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Original Research Article

Antioxidant activity and hepatoprotective effect of *Cichorium intybus* (Kasni) seed extract against carbon tetrachloride-induced liver toxicity in rats

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Abstract

Purpose: *To assess the antioxidant and hepatoprotective activity of the aqueous-methanol extract of Cichorium intybus seeds (C. intybus) against carbon tetrachloride (CCl_4)-induced liver toxicity in albino Wistar rats.*

Method: *The seed extract of C. intybus was prepared in aqueous methanol (20:80) via Soxhlet solvent extraction process. CCl_4 (0.8 mL/kg) was administered to induce hepatic damage in Wistar rats. The seed extract (100, 250 and 500 mg/kg doses) and a 25 mg/kg dose of silymarin (as standard drug) were administered orally to separate groups of albino Wistar rats for 14 days. Blood samples from the rats were analyzed for biochemical markers for hepatic injury. The tissue samples of the rats were subjected to histopathological studies as well as analyzed for liver antioxidants.*

Results: *The results for biochemical markers revealed that the rats treated with the extract (500 mg/kg dose) showed a maximum elevation of catalase (48.90 µmole of H_2O_2 consumed/min/mg protein), glutothione peroxidase (22.1 mg GSH consumed/min/mg protein), superoxide dismutase (14.2 units/min/mg protein), and a reduction in glutathione (18.1 µmole of GSH/mg protein). Serum biochemical parameters including serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphate (ALKP), and direct bilirubin were significantly ($p < 0.01$) increased in the treated groups. Oral administration of different doses of C. intybus seed extract significantly ($p < 0.01$) protected the hepatic cells from impairment. The biochemical markers and hematological parameters were also normal in extract-treated rats in contrast to the standard (silymarin) and control groups.*

Conclusion: *The results show that C. intybus plant is potential a good natural source of natural hepatoprotective and antioxidants agents.*

Keywords: *Cichorium intybus, Antioxidant, Hepatoprotective Biomarkers, Silymarin, Hematological parameters*

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INTRODUCTION

Liver disorders (jaundice, fatty liver, cirrhosis) are commonly affecting human health worldwide. Approximately 70–75% of the world's population depends on herbal medicines for curing diseases because they are cost- effective, less toxic, and easily available [1]. Major metabolic activities of body are taking place in liver [2]. Hepatic disorders usually develop during the process of removal of toxic and harmful chemicals from the liver [3]. Plants and their various parts (stem, roots, leaves, flowers, and fruits) are well known for the treatment of hepatic disorders [4,5].

Approximately only 1 –2 % of plant species have been explored properly [6]. *C. intybus* (Family: Asteraceae) is a small perennial herb that is usually bushy in nature; its common name is chicory [7]. The colors of the flowers of *C. intybus* are usually blue, light purple, or lavender, but white or pink flowers have also been reported but very rarely [8].This plant is usually found as a wild plant grown along roadsides in Europe. *C. intybus* has different names when grown for its leaves such as leaf chicory, endive, or French endive [7]. Chicory has been used in conventional medicines throughout the world for hundreds of years [8]. In Iran, the parts of *C. intybus* other than the roots are mostly used to purify blood [9]. Extracts of seeds of chicory have also been used in Ayurvedic medicines for the treatment of hepatic disorders [9,10].

C. intybus is also enlisted as a domestic plant usually grown for food, animal fodder, and to make traditional medicines [11]. Several reports are available on the chemical composition of *C. intybus* seeds, but little attention has been given to their hepatoprotective effects [11]. Therefore, we designed this research to evaluate the hepatoprotective and antioxidant potential of *C. intybus* seeds against CCl_4 (carbon tetrachloride)-induced hepatic damage in rats.

