b	16.59 \pm 0.81 ^b
Silymarin (25 mg/kg b.wt) + t-BHP	5.95 \pm 0.11 ^b	10.34 \pm 0.32 ^b	5.26 \pm 0.27 ^b	17.40 \pm 0.78 ^{ab}

Values are expressed as mean \pm SD for six animals. , Group comparison and statistical significance at $p < 0.05$: ^a: Group I vs. II, III, IV, V ^b: Group II vs. I, III, IV, V

Units: SOD - inhibition of 50% nitrite formation/min/mg protein; - μ moles of H₂O₂ consumed/min/mg protein; GPx- μ moles of glutathione oxidized/min/mg protein; GR - μ moles of glutathione utilized/min/ mg protein.

Table 3: Effects of aqueous extract of *Brassica rapa chinensis* on the levels of hepatic non-enzymic antioxidants

Groups	Vitamin C (μ g /mg protein)	Vitamin E (μ g /mg protein)	GSH (μ g/mg protein)
Control	1.74 \pm 0.03 ^b	1.25 \pm 0.07 ^b	17.17 \pm 0.75 ^b
t-BHP (0.2 mmol/kg b.wt)	0.49 \pm 0.02 ^a	0.56 \pm 0.02 ^a	6.08 \pm 0.29 ^a
BRCAE (250 mg/kg b.wt) + t-BHP	0.98 \pm 0.04 ^b	1.05 \pm 0.03 ^{ab}	9.98 \pm 0.33 ^{ab}
BRCAE (500 mg/kg b.wt) + t-BHP	1.71 \pm 0.06 ^{ab}	1.18 \pm 0.04 ^b	17.22 \pm 0.80 ^b
Silymarin (25 mg/kg b.wt) + t-BHP	1.69 \pm 0.08 ^b	1.30 \pm 0.05 ^b	16.85 \pm 0.58 ^b

Values are expressed as mean \pm SD for six animals. , Group comparison and statistical significance at $p < 0.05$: ^a: Group I vs. II, III, IV, V ^b: Group II vs. I, III, IV, V

DISCUSSION

Tert-butyl hydroperoxide (t-BHP), an organic lipid hydroperoxide analog, that has been demonstrated to exert pro-oxidant effects to evaluate mechanisms involving oxidative stress in hepatocyte cells and rat liver [22]. In the present study t-BHP was used as a free radical generator. After metabolic activation, metabolites such as *t*-butoxyl and *t*-methyl radicals may cause permeabilisation of cell membranes, DNA damage, and depletion of glutathione via a variety of mechanisms [23-25]. These radicals initiate and propagate lipid peroxidation in cells which are susceptible to oxidative stress. Hepatocytes have a particularly high probability of being subjected to ROS-induced toxicity as they play a potent role in the detoxification process.

The observed elevated levels of hepatic peroxides in group II rats (administered t-BHP alone) in the present study could be attributed to the increase oxidative stress induced by t-BHP. Thus, the maintenance of near normal levels of peroxidation as observed in group III and IV rats (administered BRCAE extracts at 250mg/kg b.wt and 500mg/kg.bwt respectively) suggests that BRCAE was effective in protecting the cells against the oxidative stress imposed by t-BHP. Choi *et al* [26] reported the significant increase in the levels of lipid peroxidation on t-BHP induction was reduced on treatment with coffee diterpenes.

The innate antioxidant defence mechanisms include the antioxidant enzymes as catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (Gpx). A reduction in the activities of these enzymes is associated with the accumulation of highly reactive free radicals, leading to deleterious effects such as loss of integrity and function of cell membranes [27,28].

Administration of t-BHP leads to generation of free radicals which is associated with inactivation of antioxidant enzymes. This probably explains the significantly reduced activities of the antioxidant enzymes observed in rats challenged with t-BHP (group II). The decreased activity of antioxidants viz., SOD, CAT, GPx, and GR may be due, in part, to an overwhelming oxidative modification of the enzymatic proteins by excessive ROS generation. More so, reduction in the activities of these enzymes may stem from decrease in their rate of synthesis. Similar reduction in the activity of GR and GPx was reported by Yen *et al* [29] on t-BHP induction.

In rats receiving t-BHP and BRCAE (group III and IV) the activities of the antioxidant enzymes were significantly higher than in group II rats, and very similar to the values noted in normal (group I rats) . In the current study, the observed modulation by BRCAE treatment is a very encouraging finding. The result could be attributed to the natural antioxidants present in the extract. that possibly had quenched the free radicals generated by t-BHP and as well or up-regulated the transcription of antioxidant enzyme genes.

Similar elevation of these enzyme levels by supplementation of antioxidant source as extract of *Graptopetalum paraguayense* has already been reported [30].

GSH is the major non-enzymatic antioxidant and regulator of intracellular redox homeostasis. GSH is ubiquitously present in all cell types [31]. Studies with a number of models show that hepatotoxicity induced by xenobiotics often is produced by GSH depletion [32].t-BHP, a substrate of GPx and is known to interfere with the glutathione-dependent antioxidant defences of the cell[33]. Thus the interference with the enzyme would have resulted in the decreased levels of GSH as evidenced in the levels of GSH in the hepatic tissue of the animals induced with t-BHP (group II). Thus, increase levels of GSH and the activities of SOD and CAT in the rats treated with BRCAE is suggestive of the capacity of the extract in countering the oxidative stress imposed by t-BHP.

Non- enzymic antioxidants - vitamin C and E operate synergistically to scavenge the free radicals formed in the biological systems. GSH acts along with vitamins E and C in inhibiting oxidative stress and acts against lipid peroxidation [34]. The decreased levels of vitamin C and E observed during t-BHP administration might be due to the excessive utilization of these vitamins in scavenging the free radicals. The marked improvement seen in the levels of vitamins C

and E in the hepatic tissue of the group III and IV rats that were treated with BRCAE at 250mg/kg b.wt and 500 mg/kg.bwt suggest the protective nature of the extract against the free radicals generated due to t-BHP induction. The standard drug silymarin also significantly improved the antioxidant status.

The extract of *Mesona procumbens* was found to increase the Vit C and GSH levels in rats that were induced with t-BHP [29] Berberine was found to increase hepatic GSH content in tert-butyl hydroperoxide- induced oxidative damage in rats [35]. Similar results were reported by Hwang *et al* [36]. Studies have reported that the secondary metabolites derived from plant extracts have potential to combat the free radicals generated upon t-BHP induction [37-39].

The results of the present study strongly suggest the protective efficacy of the extract could be due the antioxidant potential of BRCAE against t-BHP. The activity may well be due to the phytochemicals present in the aqueous extract. The extract could be explored further to derive the mechanism of action.

In conclusion, the results of this investigation demonstrate that the decrease of lipid peroxidation levels and significant improvement in the antioxidant enzyme activities and the levels of GSH, vitamin E and C in the hepatic tissue contribute to hepatoprotective effects of BRCAE against t-BHP-induced oxidative stress.

The results observed suggests the antioxidant activity of the extract in neutralizing the free radical induced oxidative stress by t-BHP. Thus the intake of bokchoy, *B. rapa chinensis* may be beneficial to human health and may lessen liver damage induced by environmental and dietary toxicants as well.

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CONFLICT OF INTEREST

None

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