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Original article

Pharmacognostic standardization, antioxidant and free radical scavenging activity of the seeds of *Triticum aestivum* L – A dietary staple

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ABSTRACT

Aims: To establish the Quality standards of *Triticum aestivum* L, seeds as per WHO guidelines. To study the antioxidant and hepatoprotective profile of *T. aestivum* L. seeds.
Methods: Pharmacognostic studies like morphological, microscopical, physico-chemical, phytochemical evaluation, fluorescence analysis, TLC, HPTLC, phytochemical analysis etc. of various extracts of the seeds of *T. aestivum* were carried out as per established methods. The ethanolic extract was evaluated for antioxidant and hepatoprotective activity using rat model.
Results: Preliminary phytochemical analysis mainly revealed the presence of carbohydrates, phenolics, proteins, resins, lipids and flavonoids. *T. aestivum* at different doses, i.e. 5–45 μg/ml showed free radical scavenging activity in dose dependent manner. The amount of phenolic components was found to be 313.5 μg/mg indicating considerable antioxidant activity. The ethanolic extract of *T. aestivum* was administered at dose level of 100 mg/kg/day, every day for 21 days along with CCl4. Biochemical and histopathological results conclude that the seeds have hepatoprotective activity.

1. Introduction

Triticum aestivum (wheat) is an annual grass belonging to family Poaceae, commonly 60–150 cm in height and is the most widely grown wheat species in India. Since wheat (*T. aestivum*) is a dietary staple, the government bodies cannot compel the manufacturer to test the safety of these materials. Given their potential benefits, an independent demonstration of their safety appears warranted. Antioxidants of natural origin have attracted special interest because they can protect human body from free radicals without producing toxic effects.[1] It is already reported that natural antioxidants, especially phenolics and flavonoids, found in plants are the most bioactive.[2] Wheat bran is a rich source of various natural antioxidants that possess health benefits for humans, such as preventing cardiovascular disease and certain cancers.[3,4] Studies have suggested that compounds of wheat bran exhibit significant capabilities in scavenging free radicals, chelating metal ion oxidants, and reducing lipid oxidation at different conditions[5,6]. Because of the antioxidant potential of wheat its seeds can be exploited to study antioxidant defense of hepatocytes exposed to oxidative stress. So, the present study was conducted to determine the quality standards, antioxidant and hepatoprotective properties of the seeds of *T. aestivum*.

2. Materials and methods

2.1. Plant material

Wheat seeds were bought from a local supermarket (Khari bavli) in Delhi. They were dried for 1 h at 60 °C in the oven. Seeds were identified as *T. aestivum* L. at the Department of Botany Jamia Hamdard University, New Delhi.

2.2. Quality standards

2.2.1. Macroscopic & microscopic analysis

Proper examination of the untreated sample of seeds of the chosen plant was carried out under diffused sunlight and artificial source similar to daylight. The colour, size, odour, taste, shape of the seeds were determined. Powder (#60) of the dried seeds was used for the observation of powder microscopical characters. The powdered drug was separately treated with phloroglucinol – hydrochloric acid (1:1) solution, acetic acid, and iodine solutions to determine the presence of lignified fibers, calcium oxalate crystals and starch grains respectively.[7]

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2.2.2. Physico-chemical analysis

Physico-chemical parameters of the powdered material such as total ash, water-soluble ash, acid-insoluble ash were determined. The powered material was subjected to Cold extraction, Hot extraction and Successive extraction using petroleum ether, chloroform, acetone, ethanol, water and ethanol–water as solvents and respective extractive values were determined. The moisture content was detected by loss on drying method.[8] Bitterness value, swelling index, foaming index resin content and fat content were also determined.[9]

