Sea buckthorn berry seed oil can replenish the depleted cellular antioxidants in cyclophosphamide-induced hepatotoxicity in BALB/c mice

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Introduction

Cancer is the second leading cause of death globally and was responsible for an estimated 9.6 million deaths in 2018, according to WHO website on cancer, published in September 2018 (Bray.et al 2001). Cyclophosphamide (CP), a widely used chemotherapeutic and immunosuppressive drug, is a synthetic alkylating (Baumann and Preiss 2001). Optimal use of CP is limited by its side effects, which are mediated through generation of generation of reactive oxygen species and oxidative stress (Stankiewicz, Skrzydlewska and Makiela 2002). Supplementation of antioxidants may help liver recover its endogenous antioxidant systems (reduced glutathione- GSH) and thus counter the hepatotoxic potential of various anti-cancer drugs and lessen free radical-induced tissue injury (Sheweita, El-Hosseiny and Nashashibi 2016). Sea buckthorn berry seed oil (SBO) is a natural source of antioxidants and offers protection against oxidative damage through its free radical scavenging abilities (Krejcarová et al. 2015). It is documented to contain a large number of antioxidants like vitamins A, C and E, flavonoids and tannins (Zeb 2004). Trace elements like zinc, selenium, iron, sulphur and copper present in sea buckthorn extract may help in biosynthesis of endogenous antioxidants like glutathione peroxidase and catalase, which are crucial for the degradation of lipid hydroperoxides (Suryakumar and Gupta 2011).

Methods and Materials

It was an 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 b.w) intraperitoneally for 10 consecutive days. Group-3 was the experimental group and was co-administered CP (same dose) with SBO (40 mg/kg b.w) orally for ten days. All animals were sacrificed on 11th day, and dissection was carries out for removal of liver. Liver tissue was put in labeled tissue sample bottles containing 25 mls phosphate buffer saline. The liver tissue samples were manually ground, using mortar and pestle. Homogenization was done by means of an electric whisk and subsequently samples were centrifuged at room temperature at 4000 rpm for 15 minutes. The supernatant was transferred into labeled 1.5 ml eppendorf tubes and levels of liver tissue antioxidant enzymes (SOD, CAT, GPx) and lipid peroxidation marker MDA were assessed by enzyme-linked immunosorbent assay (ELISA).

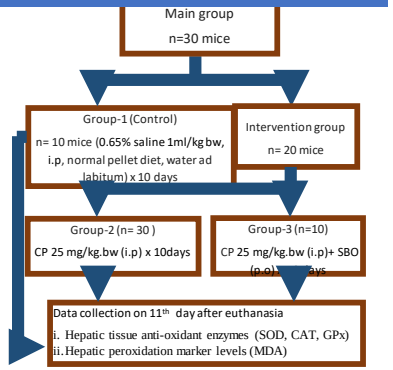
Table 1. The effects of cyclophosphamide (CP), and cyclophosphamide plus sea buckthorn berry seed oil (CP+SBO) on liver tissue levels of Malondialdehyde (MDA), glutathione peroxidase (GSH-Px), superoxide dismutase (SOD) and catalase (CAT)

	Malondialdehyde (MDA) ng/ml	Glutathione Peroxidase (GSH-Px) ng/ml	Superoxide Dismutase (SOD) pg/ml	Catalase (CAT) ng/ml
Group-1(control) n=30	8.66 ± 0.41	4.61± 0.28	1228.80±9.52	0.49±0.02
Group-2 (CP) n=30	10.97 ± 1.80[a]	2.47±0.29[a]	756.49±12.96[a]	0.37±.02[a]
Group-3(CP+SBO) n=30	9.12 ± 0.15[b]	3.76± 0.25[a,b]	920.11±7.96[a,b]	0.47±.03[b]

The results are means SD. The group means were compared with one-way ANOVA with post-hoc Tukey HSD test.

a = $p < 0.05$ vs. Group-1 (control), b= $p < 0.05$ vs. Group-2 (cyclophosphamide)

Schematic flow chart of the study

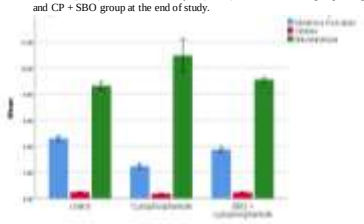


Results

The statistical analysis was done by using SPSS version 24. Values were expressed as means ± SD. The statistical significance of the difference of various quantitative changes between the groups was evaluated by one-way ANOVA followed by Tukey HSD. The difference was regarded statistically significant if the '*p*' value was equal to or less than 0.05.

