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Pak J Med Res
Vol. 63, No. 3, 2024

Original Article

Sea Buckthorn Berry Seed Oil: A Remedy for Depleted Cellular Antioxidants in Cyclophosphamide-induced Oxidative Stress in BALB/c Mice

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Abstract

Background: Cyclophosphamide is a chemotherapeutic and immunosuppressive drug, however, its usage is constrained by the development of dose-dependent side effects, like hepatotoxicity and hemorrhagic cystitis. The proposed mechanism of these side effects is oxidative stress. This study focuses on the role of sea buckthorn seed oil, which is high in antioxidants, in reducing oxidative stress caused by cyclophosphamide.
Objective: To ascertain how sea buckthorn berry seed oil affects the serum levels of antioxidant enzymes and lipid peroxidation marker in BALB/c mice under oxidative stress brought on by cyclophosphamide.
Study type, settings & duration: This quasi-experimental study was carried out at the Department of Physiology, Foundation University School of Health Sciences, Islamabad from February to April 2018.
Methodology: It was a quasi-experimental animal study. Thirty healthy male BALB/c mice were divided into three groups of 10 each. Group-1 served as negative control, group-2 was positive control and received cyclophosphamide (25 mg/kg body weight) intraperitoneally for 10 consecutive days. Group-3 was co-administered cyclophosphamide (same dose and route) with sea buckthorn berry seed oil (40 mg/kg body weight) orally for ten days. All animals were sacrificed on the 11th day. Serum levels of antioxidant enzymes and malondialdehyde as stress biomarkers were assayed in all three groups.
Results: Malondialdehyde levels rose while antioxidant enzyme levels, including glutathione peroxidase, superoxide dismutase, and catalase, fell in group-2 receiving cyclophosphamide (p <0.05). The deviation from the control group values was partially mitigated by the co-administration of sea buckthorn berry seed oil in group-3.
Conclusion: In the cyclophosphamide-induced oxidative stress, co-administration of sea buckthorn berry seed oil reduced both the reduction in antioxidant enzymes and the rise in the lipid peroxidation marker malondialdehyde.
Key words: Antioxidant, biomarkers, cyclophosphamide, lipid peroxidation, oxidative stress, sea buckthorn berry seed oil.

Introduction

Cyclophosphamide (CP) is a synthetic alkylating agent frequently used in cancer chemotherapy to target the body's rapidly proliferating cells.[1] It is effective against numerous cancers, including breast, ovarian, prostate and lung cancers, malignant lymphomas and leukaemias. It is also used as an immunosuppressive medication to treat connective tissue disorders such as rheumatoid arthritis, scleroderma, multiple sclerosis, and systemic lupus erythematosus.[2] CP is an inactive cytostatic, which undergoes metabolic activation in liver, catalyzed by the hepatic cytochrome P450 (CYP450) monooxygenase systems into active metabolites, acrolein and phosphoramide mustard. Phosphoramide exerts the anti-tumour effects by modifications and cross-linking of purine bases in DNA, thereby preventing the production of deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and proteins and killing

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Received: 23 May 2022, **Accepted:** 23 July 2024
Published: 08 October 2024

Authors Contribution

GNS & SA conceptualized the project along with the drafting, revision & writing of manuscript. GNS & MS did the data collection and statistical analysis. GNS also did the literature search.

rapidly dividing cells. Acrolein obstructs the tissue antioxidant defense system, produces reactive oxygen species (ROS), and interacts with protein amino acids causing structural and functional changes.[1] Generation of ROS with depletion of cellular antioxidants and the resultant oxidative stress are the major limitations for using CP.

CP-induced oxidative stress causes oxidative damage to a number of organs by escalating lipid peroxidation and causing a decline in activity of endogenous antioxidants [glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT)].[3] These adverse reactions include sterile haemorrhagic cystitis, pulmonary fibrosis, gastrointestinal haemorrhage, permanent azospermia in men, and more. Additionally, bone marrow suppression is observed along with decreased haemoglobin and reduced red and white blood cells counts, as well as weakened cell-mediated and humoral immunity.[4]

According to research, antioxidant supplements may assist tissues regain their natural antioxidant mechanism (reduced glutathione-GSH) and diminish tissue damage caused by free radicals. These supplements may also help reduce the cytotoxic potential of anti-cancer medications.[5]