2.2.3. pH, microbial load and heavy metal analysis

The microbial load was determined as per WHO guidelines using nutrient agar medium. 1% and 10% solutions of the wheat power in distilled water were prepared and the pH of their filtrate was measured with a standardized glass electrode. Heavy metal analysis for wheat (*T. aestivum*) seeds was performed at ITL Labs Pvt. Ltd, New Delhi, India, under the analysis No. ITLR093-080307.[10]

2.2.4. Fluorescence analysis

The fluorescence character of the plant powders (40 mesh) was studied both in daylight and UV light (255 and 366 nm) and after treatment with different reagents like sodium hydroxide, picric acid, acetic acid, hydrochloric acid, nitric acid, iodine, ferric chloride.[11]

2.2.5. Preparation of extracts and preliminary phytochemical analysis

The powdered plant material was passed through sieve no. 40 and used for extraction. Petroleum ether, acetone, chloroform, alcoholic, hydro alcoholic and aqueous extracts of the powdered plant material were prepared.[12] Preliminary phytochemical analysis of these different extracts was done using standard methods.[13]

2.2.6. TLC and HPTLC profile

Petroleum ether, chloroform and methanolic extracts of *T. aestivum* were subjected to TLC with the aim of identifying the individual substances in a mixture and also for testing the purity. Solvent systems used for petroleum ether extract was Hexane: Ethyl acetate as 8:1, for chloroform extract it was Hexane: Ethyl acetate: Glacial acetic acid (GAA) in the ratio of 20:5:2 and for methanolic extract the solvent system used was Toluene:Ethyl acetate:Formic acid as 14:4:1. Anisaldehyde sulphuric acid was used as the visualizing agent.[14] The methanolic extract were then taken and HPTLC was done with Toluene:Ethyl acetate:Formic acid (7:2:5) as solvent system using CAMAG Linomat 5.[15]

2.3. Antioxidant activity

2.3.1. Quantitative estimation of total phenolic contents

The total phenolic content of *T. aestivum* seeds was determined with Folin-Ciocalteu method reagent in alkaline medium using gallic acid as standard.[16] The total phenolic content was expressed in μg of gallic acid equivalents/mg of extract.

2.3.2. Free radical scavenging activity

Free radical scavenging activity was measured by 1, 1-diphenyl-2-picryl-hydrazyl (DPPH). The antioxidant activities of the ethanolic extract of *T. aestivum* seeds were compared with standard vitamin C, butylated hydroxy anisole (BHA) and butylated hydroxy toluene (BHT). The activities were assessed on the basis of the radical scavenging effect of the stable DPPH free radical.[17]

2.4. Hepatoprotective activity

The hepatoprotective activity of the ethanolic extract of the *T. aestivum* seeds was studied using carbon tetrachloride as the hepatotoxic agent.[18] The study was carried out for 21 days.[19]

2.4.1. Animals and treatments

Swiss albino rats of either sex were selected for performing the biological studies.[20] The weight of the animals ranged from 125 to 150 g.[21] The animals were kept in polypropylene cages (6 in each cage) under standard laboratory conditions (12 h light and 12 h dark: day and night cycle) and had a free access to commercial pelleted diet and tap water *ad libitum*. All studies were performed in accordance with the guidelines of Institutional Animal Ethics Committee (IAEC), that is fully accredited by the Committee for Purpose of Control and Supervision on Experiments on Animals (CPCSEA) Chennai, India. The animals were provided by "Central Animal House Facility, Jamia Hamdard", whose registration number and date of registration are 173/CPCSEA and 28th Jan 2000. Approval ID/project number for this study is 710.

