HEPATOPROTECTIVE ACTIVITY OF *BRASSICA JUNCEA* (L) CZERN AGAINST CARBON TETRACHLORIDE INDUCED HEPATOTOXICITY IN ALBINO RATS

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Summary

The present study is aimed to evaluate the hepatoprotective activity of aqueous extract of *Brassica juncea* (L) Czern against carbon tetrachloride (CCl_4) induced hepatic damage in albino rats. The different groups of animals (Groups II, III, IV and V) were administered with CCl_4 at a dose level of 0.5 ml/150 g b.w. in olive oil (1:1 v/v) for 3 days. The aqueous extract of *Brassica juncea* (L) Czern at the doses of 100 and 200 mg/kg b.w. and silymarin (25 mg/kg b.w.) were administered orally to the CCl_4 treated Group III, IV and V rats. Group VI animals were alone administered with aqueous extract of *Brassica juncea* (L) Czern at the dose of 200 mg/kg b.w. Hepatoprotective effects were studied by assaying the activity of liver marker enzymes like glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT), alkaline phosphatase (ALP), and Gamma glutamyl transferase (GGT). The Lipid peroxide (LPO) and antioxidants like Superoxide dismutase (SOD) and reduced glutathione (GSH) were also estimated in liver tissue homogenate. Hepatotoxicity induced rats showed increased level of LPO and decreased levels of antioxidants like SOD and GSH. The activities of liver marker enzymes such as GOT, GPT, ALP, and GGT in serum were increased. On treatment with *Brassica juncea* (L) Czern extracts, all the biochemical changes observed in the hepatotoxicity induced rats were reversed in a dose dependent manner. The efficiency of the plant *Brassica juncea* (L) Czern is comparable with a standard drug silymarin. The phytochemical screening of the plant extract showed the presence of terpenoids and flavonoids which brought possible mechanism of hepatoprotective activity due to their free radical scavenging and antioxidant properties. These results revealed that aqueous extract of *Brassica juncea* (L) Czern has significant protection against CCl_4 induced hepatocellular injury.

Keywords: *Brassica juncea* (L) Czern, Carbon tetrachloride, Hepatotoxicity, Silymarin, Terpenoids and Flavonoids

Introduction

Liver is responsible for metabolism of chemicals and foods for the regulation of internal environment. The major functions of the liver are carbohydrate, protein, fat metabolism and detoxification, secretion of bile and storage of vitamins [1]. Liver disorders are mainly caused by toxic chemicals, excessive consumption of alcohol, infections and autoimmune disorders. Most of the hepatotoxic chemicals damage liver cells mainly by inducing lipid peroxidation and other oxidative damages [2]. Carbon tetrachloride (CCl_4) is widely used for experimental induction of liver injury. The injury produced depends on CCl_4 metabolism to a highly reactive of free radicals which initiate lipid peroxidation [3]. The resulting hepatic injury was characterized by leakage of cellular enzymes into the blood stream and by centrilobular necrosis [4].

Brassica juncea belongs to Crucifereae (or) Brassicaceae family. It is a perennial herb. It is widely cultivated in many parts of India. Its constituent's seeds and oil which contain about 20 – 25 percent of oil. The seeds of the plant have been used traditionally for the treatment of muscular rheumatism, inflammatory neuralgic affections, abdominal colic and obstinate, vomiting, emetic, dengue. The present study was aimed to evaluate the effect of aqueous extract of seed parts of *Brassica juncea* (L) Czern. against CCl_4 induced hepatic damage in albino rats.

Materials and methods

Collection of seed

Plant source selected for the present study is *Brassica juncea*. The seeds of *Brassica juncea* (L) Czern. were collected from market and authenticated with the specimen deposited at RAPINAT Herbarium, Department of Botany, St. Joseph's college, Trichy, Tamil Nadu.