Sea buckthorn berry seed oil (SBO), rich in polyphenols, vitamins, essential fatty acids and amino acids, is a natural source of antioxidants with documented free radical scavenging abilities. It is extracted from sea buckthorn (SBT) berries and is prized for its benefits which include anti-inflammatory, anti-carcinogenic, immunomodulatory, antiviral, antibacterial, and hepatoprotective properties.[6] Vitamins A, C, and E, tannins, flavonoids, and trace minerals like zinc, iron, copper, and sulphur are among the many antioxidants that have been observed to be present in SBO. In addition, sea buckthorn oil and extract also contain selenium, which may aid in the formation of glutathione peroxidase, an enzyme essential for the breakdown of lipid hydroperoxides.[7] The seed oils are also abundant in omega-6 and omega-3 polyunsaturated essential fatty acids, as well as omega-7 and omega-9 monounsaturated non-essential fatty acids.[8]

Previous studies have shown that sea buckthorn extract significantly reduces the formation of free radicals, oxidative stress, and lipid peroxidation caused by acetaminophen, chromium, sodium nitroprusside, nicotine, carbon tetrachloride (CCL4), hypoxia, and radiation.[9,10] The goal of the current study was to determine how sea buckthorn berry seed oil affects the serum levels of antioxidant enzymes and lipid peroxidation marker in BALB/c mice under cyclophosphamide-induced oxidative stress.

Methodology

The study used healthy male BALB/c mice from an inbred colony kept at the Animal House at Veterinary Farms Managements Sub-Division (VFMS) of National Institutes of Health (NIH) Islamabad. Since this was an exploratory animal study, Resource Equation method was used to determine the sample size.[11] Thirty adult animals with average weight of 30 ± 5 g were maintained in well-ventilated polypropylene cages (with five mice each) containing wood shavings. Animals were kept at 25 ± 2 ^{o} C with a relative humidity of 50-60% and on 12-hour light/12-hour dark cycle. The duration of this acclimatization period was one week. Rodent feed was purchased from Animal House of NIH. The pellet diet provided 65% as carbohydrate, 25% as protein and 10% of calories as fat. Feed and tap water were given ad libitum. The Guide for the Care and Use of Laboratory Animals was followed when handling the animals (8th edition, 2010).[12]

Injection cyclophosphamide 1gm (Cyclomide 1000 mg) was procured from Pharmedic Laboratories, Pakistan. Commercially available preparation of sea buckthorn berry seed oil was procured from SIBU Sea Berry Therapy, USA. Phosphate-buffered saline (PBS) (0.1M, pH 7.4) containing KCl (1.17%w/v) was freshly made in physiology lab at Foundation University School of Medical Sciences (FUSH). All other chemicals utilized in the experiment were of analytical grade.
Three groups of ten mice each were created from a total of 30 healthy male BALB/c mice.

Group 1: (Control group) Mice were given normal saline (0.65%) 1ml/kg.bw intraperitoneally daily for 10 days.
Group 2: (cyclophosphamide group) Mice received CP 25 mg/kg.bw intraperitoneally daily for ten days.[13, 14]
Group 3: (cyclophosphamide + sea buckthorn berry oil) Mice were given CP 25 mg/kg.bw intraperitoneally and SBO 40 mg/kg orally daily for ten consecutive days.

On 11th day of intervention, intra-cardiac blood sampling was done after ensuring proper euthanasia for assessments of serum levels of antioxidant enzymes and malondialdehyde. Samples were centrifuged at room temperature at 4000 revolutions per minute for 15 minutes. The separated serum was used to assess lipid peroxidation marker MDA and antioxidant enzymes

[glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT)] by enzyme-linked immunosorbent assay (ELISA).[15]

Statistical Package for the Social Sciences (SPSS) version 24 was used to perform statistical analysis. Values were expressed as means ± SD. The statistical significance of differences in various quantitative changes between experimental and control groups was assessed by one-way analysis of variance (ANOVA), followed by Tukey HSD (Honestly Significant Difference) post hoc test for multiple comparisons. Tukey HSD post hoc test is used to assess the significance of differences between pairs of group means. Differences are considered statistically significant if the "*p*" value is less than or equal to 0.05.

The ethical approval was obtained from the Ethical Review Board of Foundation University Medical College, Rawalpindi vide letter no. 217/FF/FUMC/ERC.

Results

The effect of CP, and CP+SBO co-administration on serum antioxidant enzymes levels Cyclophosphamide administration resulted in a decrease in antioxidant enzymes in group-2, but this decrease was less in group-3 where SBO was co-administered.