EXPERIMENTAL

Chemicals

Chemicals and reagents such as butylated hydroxytoluene (BHT; 99.07 % pure); 2, 2-diphenyl-1-picrylhydrazyl (DPPH, 90.0 % pure), linoleic acid, carbon tetrachloride (CCl_4), standard antibiotic drug (silymarin), standard kits for the determination of levels of serum enzymes such as serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate (SGPT), alkaline phosphate (ALKP), and direct bilirubin (D. Bil) were purchased from Atlas Chemicals Co. (United Kingdom). Other reagents like normal saline, formalin, olive oil, and alcohol were of high quality and purchased from Sigma brands.

Collection of plant material

The seeds of *C. intybus* were collected from Lahore (Punjab, Pakistan) in March 2017. Prof Sohail Sheikh, Department of Botany, Govt. M.A.O College, Lahore, Pakistan, identified the plant seeds. A voucher specimen (no. GC-HERB-570) was submitted to the herbarium of Govt. M.A.O College, Lahore, Pakistan for future reference.

Sample and extract preparation

First, plant seeds were washed to remove dust; after that, they were shade dried at a temperature of 25–30 °C [12]. With the help of a commercial blender (FTA-788, West point, Germany), seeds were pulverized and then sieved (150 mesh sieve (0.065 mm)) [13]. The resultant seed powder material was stored in an airtight container. The powder of plant seeds was placed in a Soxhlet extractor for hot extraction using water and methanol in the proportion of 20:80 as a solvent respectively [14]. The Soxhlet extraction process was performed continuously at a temperature of 70–75 °C for 6 hr. An aqueous-methanol seed extract was concentrated by the removal of excess quantity of solvent (water and methanol) using a rotary evaporator. The crude extract was refrigerated in an airtight bottle in a refrigerator for future use [15].

Animals

Wistar albino rats (male) weighing 150–180 g were used in experimental work. They were acquired from the Department of Zoology, GC University Lahore, Pakistan. The rats were housed for seven days before pharmacological experiments in the chemistry lab of the University of Management and Technology Lahore. The Wistar rats were kept in a 12 h light/dark cycle under appropriate climatic conditions. These conditions include ac temperature of 23 ± 2 °C and humidity of 40–45 %. All the animals were grouped into five groups and each group consisted of six rats. Each group was provided a standard normal diet and water. Before the start of the experiment, all the animals were adapted to their surroundings for almost 7 days. For the animal experiments, approval (reg no. KMCRET/MS/03/2017) was obtained from the Institutes of Animal Ethics Committee (IAEC) and the studies followed international guidelines [16].

Determination of acute oral toxicity

By following the guideline of OECD for the testing of chemicals, (Test No. 423 (OECD); acute oral toxicity-acute toxic class method), an acute oral toxicity study was carried out. Five rats (n = 5) were used for this study [17]. The animals were kept under observation continuously for 24 h and allowed access to water, but food was not allowed. Animals (rats) were administered aqueous-methanol seed extract orally at dosage levels of 100, 250, and 500 mg/Kg body weight and kept under examination for 24 h continuously. The treated animals were observed for the first 2 h for morbidity and further for mortality for the next 24 h.

If mortality from 2 out of 3 treated animals was observed, the administered dosage was then recognized as a toxic dosage. If mortality was perceived in one animal, the same amount of dosage was repeated again to confirm the toxic dose. If mortality was detected again, the procedure was repeated in lower dosages.

Hepatoprotective property of *C. intybus*

Six groups of albino Wister rats (Group I, II, III, IV, V, VI) were made comprising six healthy animals of the same weight. The hepatotoxicity was induced by the administration of CCl_4 at the dosage level of 0.8 mL/Kg according to the induced liver damage-model reported by [18] in all the groups (II–VI) except Group I. Group I was labeled a control that was only provided with a normal diet and was not treated with CCl_4, while Group II acted as a negative control. Group III was kept as a positive control and provided with silymarin (standard drug) with the dosage of 25 mg/kg, while groups IV, VI, and VI were provided with plant's aqueous-methanol extract. All the doses were given to the animals orally with the help of gastric tubes. Group I rats were given only normal diet for 14 days. Group II animals were given 14 doses of silymarin at 24 hourly intervals. Similarly, 14 doses of test extracts in the concentration range of 100, 250, and 500 mg/Kg were given to animal groups III, IV, and V respectively at 24 h intervals. All the administered doses of the aqueous-methanol extract were given orally to the animals with the aid of gastric tubes. After the 15th day, the samples of blood were collected by piercing the retro-orbital plexus of all animals under mild ethereal anesthesia. Further, liver tissues of the entire treated animal were acquired by scarifying them. The estimation of liver antioxidants as well as histopathological studies were done through the analysis of tissue samples of treated animals. Moreover, the blood samples of treated animals were investigated for their biochemical markers of hepatic injury.