Preparation of plant extract

The plant materials were shade dried and coarsely powdered with electrical blender. 500 gm seed powder of *Brassica Juncea* (L) Czern. was mixed with 3000 ml of water. Then it was boiled until it was reduced to one third and filtered. The filtrate was evaporated to dryness. Paste form of the extract obtained was subjected to pre- clinical screening.

Experimental Animals

Healthy adult wistar strain of albino rats of both sexes, two to three months old and weighing 150-200 g were obtained from Tamilnadu Veterinary and Animal Sciences University, Chennai. The animals were allowed to acclimatize to laboratory conditions for a period of 5 days prior to the experiment. Animals were housed in standard polupropylene cages. Six animals were housed per cage, so as to provide them with

sufficient space, and to avoid unnecessary morbidity and mortality. Animals were maintained under standard condition of 12- hour's light/ dark cycle and at an ambient temperature at 23 ± 2°C, with 65 ± 5 % humidity. Animals were fed with standard rat chow pellet obtained from Sai Durga Feeds and Foods, Bangalore, India and water *ad libitum*. All the studies were conducted according to the ethical guidelines of CPCSEA after obtaining necessary clearance from the committee (Approval No: 790/03/ac/CPCSEA).

Preliminary Phytochemical Screening [5]

The preliminary phytochemical screening of ethanolic and aqueous extract were carried out.

Assessment of hepatoprotective activity

Wistar strains of albino rats were divided into six groups, each comprising of six rats. Group I served as normal control. Group II received at a dose of 0.5 ml/150 g b.w. in olive oil (1:1 v/v) for 3 days. Group III and IV CCl_4 induced hepatotoxic rats treated with aqueous extract of *Brassica juncea* (L) Czern at the doses of 100 and 200 mg/kg b.w. Group V CCl_4 induced hepatotoxic rats received standard drug silymarin (25 mg/kg b.w.). Group VI animals received 200 mg/kg b.w. of *Brassica juncea* (L) Czern alone. After the experimental period (30 days) animals were sacrificed by cervical decapitation. Blood was collected and serum was separated by centrifuging at 3000 rpm for 10 minutes and subjected for the determination of biochemical parameters like SGOT[6], SGPT[6], ALP[6], GGT[7], Bilirubin[8], A/G ration[9], Urea[10], Cholesterol[11], Triglycerides[12] and Protein[13]. Liver were dissected out and washed in ice-cold saline. Liver tissues were homogenized in 0.1 M phosphate buffer, P^H 7.4 and used for studying LPO[14], SOD[15], GSH[16], Na^+ / K^+ ATPase[17], Mg^{2+} ATPase[18], Ca^{2+} ATPase[19], Phospholipid[20] and glycogen[21].

Histopathological Studies[22]

Liver tissue collected were used for the preparation of histopathological slides by using microtome, were suitably stained and observed under light microscope for architectural changes seen during CCl_4 challenge in aqueous extract of *Brassica juncea* (L) Czern treated and control groups.

Statistical analysis

All the results were expressed as mean ± S.E. The data were statistically analyzed by one- way analysis of variance (ANOVA) and P values <0.01 were considered as significant.

Results

The phytochemical analysis showed the presence of phytoconstituents such as reducing sugar, cardioglycosides, quinine, tannin, terpenoid, coumarin in the aqueous extract of *Brassica juncea* (L) Czern. In ethanolic extract carbohydrate, reducing sugar, cardioglycosides, steroid, tannin, terpenoid, coumarin are present in the *Brassica juncea* (L) Czern. **(Table 1).**

Table 1: Analysis of phytoconstituents in aqueous extracts of *Brassica juncea* (L) Czern.