GPx levels were significantly decreased in group-2 and group-3 compared to group-1 ($p < 0.001$), and the difference between group-2 and group-3 was also statistically significant ($p < 0.001$) The SOD levels in group-2 and group-3 were significantly lessened compared to the group-1($p < .001$); and the difference between group-2 and group-3 was also statistically significant ($p < 0.001$). The decrease in CAT levels in group-2 compared to group-1 was statistically significant ($p < 0.001$), but the difference between group-1 and group-3 was insignificant ($p < 0.223$), implying that the CP-induced decrease in CAT was mitigated to a large extent by SBO co-administration in group-3. The difference between group-2 and group-3 was statistically significant ($p < 0.001$).

Actual values are given in the accompanying Table-1 and *p* values as significance of difference between the groups are given in Table-2.

The effect of CP, and CP+SBO co-administration on serum MDA levels CP treatment in group-2 significantly raised the levels of lipid peroxidation marker MDA, relative to the control group-1 ($p < 0.001$). This increase in lipid peroxidation was mitigated by SBO co-administration in group-3, with insignificant difference in MDA levels between Gp-1 and Gp-3 ($p = 0.603$) and significant difference between group-2 and group-3 (0.001). (Values given in the accompanying Table-1 and *p* values as significance of difference between the groups are given in Table-2).

Discussion

The optimal use of CP, a cytotoxic alkylating chemotherapeutic agent, is often constrained because of its multiple adverse side effects and toxicity. Drugs that could reduce

Table-1: The effects of cyclophosphamide, and cyclophosphamide plus sea buckthorn berry seed oil on serum levels of malondialdehyde, glutathione peroxidase, superoxide dismutase and catalase.

Groups (n=10)	*Parameters*			
	Malondialdehyde (MDA) ng/ml	*Glutathione Peroxidase (GPx) ng/ml*	*Superoxide Dismutase (SOD) pg/ml*	*Catalase (CAT) ng/ml*
Group-1	8.66±0.41	4.61±0.28	1228.80±9.52	0.49±0.02
Group-2	10.97±1.80	2.47±0.29	756.49±12.96	0.37±.02
Group-3	9.12±0.15	3.76±0.25	920.11±7.96	0.47±.03

Note: The results are means ± SD. The group means were compared with one-way ANOVA
Group-1: control, Group-2: cyclophosphamide, Group-3: cyclophosphamide plus sea buckthorn berry seed oil

Table-2: The significance of difference between the groups stated as *p* value.

	Group-1 vs. Group-2	*Group-1 vs. Group-3*	*Group-2 vs. Group-3*
Glutathione Peroxidase (GPx)	< 0.001	< 0.001	< 0.001
Superoxide Dismutase (SOD)	< 0.001	< 0.001	< 0.001
Catalase (CAT)	< 0.001	0.223	< 0.001
Malondialdehyde (MDA)	< 0.001	0.603	0.002

Note: Multiple comparisons between groups were carried out with post-hoc Tukey HSD test. p value < 0.05 was considered significant.
Group-1: control, Group-2: cyclophosphamide, Group-3: cyclophosphamide plus sea buckthorn berry seed oil

Drugs that could reduce these side effects will be of great help in maximizing the therapeutic effects of cancer treatment approaches. Recently there has been a revival of interest in chemoprotective and antioxidant activities of plant extracts used in traditional medicine.[5] According to Nafees et al., plant extracts provide protection by inducing the production of antioxidants, which reduce the quantity of oxidants (ROS) generated by CP.[16]

In the present study, SBO was evaluated for the antioxidant activity against the oxidative stress brought on by CP in male BALB/c mice. The outcomes showed that the SBO significantly shielded the mice from the oxidative damage resulting from CP administration. Lower levels of GPx, SOD, and CAT and higher levels of MDA, a lipid peroxidation marker in serum, were signs of the oxidative stress brought on by CP. The change in all these parameters seen after CP administration was significantly mitigated when SBO was co-administered.

Review of phytochemical composition of SBT has shown it to be a rich source of vitamin C, carotenoids such as α-carotene and β-carotene, tocopherols such as α-tocopherol and β-tocopherol, and flavonols like quercetin and isorahmnetin.[17] By replenishing the cellular antioxidant enzymes, these components of SBT appear to account for the antioxidant actions of SBO to counteract CP-induced oxidative stress.

In 2020, a study by Mohamed and colleagues confirmed similar findings. Their research aimed to evaluate the antioxidant effects of sea buckthorn (Hippophae rhamnoides) and grape extract in countering oxidative stress in hyperlipidemic rats treated with atorvastatin.[18] Antioxidant potential was assessed by measuring total antioxidant capacity (TAC's) values by using cupric ion reducing antioxidant capacity (CUPRAC) assay. The statistics (ANOVA) revealed that sea buckthorn diminished significantly ($p < 0.001$) the oxidative stress in the heart, liver, and kidney.