The animals were divided into four groups, each group had six animals. Group first served as control animals and received orally a single daily dose of normal saline (0.3 ml). Group two animals received carbon tetrachloride (0.7 ml/kg body weight, i.p. 1:1 v/v mixture of CCl_4 and liquid paraffin) alone on first day.[22] Third group served as standard control and the animals received a single dose of CCl_4 (as in group 2 animals) on first day along with a single dose of standard Silymarin (Sivylar-140, Ranbaxy) (25 mg/kg p.o.) daily for 20 days.[23] Group four animals received a single dose of CCl_4 (0.7 ml/kg i.p. in liquid paraffin) and a daily single dose (100 mg/kg b.w.) of ethanolic extract of *T. aestivum*. Animals were sacrificed 48 h after the last dose of the drug, blood samples were collected through retro-orbital plexus and liver of each animal was excised.[24]

2.4.2. Estimations in serum

The collected blood was allowed to clot and serum was separated at 2500 rpm for 15 min and the biochemical parameters like serum enzymes: aspartate aminotransferase (AST, U/L), serum glutamate pyruvate transaminase (ALT, U/L),[25] serum alkaline phosphatase (ALP, IU/L)[26] and total bilirubin (mg/dL)[27] were assayed using assay kits.

2.4.3. Estimations in liver

2.4.3.1. Assessment of lipid peroxidation (LPO). The dissected out liver samples were washed immediately with ice cold saline to remove as much blood as possible. 10% w/v tissue homogenate was prepared in ice cold 0.15 M KCl for TBARS. 1 ml of suspension medium was taken from the 10% tissue homogenate. 5 ml of 30% TCA was added to it, followed by 0.5 ml of 0.8% TBA reagent. The tubes were then covered with aluminium foil and kept in shaking water bath for 30 min at 80 °C. After 30 min tubes were taken out and kept in ice cold water for 30 min. These were then centrifuged at 3000 rpm for 15 min. The absorbance of the supernatant was read at 540 nm at room temperature against blank. Blank consisted of 1 ml distilled water, 0.5 ml of 30% TCA and 0.5 ml of 0.8% TBA.[28]

2.4.3.2. Assessment of reduced glutathione (GSH) activity. This spectrophotometric procedure is based on the method of Ellman i.e. 5,5′-dithiobis-(2-nitrobenzoic acid) or DTNB, is reduced by SH groups to form 1 mol of 2-nitro-5-mercaptobenzoic acid per mole of SH. The reaction mixture contained equal volumes of 4% sulfosalicylic acid and tissue samples homogenized in 4 vol. of ice cold 0.1 m/l phosphate buffer (pH 7.4). The method used for estimating GSH in this study also measures non-protein sulfhydryl concentration inclusive of GSH. However, 80–90% of the non-protein sulfhydryl content of the cell represents free endogenous GSH. Enzyme activity was expressed as milligram per hundred grams.[29]

Table 1
Extractive values of *Triticum aestivum* seeds.

Extract	Individual extractive value (% w/w)		Successive extraction (% w/w)
	Hot extraction	Cold extraction	
Petroleum ether	0.52	0.48	0.42
Chloroform	1.98	2.66	1.43
Acetone	1.75	2.08	1.87
Alcoholic	0.16	1.48	0.72
Hydro alcoholic	3.95	3.25	3.37
Aqueous	7.01	3.33	2.00

Table 2
Fluorescence analysis of powdered seeds of T. *aestivum*.

Reagents	Colour observed in ordinary light	Colour observed under ultraviolet light	
		254 nm	365 nm
Powder as such	Light brown	Light green	Light orange
Distilled water	Light brown	Light green	Black
5% NaOH	Light yellow	Light green	Dark brown
1N NaOH	Light yellow	Green	Dark brown
Conc. H_2SO_4	Light brown	Light green	Black
Conc. HCl	No change	Off white	Black
Acetone	Light brown	Light green	Dark brown
Chloroform	Off white	Light brown	Brown

2.4.3.3. Protein estimation. Protein reacts with Folin's ciocalteau phenol reagent to give a coloured complex. The colour so formed is due to the reaction of alkaline copper with protein as in Biuret test and the reduction of phosphomolybdate by tyrosine and tryptophan present in the protein.[30]

500 mg of liver tissue was homogenized in 5 ml 0.15 M KCl and centrifuged at 10,000 rpm for 10 min 1 ml of supernatant was mixed with 5 ml of alkaline copper solution and allowed to stand at room temperature for 10 min. 0.5 ml of Folin's reagent (1:2) was added and tubes were shaken to mix the solution. After 30 min the absorbance was read at 750 nm against appropriate blank. The protein content was expressed in mg.