S.No	Test For	Observation	
		Water extract	Ethanolic extract
1.	Carbohydrate	Present (trace)	Present (trace)
2.	Reducing Sugar	Present (trace)	Present (trace)
3.	Cardioglycosides	Present	Present
4.	Anthraquinone	Absent	Absent
5.	Saponin	Absent	Absent
6.	Protein	Absent	Absent
7.	Tannin	Present	Present
8.	Steroid	Absent	Present
9.	Terpenoid	Present	Present
10.	Flavonoid	Absent	Absent
11.	Coumarin	Present	Present
12.	Quinone	Present	Present
13.	Alkaloid	Absent	Absent

Table 2: Activities of liver marker enzymes in hepatotoxic rats and aqueous extract of *Brassica juncea* (L) Czern treated rats

Groups	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	GGT (IU/L)
I	33.87 ± 1.95*	37.90 ± 1.52*	82.77 ± 1.81*	15.76 ± 1.86*
II	175.49 ± 1.04*	194.03 ± 1.53*	335.16 ± 1.78*	67.57 ±1.85*
III	98.11 ± 1.50	107.15 ± 2.28	236.66 ± 1.45	50.94 ± 1.79
IV	36.83 ± 1.97*	55.06 ± 1.13*	120.50 ± 2.20*	32.85 ± 1.74*
V	41.20 ± 2.48	41.87 ± 1.68	100.78 ± 1.61	13.94 ± 1.92
VI	34.72 ± 1.99	39.42 ± 3.42	81.62 ± 1.87	16.80 ± 1.86

Values are mean ± SEM n=6, $p<0.01$*

Table 3: Activities of membrane bound enzymes in hepatotoxic rats and aqueous extracts of *Brassica juncea* (L) Czern treated rats

Groups	Na^+/K^+ ATPase (µg of pi liberated/min/mg)	Mg^{2+}ATPase (µg of pi liberated/min/mg)	Ca^{2+}ATPase (µg of pi liberated/min/mg)
I	1.17 ± 0.04*	1.92 ± 0.005*	1.88 ± 0.004*
II	0.79 ± 0.05*	4.07 ± 0.004*	3.43 ± 0.02*
III	0.98 ± 0.004	2.44 ± 0.004	2.43 ± 0.006
IV	1.11 ± 0.01*	1.77 ± 0.009*	1.81 ± 0.06*
V	1.19 ± 0.05	1.86 ± 0.04	1.55 ± 0.04
VI	1.24 ± 0.005	1.94 ± 0.05	1.96 ± 0.05

Values are mean ± SEM n=6, p<0.01*

Table 4: Levels of LPO, SOD and GSH antioxidants in hepatotoxic rats and aqueous extracts of *Brassica juncea* (L) Czern treated rats

Groups	LPO (ng of MDA/g of tissue)	SOD (mM of epinephrine oxidised/min/mg)	GSH (µg of GSH/g of tissue)
I	7418.64 ± 9.53*	5.12 ± 0.08*	5570.8 ± 11.4*
II	12362.50 ± 14.95*	1.06 ± 0.05*	2794.30 ± 12.36*
III	9889.28 ± 10.01	3.08 ± 0.06	3486.95 ± 10.84
IV	8662.79 ± 14.31*	4.10 ± 0.07*	4180.84 ± 10.44*
V	7184.81 ± 7.80	5.54 ± 0.26	5875.90 ± 9.04
VI	7037.40 ± 7.30	5.18 ± 0.12	5960.82 ± 11.39

Values are mean ± SEM n=6, p<0.01*

Table 5: Levels of Biochemical parameters in hepatotoxic rats and aqueous extracts of *Brassica juncea* (L) Czern treated rats

Groups	Triglycerides (mg/dL)	Cholesterol (mg/dL)	Liver Glycogen (mg/g of tissue)	Serum protein (g/dL)
I	227.5 ± 1.87*	150.08 ± 1.68*	5.41 ± 0.06*	8.38 ± 1.91*
II	54.46 ± 1.66*	54.19 ± 1.74*	3.31 ± 0.04*	4.7 ± 1.18*
III	113.86 ± 1.70	76.05 ± 2.36	3.53 ± 0.03	5.59 ± 1.85
IV	170.84 ± 1.83*	138.08 ± 1.42*	4.14 ± 0.03*	6.98 ± 1.83*
V	210.88 ± 1.92	149.71 ± 1.70	3.34 ± 0.02	8.22 ± 1.85
VI	229.15 ± 3.23	151.81 ± 1.96	4.47 ± 0.02	8.42 ± 1.90