These findings are in accordance with our results, however the main difference with our study is that they assessed total antioxidant capacity (TAC) instead of individual antioxidant enzymes.

Similarly, in a study by Suchal et al., authors attempted to investigate the association of ischemia–reperfusion (IR) injury with oxidative stress, inflammation and apoptosis, and whether pretreatment with SBT pulp oil possess any protective effect on these parameters. The SBT pulp oil (in doses of 5, 10, and 20 ml/kg) was administered orally to the rats for a period of 30 days. IR injury resulted in oxidative stress which caused significant reduction in the activities of antioxidant enzymes SOD and CAT, and GSH content as compared to sham group ($p < 0.01$ for CAT and $p < 0.001$ for SOD and GSH). SBT pulp oil augmented the activities of these antioxidants and attenuated the deleterious effect of IR injury on myocardium in a dose-dependent manner.[19] These findings are in line with our study with higher doses and longer duration of supplementation than ours resulting in a greater recovery of the anti-oxidant enzymes in heart tissue.

Another study assessed the alveolar oxidative stress induced by hypoxia by measuring antioxidant enzyme, GPx, in the hypoxic lung tissue before and after treatment with SBT leaf extract (100 mg/kg bw). The authors reported a marked increase in GPx levels as result of pretreatment of animals with SBT leaf extract as compared with the hypoxic animals with no pretreatment, thus offering significant protection against the oxidative damage that hypoxia induced.[20] This positive result agrees with our result of increased GPx levels with SBO treatment compared to CP group.

One of the main signs of oxidative stress is lipid peroxidation, which has been associated with impaired membrane function, reduced fluidity, amplified permeability to ions and inactivation of membrane-bound enzymes and receptors.[21]

The current study found that mice treated with CP had increased levels of MDA, which is an indicator of lipid peroxidation occurring as a result of cellular redox imbalance.

Decreased levels of MDA seen in SBO-supplemented group-3 may result from selenium present in sea buckthorn extract, which may help in biosynthesis of glutathione peroxidase, a selenoprotein enzyme crucial for the degradation of H_2O_2 as well as lipid hydroperoxides while oxidizing glutathione.[22] Moreover, phenolic compounds present in SBO may act as chelators for the transition of metal ions Fe^{2+}, Fe^{3+}, and Cu^{2+} that are involved in the conversion of H_2O_2 into $OH\cdot$ and stimulation of lipid peroxidation. [23]

A number of studies support the potential of sea buckthorn (SBT) to curtail lipid peroxidation. In a study by Olas et. al, antioxidative activities of the phenolic fraction from SBT fruits (at a dose range 0.5–50 μg/mL; incubation time: 15 and 60 min) on the production of thiobarbituric acid reactive substances (TBARS, a marker of lipid peroxidation) and the generation of superoxide anion (O2 −·) in human blood platelets (resting platelets and platelets stimulated by a strong physiological agonist, thrombin) were studied in vitro. After a 15 min pre-incubation of platelets with the SBT phenolic fraction, the amount of TBARS in resting platelets and thrombin-activated platelets

diminished in a concentration-dependent manner. At the highest concentration of the tested fraction (50 μg/mL), production of TBARS in resting and activated platelets was reduced by about 60%.[23] Their results support the antioxidant activity of SBO and its ability to reduce CP-elevated ROS formation seen in our study.

Our research highlights SBO's antioxidant properties and demonstrates its potential to protect against oxidative damage brought on by CP. Decreased serum levels of antioxidant enzymes and increased MDA levels caused by CP were successfully mitigated by SBO co-administration. Furthermore, our study demonstrates that SBT (H. rhamnoides) fruits may be used as a natural source of antioxidants and compounds to prevent and/or cure disorders associated with oxidative stress. Further research is required to elucidate fully the therapeutic potential of SBT. Attention should be paid to its cultivation and global distribution, given the promising results seen in numerous scientific studies as well as in the traditional system and cosmeceuticals. In conclusion, the promising results derived from this study will open up a new horizon for adjuvant cancer treatment and give a fresh impetus to the alternative medicine.

Acknowledgment

This study was funded by the Foundation University, Islamabad (FUI). The role of FUI in promoting the culture of research and development in the medical sciences is greatly appreciated and duly acknowledged.

The study protocol was carried out in the Animal House of National Institute of Health (NIH), Islamabad. The authors are grateful to the management of the NIH for their consent and cooperation. Special thanks are due to Dr. Hussain Ali, Head of Veterinary Farms Managements Sub-Division (VFMS) NIH, for his generous and competent help in animal handling, feed preparation, administration of drugs and blood sampling procedures.

Conflict of interest: None declared.

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