Serum preparation from blood

The blood of treated animals was taken. The samples were collected in plain sample bottles for biochemical examination, while the whole blood was collected in bottles already having anticoagulant and ethylene diamine tetra-acetic acid (EDTA) for hematogram use [14]. The serum separation was performed by the centrifugation process at the rate of 700×g for 20 min. and further examined for biochemical parameters. Before further analysis, the serum was stored at -80 °C. With the help of an automatic hematology analyzer (Sysmex F-800, Japan) the erythrocytes, leucocytes, and platelets were determined. Furthermore, biochemical parameters such as serum glutamic oxaloacetic transaminase (SGOT), lipid profiles like total cholesterol (TC), alkaline phosphatase (ALP), serum glutamic pyruvic transaminase (SGPT), triglycerides (TG), bilirubin, and creatinine were also estimated by employing the Span Diagnostics Limited kit, Pakistan.

Liver homogenate preparation

The hepatic tissues of animals were homogenized in a 0.2 M phosphate buffer or 0.2 M tris buffer (pH 7.1) and centrifuged at 3,000×g for 15 min. Liver enzymatic as well as non-enzymatic antioxidants were measured by using a supernatant.

Evaluation of antioxidant enzymes

A standard protocol was followed to determine the total protein content of the tissues 14]. Enzymatic antioxidants were assayed by determining glutathione peroxidase (GPx) [15], catalase (CAT) [17], and superoxide dismutase (SOD) [18], while the estimation of non-enzymatic anti-oxidants was done through reduced glutathione (GSH) [14] and lipid peroxidation (LPO) [15].

Histopathological studies

Highly active animals (alive) from each group were selected for histopathological studies. Each selected animal was dissected and liver was removed by the normal process. The removed liver from each animal was preserved in 10% formalin for 72 h, then the liver was washed with distilled water and then with alcohol and xylene; it was then embedded in paraffin wax. A small section was cut and stained with haematoxylin and eosin for histopathological investigations

[14]. Liver samples were then sent to UVAS Laboratories Pakistan, for histopathological studies. The liver sections were studied under a fluorescent microscope, and histopathological changes in its structure were observed. Bright and vivid photographs of liver sections were also taken through a fluorescent microscope to support histopathological findings. As a high-resolution fluorescence microscope was used for this purpose, photographs clearly showed the changes in hepatocytes due to mortification and inflammation.

Evaluation of antioxidant activities

DPPH free radical scavenging assay

Antioxidant evaluation of plant extract was done by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity following the procedure described [19-22].

Ferric thiocyanate (FTC) assay

Antioxidant activity of the plant extract in terms of the inhibitory effect on linoleic acid peroxidation was assessed by thiocyanate procedure [23-26].

Statistical analysis

All data obtained from this study was expressed in terms of mean standard deviation and presented as ±SD. ANOVA was applied to estimate the variance between different groups. The significant value was taken as $p < 0.045$.

RESULTS

Acute toxicity

Results showed that the aqueous-methanol seed extracts of *C. intybus* did not show any mortality until the concentration of 500 mg/kg. When extracts of *C. intybus* were employed in different concentrations (100, 250, and 500 mg/kg), no impairment was developed in the livers of tested animals; therefore, no mortality was perceived in any experimental group of mice.