Values are mean ± SEM n=6, p<0.01*

Table 5: Levels of Biochemical parameters in hepatotoxic rats and aqueous extracts of *Brassica juncea* (L) Czern treated rats

Groups	Phospholipid (mg/g of tissue)	Triglycerides (mg/g of tissue)	Cholesterol (mg/g of tissue)	Urea(mg/dL)
I	17.5 ± 0.09*	3.72 ± 0.06*	4.13 ± 0.61*	19.88 ± 1.90*
II	5.76 ± 0.16*	11.47 ± 0.27*	12.04 ± 0.51*	62.59 ± 1.82*
III	8.74 ± 0.13	7.36 ± 0.21	9.07 ± 0.55	54.87 ± 1.82
IV	14.69 ± 0.12*	3.83 ± 0.14*	5.97 ± 0.48*	32.64 ± 1.96*
V	16.15 ± 0.12	3.41 ± 0.02	4.46 ± 0.32	24.77 ± 1.92
VI	19.04 ± 0.04	4.24 ± 0.02	4.26 ± 0.11	19.11 ± 3.90

Values are mean ± SEM n=6, $p<0.01$*

Table 5: Levels of Bilirubin and Albumin/Globulin ratio in hepatotoxic rats and aqueous extracts of *Brassica juncea* (L) Czern treated rats

Gps	Bilirubin (mg/dL)		Albumin/Globulin ratio		
	Total bilirubin	Direct bilirubin	Direct bilirubin	Albumin (g/dL)	Globulin (g/dL)
I	0.63 ± 0.05*	0.45 ± 0.02*	8.02 ± 0.64*	5.20 ± 0.40*	2.67 ± 0.39*
II	1.48 ± 0.02*	1.08 ± 0.02*	2.56 ± 0.35*	1.17 ±0.02*	0.95 ± 0.02*
III	1.19 ± 0.02	0.92 ± 0.49	3.51 ± 0.03	2.28 ± 0.03	1.29 ± 0.04
IV	0.67 ± 0.02*	0.49 ± 0.02*	7.19 ± 0.09*	5.42 ± 0.29*	2.03 ± 0.18*
V	0.67 ± 0.05	0.50 ± 0.02	8.61 ± 0.03	5.53 ± 0.08	3.28 ± 0.36
VI	0.55 ± 0.02	0.40 ± 0.02	8.16 ± 0.15	5.31 ± 0.28	2.61 ± 0.42

Values are mean ± SEM n=6, $p<0.01$*

The administration of CCl_4 in rats resulted in significant hepatic damage as observed from the elevated levels of SGOT, SGPT, ALP, GGT, Mg^{2+} ATPase, Ca^{2+} ATPase, LPO, bilirubin, urea, tissue triglycerides, tissue cholesterol and reduced levels of antioxidant enzymes, Na^+ / K^+ ATPase, total protein and albumin, serum cholesterol, serum triglycerides, tissue phospholipids, glycogen when compared to normal group.The levels of LPO, marker enzyme, Mg^{2+} ATPase, Ca^{2+} ATPase, tissue triglycerides, tissue cholesterol were decreased AEBJ treated animals when compared to CCl_4 induced hepatotoxic animals. Treatment with AEBJ brought the altered levels of the above biochemical parameters to the near normal levels thereby causing significant protection against CCl_4 induced liver damage. However, highly significant effect was seen with higher dose of the extract (200 mg/kg b.w.)

Histopathological studies

Histopathological examination of liver sections of control group showed normal cellular architecture with distinct hepatic cells, sinusoidal spaces and central veins **(Fig 1)**. Disarrangement of normal hepatic cells with centrilobular necrosis, vacuolization of cytoplasm and fatty degeneration were observed in CCl_4 intoxicated animals **(Fig 2).** The liver sections of the CCl_4 induced hepatotoxic rats treated with aqueous extract of *Brassica juncea* (L) Czern. at the dose level of 200 mg/kg b.w **(Fig 3 and 4)** showed a sign of protection as it was evident that absence of necrosis and vacuoles.

