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Brassica oleracea var. italica Extract Protection Against Cyclosporine A-Induced Erythropoiesis Toxicity and Hepatotoxicity in Mice

Conflict of interest: nothing to declare.
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Abstract

Introduction. Cyclosporine A (CsA) is a potent immunosuppressive agent extensively utilized in organ transplantation and autoimmune disorders. However, its therapeutic use is frequently linked to adverse outcomes, such as hepatotoxicity and hematological disturbances attributable to oxidative stress. Antioxidant-rich natural plant extracts may offer a means to counteract these toxic effects.
Purpose. This study investigates the protective efficacy of the ethanolic extract of Brassica oleracea var. italica (cauliflower) against CsA-induced hepatotoxicity and erythropoiesis toxicity in a murine model.
Materials and methods. Adult male Mus musculus mice were divided into four groups (n=5), with one control group and three experimental groups subjected to CsA and varying doses of cauliflower extract (100 mg/kg and 200 mg/kg body weight) over seven days. Hematological parameters, including WBC, RBC indices (MCV, MCH, MCHC, HCT), and platelet metrics (PLT, MPV, PDW, PCT), as well as liver histopathology, were assessed.
Results. CsA administration induced significant hematological alterations, which were mitigated by cauliflower extract treatment, particularly at higher doses, as evidenced by improved erythrocyte indices (e.g., increased MCV, MCH, HCT) and reduced WBC counts, especially lymphocytes and granulocytes. Platelet indices showed decreased PLT and PCT with increased MPV in treated groups. Histopathological findings revealed fatty degeneration and inflammation in the CsA group, while cauliflower extract treatment, especially at 200 mg/kg, demonstrated substantial restoration of liver architecture with near-normal hepatocytes. These findings suggest that the ethanolic extract of Brassica oleracea var. italica exerts a protective effect against CsA-induced hepatic and hematological toxicity, likely attributable to its antioxidant and hepatoprotective properties.

Conclusion. The ethanolic extract of Brassica oleracea var. italica exhibits a protective effect against CsA-induced hematological and hepatic toxicity in mice, likely due to its antioxidant and hepatoprotective properties.
Keywords: Brassica oleracea var. italic, cyclosporine A, erythropoiesis toxicity, hepatotoxicity, mice

■ INTRODUCTION

Plants and their extracts and bioactive compounds resambles therapeutic potential whether in vitro or in vivo studies of its antioxidants, anti-inflammatory and anti-cancer properties all of which highlights their safety use as natural alternatives for synthetic pharmacological applications [1–3].

Brassica oleracea var. italica noun as broccoli or cauliflower, is an eaten vegetables belongs to the cabbage family: brassicaceae. With their edible flower, leaves, stalk, it has rich source of important vitamins (C, K) besides it content of glucosinolates, isothiocyanates and sulforaphane [4]. It is rich source of carotenoid (lutein, violaxanthin, B carotene, neoxanthin, antheraxanthin, which rejumbles powerful antioxidants [5]. Sulforaphane (a sulfur rich phytochemical) it is known for its potent antioxidant, anti-inflammatory and chemopreventive properties [6]. Many studies improved its mechanism in activating Nrf2-mediated pathway and modulating enzymes detoxification which contribute to its protective effect against chronic disease such as cardiovascular disorders, cancers and neurodegenerative diseases [7]. On the other hand, CsA is a broadly used immunosuppressive drug which studies revealed it has few hepatotoxic and nephrotoxic side effects and its mechanism of inducing toxicity is by oxidative stress and releasing reactive oxygen species and depletion of liver antioxidant [8].

■ PURPOSE OF THE STUDY

This study investigates the protective efficacy of the ethanolic extract of Brassica oleracea var. italica (cauliflower) against CsA-induced hepatotoxicity and erythropoiesis toxicity in a murine model.