Effect of *C. intybus* seed extract on hematological parameters

The influence of plant extract at three different dose levels (100, 250, and 500 mg/kg) on hematological parameters is presented in Table 1. The results of hematological parameters indicated that the reduction in the numbers of leucocytes (total WBC count, polymorphs, lymphocytes), erythrocytes (RBC count, hemoglobin, hematocrit, MCV, MCH, MCHC, RDW), and in platelets was observed in the tested animals due to CCl_4 induced hepatic injury. The administration of aqueous-methanol seed extract of *C. intybus* (100, 250, and 500 mg/kg) led to increased levels of hematological parameters (total WBC count 6950±0.01 cells/cm^3 RBC count 7.6±0.04 milicm^3 and platelets 430±0.05 thou/cm^3).

Effect of *C. intybus* seed extract on serum biochemical markers

Table 2 displays the effect of plant extract (100, 250, 500 mg/kg) on serum biochemical markers in CCl_4 intoxicated rats. The results indicated that hepatic impairment elevated the level of liver enzymes such as ALP (alkaline phosphatase), SGPT (serum glutamic pyruvic transaminase), and SGOT (serum glutamic oxaloacetic transaminase).

Treatment with plant extract at 100 mg/kg displayed the lesser activity but showed a comparable hepatoprotective activity in terms of decreasing the level of liver enzymes with the treatment at the dosage of 250 mg/kg in contrast to employed standard silymarin. The maximum hepatoprotective activity counter to decrease the level of liver enzymes was observed with the aqueous-methanol seed extract of *C. intybus* at the dosage of 500 mg/Kg than standard silymarin (25 mg/kg) as shown in Table 2. The aqueous-methanol seed extract of *C. intybus* decreased the liver markers (biochemical parameters) SGPT (41.21 ± 0.94 U/l), SGOT (39.29 ± 0.18 U/l), and ALP (109.24 ± 0.89 U/l).

Effect of aqueous-methanol seed extract of *C. intybus* on *in vivo* antioxidant activity

The results of *in vivo* antioxidant activity exhibited that the level of lipid peroxidation in intoxicated rats increased, while the level of enzymatic antioxidants decreased due to administration of CCl_4 at the dosage of 0.8 mL/Kg to the Wistar rats (Table 3). The aqueous-methanol seed extract of *C. intybus* (500 mg/kg) increased the total protein (9.95 µg/10 mg of liver tissue), enzymatic antioxidants SOD (14.2 units/min/mg protein), CAT (48.90 µmole of H_2O_2 consumed/min/mg protein), GPx (22.1 mg GSH consumed/min/mg protein), and GSH (18.1 µmole of GSH/mg protein).

Table 1: Effect of aqueous-methanol seed extract of *C. intybus* on hematological parameters in CCl_4-induced toxicity in rats

Parameter	Group I (Control)	Group II (CCl_4)	Group III (Silymarin)	Group IV (100mg/kg)	Group V (250mg/Kg)	Group VI (500mg/Kg)
Erythrocytes						
RBC count (mili/cm^3)	7.1±0.07	4.1±0.07	6.7±0.05	5.4±0.08	6.9±0.08	7.6±0.04
Hemoglobin (%)	13.5±0.06	7.5±0.0.6	12.8±0.03	9.9±0.09	11.5±0.09	13.8±0.06
Hematocrit (%)	32.3±0.05	16.9±0.04	31.3±0.04	25.9±0.09	29.8±0.07	32.9±0.04
MCV (fl)	47.8±0.08	55.3±0.08	45.9±0.08	53.9±0.04	50.1±0.04	47.5±0.03
MCH (pg)	19.8±0.09	22.9±0.05	17.2±0.04	21.1±0.04	19.9±0.03	19.1±0.01
MCHC (%)	39.3±0.08	43.1±0.08	36.9±0.01	42.3±0.06	41.9±0.09	38.9±0.07
RDW (%)	12.4±0.04	14.1±0.03	10.9±0.02	13.9±0.05	12.1±0.01	12.6±0.04
Leucocytes						
Total WBC count	7300±0.09	2650±0.09	5500±0.07	6000±0.04	6660±0.09	6950±0.01
(cells/cm^3).	8.8±0.04	10.5±0.02	9.99±0.05	10.1±0.08	9.50±0.08	9.10±0.04
Polymorphs (%)	90.1±0.09	92.9 ±0.01	80.9±0.04	91.9±0.01	91.1±0.03	89.8±0.07
Lymphocytes (%)	536±0.01	84.9±0.06	200±0.06	175±0.03	250±0.06	350±0.05
Platelets (thou/cm^3)	460±0.01	94±0.01	400±0.03	190±0.07	250±0.06	430±0.05