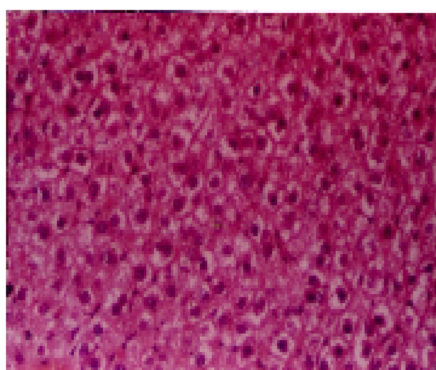

Fig 1

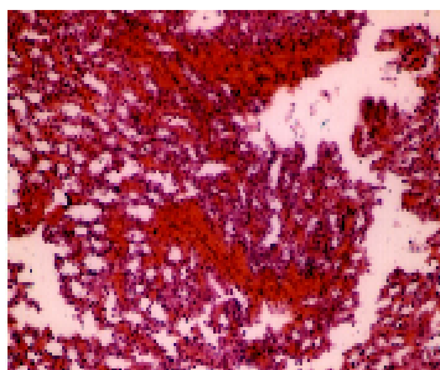

Fig 2

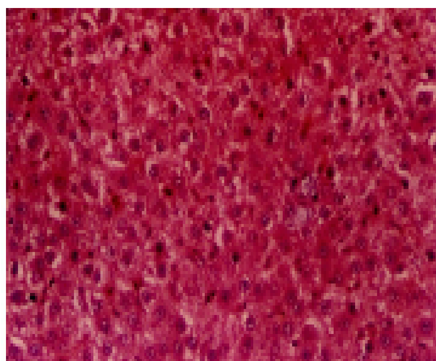

Fig 3

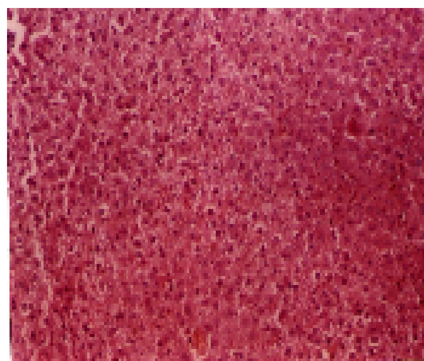

Fig 4

Figure 1-4: Histology of liver sections of experimental animals

Discussion

The experimental intoxication induced by CCl_4 is widely used for modeling liver injury in rats. Hepatotoxicity is connected with severe impairment of cell protection mechanisms [23]. The location of liver injury is defined mainly by the biotransformatin of CCl_4, which is cytochrome P-450 dependent. Free radicals initiate the process of lipid

peroxidation, which is generally caused by inhibition of enzyme activity[24]. It is now generally accepted that the hepatotoxicity of CCl_4 is the result of reductive dehalogenation, which is catalyzed by P450, and which forms the highly reactive trichloromethyl peroxy radical. Both trichloromethyl and its peroxy radical are capable of binding to proteins or lipids, or of abstracting a hydrogen atom from an unsaturated lipid, intiating lipid peroxidation and liver damage and by doing so playing a significant role in pathogenesis of diseases[4].

Prelininary phytochemical studies reveal the presence of Coumarin [25, 26, 27], Triterpenoids [28], Glycoside [29] which are hepatoprotectives.