■ MATERIALS AND METHODS

Study Design and Setting

Brassica oleracea var. italic flowers has been collected from local markets and an 70% ethanolic extract was made by weighting 309 g of fresh cauliflower and immersed it in 1 liter of 70% ethanol (1 : 5) for 5 days in sealed flasks in room temperature then it was filtered and dried it with rotary evaporator [7].

20 Mus musculus mice were orally treated daily for a week then whole blood was collected for blood tests and liver were collected for histopathological examination. We used in 4 groups, the control group, CsA (Cyclo) group were had 0.2 ml of 20 mg of CsA, (Cyclo+Bro 100 mg) group were had 0.2 ml of 20 mg of CsA then treated with CEE for a week, (Cyclo+Bro 200 mg) group were had 0.2 ml of 20 mg of CsA then treated with CEE for a week, Cyclo+Bro 100 mg) group had 0.2 ml of 20 mg of CsA then treated with CEE for a week.

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Statistical Analysis

The Statistical Packages of Social Sciences-SPSS (2019) program was used to detect the effect of difference factors in study parameters. Least significant difference-LSD was used to significant compare between means (ANOVA/ One way) in this study.

■ RESULTS

The results shown reduction in WBC counts especially lymphocytes and granulocytes (Table 1). Moreover, results revealed that CEE elevates MCV, MCH and HCT in Cyclo+Bro groups comparing to CYCLO groups (Table 2). MCHC on the other side showed high significant increase in CYCLO group compared to control (Table 2), while MCHC highly dropped after treatment with CEE in the Cyclo+Bro. Additionally, PLT, PCT was shown to be reduced and MPV was increased by CEE in Cyclo+Bro group compared to Cyclo group (Table 3).

The histological examination of the liver showed in Figure. The Cyclo group showed mild fatty changes with microvesicular fat accumulation indicates a disruption in hepatocytes

Table 1
Comparison between difference groups in WBC and its differential

Group	Mean±SE						
	WBC ($\times 10^3$)	Lymph – No.	Mid – No.	Gran – No.	Lymph – (%)	Mid – (%)	Gran – (%)
Control	8.48±0.99a	5.53±0.42a	1.13±0.23	3.64±1.06a	66.6±4.73	11.84±1.78	21.52±3.02
Cyclo	5.54±1.01b	3.22±0.49b	0.86±0.25	1.46±0.45b	60.67±6.31	14.52±2.76	24.82±4.56
Cyclo+Bro 100 mg	5.8±1.42b	4.2±0.69a, b	0.948±0.27	1.53±0.39b	70.04±3.68	11.63±1.53	18.3±2.15
Cyclo+Bro 200 mg	5.9±0.61b	4.48±0.43a, b	0.832±0.12	1.42±0.27b	65.62±5.51	13.04±1.85	21.3±4.01
L.S.D.	3.151*	1.567*	0.676 NS	1.878*	15.458 NS	6.114 NS	10.667 NS
P-value	0.045	0.0478	0.793	0.0498	0.646	0.738	0.648

Notes: means having with the different letters in same column differed significantly; * P≤0.05; NS – Non-Significant.