Data are mean ± SEM (n = 6)

Table 2: Effect of aqueous-methanol seed extract of *C. intybus* on biochemical parameters in CCl_4-induced toxicity in rats

Group	Treatment	SGOT (U/l)	SGPT (U/l)	ALKP (U/l)	D. Bil (mg/dL)
I	Control	35.9±0.48	40.8±0.38	110.9±0.51	0.61±0.01
II	CCl_4	58.25±1.48	56.05±1.89	139.11±3.29	0.98±0.02
III	Silymarin (25 mg/kg)	40.28±0.19	45.06±0.14	125.85±0.35	0.75±0.01
IV	*C. intybus* (100 mg/kg)	50.54±0.62	53.89±0.98	128.36±0.39	0.85±0.08
V	*C. intybus* (250 mg/kg)	45.67±0.23	47.44±0.98	118.41±0.25	0.79±0.05
VI	*C. intybus* (500 mg/kg)	39.29±0.18	41.21±0.94	109.24±0.89	0.68±0.01

Data are presented as mean ± SEM (n = 6)

Table 3: Effect of aqueous-methanol seed extract of *C. intybus* on *in vivo* antioxidant activity in CCl_4-intoxicated rats [SOD–superoxide dismutase (units/min/mg protein), CAT–catalase (µmole of H_2O_2 consumed/min/mg protein), GPx–glutothione peroxidase (mg GSH consumed/min/mg protein), GSH–reduced glutathione (µmole of GSH/mg protein)

Group	Treatment	Total Protein	SOD	CAT	GPx	GSH
I	Control	10.1±0.48	15.10±0.38	50.12±0.51	21.64±0.01	18.84±0.01
II	CCl_4	5.0±1.48	7.85±1.89	30.90±3.29	12.73±0.02	11.52±0.05
III	Silymarin (25 mg/kg)	7.10±0.19	12.0±0.14	40.10±0.35	23.53±0.01	17.98±0.09
IV	*C. intybus* (100 mg/kg)	8.10±0.62	11.50±0.98	35.90±0.39	14.76±0.08	14.15±0.06
V	*C. intybus* (250 mg/kg)	8.89±0.23	13.0±0.98	42.90±0.25	19.26±0.05	16.20±0.08
VI	*C. intybus* (500 mg/kg)	9.95±0.18	14.20±0.94	48.90±0.89	22.10±0.01	18.10±0.07

Data are presented as mean ± SEM (n = 6)

Histopathological observations

The hepatoprotective effect of the plant extract against CCl_4 induced hepatic injury confirmed from the result of histopathological investigation. The hepatocytes' impairment, which was produced due to administration of CCl_4 in rats, was presented in Figure 1(a). The standard silymarin treated rat liver exhibited portal tract inflammation with lymphocysts and displayed premature fibrosis of the perivenular region (Figure 1d), but the aqueous-methanol seed extracts of *C. intybus* shielded the liver hepatocytes by averting the oxidation in liver cells (Figure 1b and c). The histopathological studies clearly showed that an increase in the concentration of extract dose (100 to 250 and then 500 mg/Kg) prevented the liver damage caused by CCl_4.