2.4.4. Histopathological studies

For histological studies, the liver tissues were fixed with 10% phosphate buffered neutral formalin, dehydrated in graded (50–100%) alcohol and embedded in paraffin. Thin sections (5 lM) were cut and stained with routine and eosin (H & E) stain for photo microscopic assessment. The initial examination was qualitative, with the purpose of determining histopathological lesions in liver tissue.[31]

2.5. Statistical analysis

Results are presented as Mean ± S.E.M. of six animals used in each group. Data were subjected to statistical analysis through one way analysis of variance (ANOVA) taking significant at 5% level of probability followed by Student's *t*-test taking significant at $P \leq 0.05$.[32]

2.6. Results and discussion

Pharmacognostic standardization including physico-chemical evaluation is meant for identification, authentication, and detection of adulteration and also compilation of quality control of crude drugs.

2.6.1. Macroscopic characteristics

The seeds of *T. aestivum* are yellowish grey in colour and are 5–10 mm long. They have smooth surface with a crease, blunt apex, no odour, mucilaginous taste and has a shallow grove on one face.

2.6.2. Microscopic characteristics

The transverse section of the seed showed tissues like embryonal axis, endospermic cells. Seed coat is inner to the bran tissue comprises of inner hyaline and outer testa. Bran tissue comprises of many layers of cells which include pericarp, epidermis, hypodermis, cross layer and tube cells.

2.6.3. Physico-chemical results

The total, acid-insoluble and water-soluble ash values were found to be 1.42 ± 0.085 (%w/w), 1.12 ± 0.27 (%w/w) and 0.84 ± 0.16 (%w/w) respectively. Foreign matter content and moisture content of the seeds were found to be 0.55 ± 0.12 (%w/w) and 9.3 ± 0.33 (%w/w). The results of extractives values after cold extraction, hot extraction and successive extraction are given in Table 1. No bitterness value was found; swelling index was found to be 0.00; no foaming index was found of the drug with respect to *Glycyrrhiza glabra* (foaming index 100). The resin and fat contents of the seeds were found to be 1.34% (w/w) and 4.08% (w/w) respectively.

2.6.4. pH, microbial load and heavy metal analysis

At the stock dilutions of 1:1, 1:10 and 1:100, the number of microbial colonies which were visible to the naked eye were 550, 200 and 6 respectively. All these microbial colonies were round in shape and white in colour. The pH of 1% and 10% solutions of *T. aestivum* seeds were found to be 6.82 and 6.43 (at 32.5 °C) respectively. The results of heavy metal analysis showed presence of lead (4.06 ppm) and arsenic (1.14 ppm). The seeds showed no presence of cadmium and mercury.

Table 3
Preliminary phytochemical analysis of various extracts.

Chemical constituents	Petroleum ether extract	Acetone extract	Chloroform extract	Alcoholic extract	Hydro alcoholic extract	Aqueous extract
Alkaloids	−	−	−	−	−	−
Carbohydrates	−	+	+	+	+	+
Glycosides	−	−	−	−	−	−
Phenolics	−	+	+	+	+	+
Flavonoids	−	−	−	+	+	+
Proteins	−	+	+	+	+	+
Saponins	−	−	−	−	−	−
Mucilage	−	−	−	−	−	−
Resins	−	+	+	+	+	−
Lipids	+	+	+	+	−	−