Table 2
Comparison between difference groups in RBC, HGB, HCT, MCV, MCH and MCHC

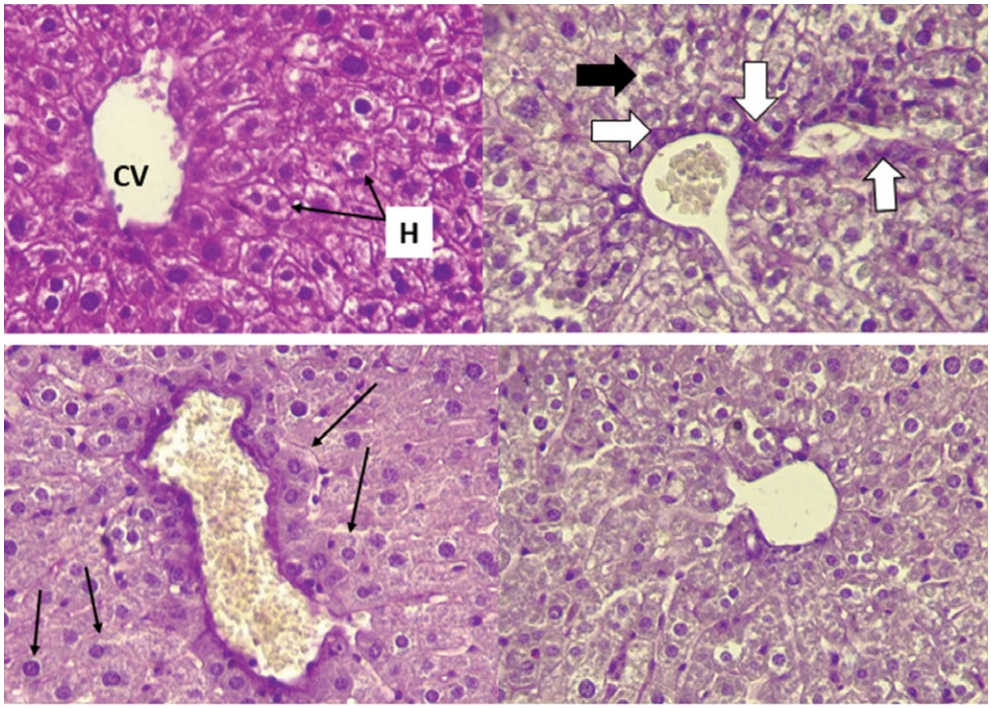
Group	Mean±SE					
	RBC ($\times 10^3$)	HGB	HCT	MCV	MCH	MCHC
Control	7.79±0.29	12.5±0.55	35.14±2.05b	45.16±2.04a, b	16.02±0.48b	169±28.26b
Cyclo	7.95±0.32	12.52±0.54	34.36±1.76b	43.14±0.63b	15.74±0.25b	365±6.46a
Cyclo+Bro 100 mg	7.96±0.28	13.16±0.84	38.46±1.27a, b	48.56±0.52a	17.32±0.31a	36.02±0.47c
Cyclo+Bro 200 mg	8.17±0.29	13±0.72	39.5±2.03a	48.14±1.07a	16.52±0.28a, b	34.42±0.82c
L.S.D.	0.894 NS	2.033 NS	3.975*	3.676**	1.027*	123.70**
P-value	0.842	0.863	0.0481	0.0097	0.0248	0.0001

Notes: means having with the different letters in same column differed significantly; * P≤0.05, ** P≤0.01.

Table 3
Comparison between difference groups in PLT, MPV, PDW and PCT

Group	Mean±SE			
	PLT (×10³)	MPV	PDW	PCT
Control	723.6±62.67[a, b]	5.7±0.36[a, b]	15.14±0.75	4.11±0.49[a, b]
Cyclo	894±51.52[a]	5.42±0.12[b]	14.48±0.12	4.86±0.35[a]
Cyclo+Bro 100 mg	551±56.26[b]	5.92±0.25[a, b]	14.92±0.25	3.17±0.26[b]
Cyclo+Bro 200 mg	611.4±142.29[b]	6.42±0.37[a]	14.32±0.76	3.60±0.82[a, b]
L.S.D.	259.62*	0.873*	1.668	1.585*
P-value	0.0466	0.0451	0.7094	0.0473

Notes: means having with the different letters in same column differed significantly; * P≤0.05; NS – Non-Significant.