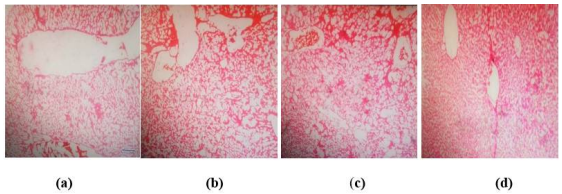

Figure 1: The Histopathological examination of CCl_4 treated and plant extract treated Wistar rats. (a) CCl_4-treated rat liver showing portal tract inflammation with lymphocyst, (b) protective effect of aqueous-methanol seed extract of *C. intybus* (250 mg/Kg) in the liver of the tested rats, (c) protective effect of aqueous-methanol seed extract of *C. intybus* (500 mg/Kg) in the

liver of the tested rats, (d) CC_4-treated rat liver showing early fibrosis of the perivenular region

Antioxidant activities

DPPH free radical scavenging activity

DPPH radical scavenging for different concentrations of aqueous-methanol seed extracts of *C. intybus* are presented in Figure 2. The maximum DPPH radical scavenging in terms of IC_{50} value (80 ± 0.22 µg/mL) was perceived with the concentration of 60 µg/mL, whereas the lowest IC_{50} value (41 ± 0.33 µg/mL) was achieved with the concentration of 1000 µg/mL (Figure 2). The other concentrations (125, 250, and 500 µg/mL) of plant extract demonstrated the IC_{50} values 74±0.53, 66±0.64, 51±0.12 µg/mL respectively in scavenging the DPPH free radical. DPPH radical scavenging increased with increasing concentration of aqueous-methanol plant extract. At all levels of concentrations from 60 µg/mL to 1000 µg/mL, the aqueous-methanol seed extract of *C. intybus* manifested the noteworthy and significant DPPH radical scavenging propensity, which was analogous to standard BHT (IC_{50} = 84±0.54 µg/mL, 76±0.81 µg/mL, 65±0.49 µg/mL, 55±0.39 µg/mL, 43±0.51 µg/mL) with the same concentration level of plant extract. This demonstrates that the aqueous-methanol seed extract of *C. intybus* holds extraordinary antioxidant potential in terms of scavenging the DPPH free radicals.

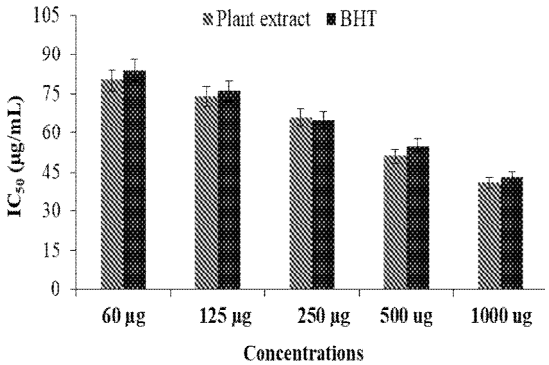

Figure 2: DPPH radical scavenging activity of the seed extract of *C. intybus*

Antioxidant activity via inhibition of linoleic acid oxidation

The antioxidant propensity at different concentrations of plant extract was estimated by means of the percentage inhibition of linoleic acid peroxidation (Figure 3). The % inhibition of linoleic acid peroxidation was 33±0.11, 45±0.28, 57±0.35, 65±0.37, and 73±0.42 at different concentrations of 60, 125, 250, 500, and 1000 µg/mL of plant extract, respectively. The highest inhibition of linoleic acid peroxidation was 73±0.42% (for 1000 µg/mL of aqueous-methanol seed extract of *C. intybus*), while the lowest percentage inhibition (33±0.11 %) was recorded for 60 µg/mL concentration of plant extract. The linoleic acid peroxidation was significantly inhibited by the aqueous-methanol seed extract of *C. intybus* at all the levels tested, and all the results for the percentage inhibition of linoleic acid were superior than that of standard BHT, which showed 30±0.27, 41±0.38 %, 53±0.45 %, 60±0.29 %, and 69±0.11 % inhibition for its different concentrations (60, 125, 250, 500 and 1000 µg/mL) respectively (Figure 3).