The determination of malondialdehyde (MDA) level is one of the most commonly used methods for monitoring lipid peroxidation [30]. Malondialdehyde (MDA) is a major reactive aldehyde resulting from the peroxidation of biological membrane polyunsaturated fatty acid. MDA, a secondary product of lipid peroxidation is used as an indicator of tissue damage involving a series of chain reactions. It reacts with thiobarbituric acid, producing red coloured products. Lipid peroxidation has been implicated in the pathogenesis of increased membrane rigidity, osmotic fragility, reduced erythrocyte survival and perturbation in lipid fluidity. It has been hypothesized that one of the principal cause of CCl_4 induced hepatotoxicity is lipid peroxidation of hepatocyte membranes by free radical derivatives of CCl_4 **[31].**

The SOD dismutates superoxide radicals O_2^- into H_2O_2 plus O_2, thus participating, with other antioxidant enzymes, in the enzymatic defence against oxygen toxicity. SOD is the most enzymatic indexes of liver injury caused by oxidative stress. SOD is one of the most abundant intracellular antioxidant present in all aerobic cells and it has an antitoxic effect against reactive oxygen species (ROS). SOD plays an important role in the elimination of ROS derived from the peroxidative process of xenobiotics in liver tissues [32]. GSH is a critical determinant of tissue susceptibility to oxidative damage and the depletion of hepatic GSH has been shown to be associated with an enhanced toxicity to chemicals including CCl_4. The significant impairment of hepatic GSH status associated with a substantial hepatocellular damage induced by CCl_4 suggested the determinant role of hepatic GSH in the development of CCl_4 toxicity [33]**.** Cell injury induced by xenobiotics occurs only if mitochondrial GSH is depleted[34]**.**

Calcium ions can decrease the lipid fluidity of hepatocyte plasma membrane by influencing membrane bound enzymes to alter the lipid composition. It has been reported that free radical enhanced calcium release from the sarcoplasmic reticulum and also they inhibited sarcolemmal Na^+/K^+ ATPase, possibly causing the activation of the Na^+- Ca^+ exchange mechanism in the hepatic cell membrane [35].

A number of hepatotoxic agents cause accumulation of fatty deposits predominantly triglycerides in the parenchyma cells of liver. This accumulation of triglycerides may be as result of an imbalance between the rate of synthesis and the rate of release of triglycerides by the parenchymal cells into the systemic circulation [36]. proposed that a block of the secretion of hepatic triglycerides into plasma is the major mechanism underlying the fatty liver induced in rats by CCl_4. Intoxication with CCl_4 also, resulted in inhibition of bile acids synthesis from cholesterol, which is synthesized in liver (or) derived from plasma lipids, leading to an increase in cholesterol levels. Suppression of cholesterol levels by CCl_4 suggests the inhibition of the synthesis of bile acids from cholesterol[37].

Bilirubin assay is a sensitive test to substantiate the functional integrity of the liver and severity of necrosis[38]. Bilirubin also measures the binding, conjugating and excretory capacity of hepatocytes and is proportional to the erythrocyte degradation rate[39]. Increase in serum bilirubin levels may be found in hepatocellular damage, haemolytic jaundice (or) hepatitis. CCl_4 injury causes significant degeneration of hepatocytes and blockade of the bile ducts which results into significant increase in the serum total bilirubin and direct bilirubin levels[40].

Liver is the vital organ, which is involved in synthesis of glycogen. Liver glycogen is used to maintain blood glucose during fasting (or) exercise. Glycogen synthesis is promoted by activation of glycogen synthetase and by the increased concentration of glucose, which enters liver cells from the hepatic portal vein[41]. The fatty infiltration of liver causes the reduction in the diffusion of glucose-6- phosphatase, and burns all the glucose to acetyl- CoA[42].

Serum SGOT, SGPT, ALP, and GGT are the most sensitive markers employed in the diagnosis of hepatic damage because these are cytoplasmic in location and are released into the circulation after cellular damage[43].

Conclusion

The results of the present study clearly demonstrate that the seed extract of *Brassica juncea* (L) Czern can be effective in the treatment of liver injury caused by toxic chemicals. This may be due to their antioxidant and free radical scavenging properties. Further profound studies are required to establish the therapeutic potential and safety of the drugs of herbal origin, in the treatment of hepatotoxicity.

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