In group-2, liver tissue antioxidant enzymes, GPx, SOD and CAT levels declined ($p \leq 0.05$). The decline was partially arrested with co-administration of sea buckthorn berry seed oil in group 3, with the levels being significantly different from group-2 ($p \leq 0.001$). CP significantly increased the levels of lipid peroxidation marker, MDA, in liver tissue by 23.53% compared to the control Gp-1($p < 0.001$). The CP-induced increase in lipid peroxidation was mitigated by SBO co-administration with insignificant difference in MDA levels between Gp-1control and Gp-3 ($p = 0.603$).

Figure 1. Comparison of mean values for liver tissue glutathione peroxidase (GPx), Catalase (CAT) and malondialdehyde (MDA) levels in control group, CP group and CP + SBO group at the end of study.



Discussion

The oxidative stress induced by CP manifested as increased levels of liver tissue MDA, which could be due to inhibition of hepatic antioxidant enzymes activity as evidenced by significantly decreased levels of GPx, SOD, and CAT in CP group. SBO-CP co-administration showed a significant recovery in the levels of antioxidant enzymes compared to CP group, along with a significant decline in MDA levels.

Previous studies have also reported the decline in the levels of antioxidant enzymes and excessive formation of hepatic MDA when oxidative stress was induced. SBT is a rich source of flavonols like quercetin, and isorahmnetin, tocopherols such as α- tocopherol and β-tocopherol, carotenoids such as α-carotene and β-carotene, and vitamin C. Theses constituents replenish the hepatic tissue antioxidant enzymes. SOD, GPx and CAT catalyze detoxification of superoxide anions and H2O2 by reduced glutathione activity and would have prevented CP-induced oxidative stress. Our results indicate the antioxidant properties of SBO and ability for reducing CP-elevated ROS formation.

Conclusions

The results demonstrate that the SBO protected the animals significantly from the CP-induced oxidative damage in liver. Co-administration of sea buckthorn berry seed oil lessens the CP-induced decline in hepatic tissue antioxidant enzymes, and mitigates the increase in MDA, the lipid peroxidation marker.

Figure 2. Intra-peritoneal injection of CP being given

Figure 3. Liver dissection being carried out at the end of study duration at NIH, Islamabad

References

1. Baumann, F, and R Preiss. "Cyclophosphamide and Related Anticancer Drugs." *Journal of Chromatography B: Biomedical Sciences and Applications* 764.1-2 (2001): 173-92.
2. Bray, Freddie, et al. "Global Cancer Statistics 2018: Globocan Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries." *CA: a cancer journal for clinicians* 68.6 (2018): 394-424.
3. Krejcarová, Jana, et al. "Sea Buckthorn (Hippophae Rhamnoides L.) as a Potential Source of Nutraceutics and Its Therapeutic Possibilities-a Review." *Acta Veterinaria Brno* 84.3 (2015): 257-68.
4. Sheweita, Salah A, Lobna S El-Hosseiny, and Munther A Nashashibi. "Protective Effects of Essential Oils as Natural Antioxidants against Hepatotoxicity Induced by Cyclophosphamide in Mice." *PloS one* 11.11 (2016): e0165667.
5. Stankiewicz, Anna, Elzbieta Skrzydlewska, and Marek Makiela. "Effects of Amifostine on Liver Oxidative Stress Caused by Cyclophosphamide Administration to Rats." De Gruyter, 2002.
6. Suryakumar, Geetha, and Asheesh Gupta. "Medicinal and Therapeutic Potential of Sea Buckthorn (Hippophae Rhamnoides L.)." *Journal of Ethnopharmacology* 138.2 (2011): 268-78.
7. Zeb, Alam. "Important Therapeutic Uses of Sea Buckthorn (Hippophae): A Review." *J Biol Sci* 4.5 (2004): 687-93.