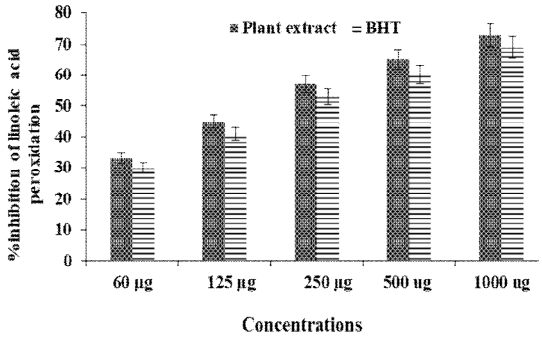

Figure 3: Antioxidant activity of seed extract of *C. intybus* by inhibition of linoleic acid peroxidation

DISCUSSION

Poisonous chemicals, toxic as well as non-toxic drugs, and viral penetrations can severely damage the liver, causing heptocellular diseases [1]. Fatty liver, necrosis, and cirrhosis are the most significant pathological features of CCl_4 that induce hepatotoxicity [7]. A free radical species which is responsible for this hepatotoxicity is formed by the free radical mechanism of CCl_4 [8]. This species is $^{\cdot}CCl_3$, which is first produced and then catalyzed by the action of an enzyme named as cytochrome p450. This enzyme is usually used to transform or catalyze the poisonous drugs, compounds or chemicals in the interconnected network of tubular membranes [9]. Hepatotoxicity induced by CCl_4 in normal rats increased the levels of biochemical framework prominently [10]. The animals treated with aqueous alcoholic extract of *C. intybus* exhibited a remarkable decrease in all the serum parameters intoxicated by CCl_4. Silymarin also exhibited the same results as test extracts. Flavonoids and phenolic compounds are responsible for the antioxidant and hepatoprotective activities of plants [14]. These natural products found in plant and seed extracts could be responsible for the hepatoprotective effects [10].

Therefore, from the present study it is concluded that aqueous-methanol extracts exhibited remarkable antioxidant and hepatoprotective activity. It can also be proposed that alcohol soluble compounds may also be present in the seeds of *C. intybus* Linn, which behave as an active ingredient. These alcohol soluble compounds are poly-phenolics or flavonoids, which may protect the liver against the damage caused by free radicals. Future research work is needed to evaluate practical usefulness of *C. intybus* by separating and isolating natural poly-phenolics or flavonoids through different techniques.

The different concentrations of aqueous-methanol seed extract of *C. intybus* were also examined for their antioxidant propensity by total antioxidant assays (inhibition of linoleic acid peroxidation and DPPH radical scavenging). All the concentrations of aqueous-methanol seed extract of *C. intybus* exhibited the comparable antioxidant activity results with standard BHT. However, in case of inhibition of linoleic acid peroxidation, all the concentrations of aqueous-methanol seed extract of *C. intybus* manifested the significant and improved antioxidant results in comparison with the standard BHT. Moreover, the results for plant extracts at different concentrations also indicate that the antioxidant activity of the aqueous-methanol seed extract of *C. intybus* was concentration-dependent [9].

The plant extract showed enhanced antioxidant properties with increase in concentration. The presence of flavonoids and phenolics in seeds of *C. intybus* has been previously reported [27]. Potent antioxidant activities of *C. intybus* seeds have been reported previously in literature, where 100% methanolic extract showed the highest antioxidant potential [27]. The results showed that plant *C. intybus* is a rich source of natural antioxidants based on flavonoids and polyphenolics [10]. The antioxidant results of *C. intybus* in current research study are in close agreement with the previously reported studies [8].

CONCLUSION

The findings of this study research demonstrate that the aqueous-methanol seed extract of *C. intybus* has potent antioxidant and hepatoprotective activities. This may be due to the presence of flavonoids or poly-phenolic compounds, which are known to exhibit hepatoprotective and antioxidant properties. Consequently, the seed extract of *C. intybus* (Linn.) has some potentials for use in pharmaceutical, nutraceutical, and cosmetic industries.

DECLARATIONS

Acknowledgement

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Conflict of interest

No conflict of interest is associated with this study.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. All authors equally contributed to this work.

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