The histological examination of the liver. Up-left: control group with normal hepatocytes and tissue structure. Up-right: The Cyclo group showed mild fatty changes with microvesicular fat accumulation. a mild focal inflammation has been shown as lymphocytes were seen near the central vein (white arrow). Moreover, ballooning lymphocytes were observed as well (black arrow). In Cyclo+Bro 100 mg group, a mild hepatocyte hypertrophy has been seen (arrows) with no inflammatory cells or fatty changes. While in Cyclo+Bro 200 mg group, normal hepatocytes had seen with no inflammatory or fatty changes

lipid metabolism. A mild focal inflammation has been shown as lymphocytes were seen near the central. Moreover, ballooning lymphocytes were observed as well. In Cyclo+Bro 100 mg group, a mild hepatocyte hypertrophy has been seen with no inflammatory cells or fatty changes. While in Cyclo+Bro 200 mg group, normal hepatocytes had seen with no inflammatory or fatty changes.

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DISCUSSION

The mechanism that explained lymphopenia in the results is that CsA inhibits T-cells by blocking calcineurin (a phosphatase essential for nuclear translocation of NFAT 'nuclear factor of activated T-cells' which will inhibit IL-2 production and then suppress CD4 and CD8, finally leads to lymphopenia. However, chronic effect of CsA may activate lymphocyte proliferation by compensatory mechanisms. CEEs anti-inflammatory effect reduces lymphocyte activation [9]. CEE also reduces Granulocytes by inhibiting chemokine signaling [10].

CEE, MCV, MCH and HCT elevation in Cyclo+Bro groups suggests improvement in erythropoiesis and RBCs health. While CsA mechanism as immunosuppressant, its oxidative stress through bone marrow toxicity and renal dysfunction (erythropoietin production) might have been mitigated by CEE antioxidants such as glucosinolates, sulforaphane and flavonoids and eventually enhancing erythropoiesis, improving liver and renal functions and reducing hemolysis of the circulating RBCs [11,12].

On the other hand, MCHC significant increase might reflect OS-induced erythrocyte dehydration, membrane toxicity or altered iron metabolism [13]. CsA generate ROS and affect Ca homeostasis which could reduce RBC volume and concentrate Hb. Moreover, liver iron in CsA induced liver may overload and increase Hb synthesis [14].

While MCHC highly drape after treatment with CEE may reflects modulating OS through Nrf2-mediated anti-oxidant pathway. CEE improved erythroid differentiation by upregulating key transcription factor like GATA-1 and enhancing HB synthesis. It reduced ROS and restored mitochondria functions in erythroid precursors. In addition, sulforaphane improves iron metabolism which is essential in HB synthesis, by regulating ferroportin and hipcidin expression [15–17]. All these mechanisms collectively contributed to restored normal MCHC levels, reflecting sulforaphane therapeutic properties in eliminating CYCLO-induced hematological toxicity.

PLT, PCT reduction and increased MPV by CEE may indicates sulforaphane role in curbing platelets dynamics as its anti-oxidant aspect reduces induced-Cyclo thrombocytopenia and platelet dysfunction thereby reducing OS and restoring megakaryocyte differentiation via Nrf2 stimulation. Increased MPV levels suggest improved platelet activation due to improved bone marrow megakaryopoiesis and reduced platelet aggregation [18].

The histological examination of the liver in the Cyclo group showed mild fatty changes with microvesicular fat accumulation indicates a disruption in hepatocytes lipid metabolism which may due to mitochondrian dysfunction or impaired beta-oxidation. A mild focal inflammation has been shown as lymphocytes were seen near the central vein implies local immune activation mediated by CsA induced hepatocyte stress [19, 20]. Moreover, ballooning lymphocytes were observed as well. In Cyclo+Bro 100 mg group, a mild hepatocyte hypertrophy has been seen with no inflammatory cells or fatty changes. While in Cyclo+Bro 200 mg group, normal hepatocytes had seen with no inflammatory or fatty changes.

CONCLUSION

This study suggest improvement in erythropoiesis and RBCs health which may mediated by the CEEs high antioxidant, hepatoprotective and anti-inflammatory properties counteracting CsA induced toxicity. Moreover, nutrient content of CEE which mainly activate erythropoiesis by enhancing iron bioavailability, decreasing oxidative stress and improving hepatic support.

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