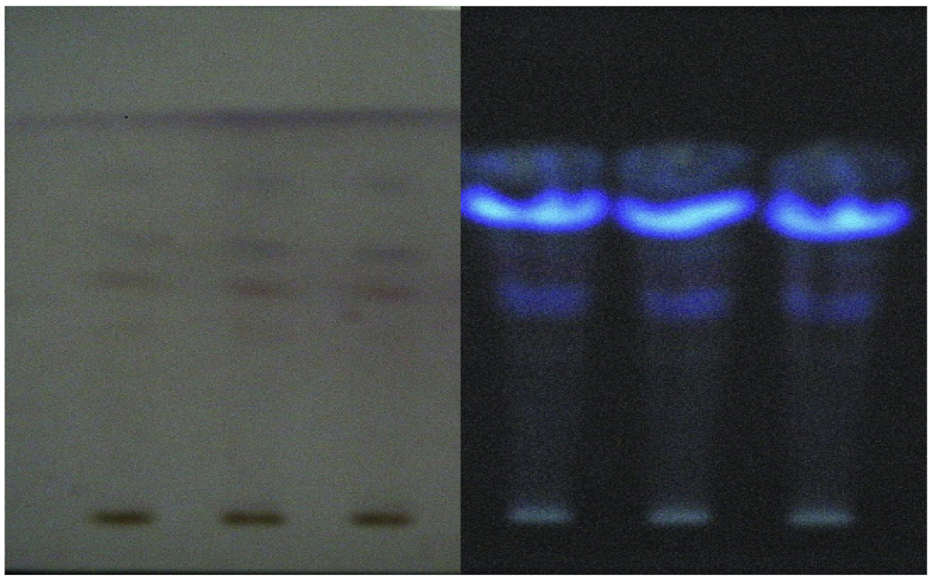

Fig. 1. High performance thin layer chromatographic (HPTLC) profile of different extracts of *Triticum aestivum* seeds 1A: Chromatogram at daylight sprayed by anisalydehyde sulphuric acid, 1B: Chromatogram scanned at 366 nm.

2.6.5. Fluorescence analysis

The powder of the seeds of *T. aestivum* (mesh size 40) was examined under daylight and UV light. The observations are recorded in Table 2.

2.6.6. Preliminary phytochemical analysis

Preliminary phytochemical analysis mainly revealed the presence of carbohydrates, phenolics, proteins, resins, lipids and flavonoids (Table 3).

2.6.7. TLC and HPTLC profile

In the last two decades HPTLC method has emerged as an important tool for the qualitative and quantitative phytochemical analysis of herbal drugs and formulations.[33] In the petroleum ether extract four spots were observed at Rf 0.34, 0.55, 0.69 and 0.74. Chloroform extract also showed four spots in the TLC profile at Rf 0.35, 0.44, 0.47 and 0.5. While the methanolic extract showed three spots at Rf 0.2, 0.56, 0.68 (Fig. 1A).

In HPTLC profile of the methanolic extract of the *T. aestivum* seeds, it was observed the constituents of methanolic extract appear at the Rf 0.55 and 0.68 (Fig. 1B).

2.6.8. Antioxidant results

DPPH assay is widely used as a free radical to evaluate the antioxidant activity of natural compounds. The antioxidant activity of *T. aestivum* seeds was carried out using DPPH dye. *T. aestivum* at different doses, i.e. 5–45 μg/ml showed free radical scavenging activity in dose dependent manner. Maximum percentage inhibition of DPPH radicals was about 93% at 45 μg/ml concentration. The IC50 value of TA was found to be 20 μg/ml and that of Vitamin C was 8.46 μg/ml, BHA was 2.82 μg/ml and BHT 7.47 μg/ml. The amount of phenolic components was calculated as gallic acid equivalents and was found to be 313.5 μg/mg indicating considerable free radical scavenging activity.

2.6.9. Hepatoprotective results

2.6.9.1. Serum enzymes. Levels of the serum marker enzymes of hepatic damage, AST, ALT, ALP, bilirubin and albumin increased significantly (while level of total serum proteins (TSP) decreased significantly) in CCl_4 treated rats compared to the control group. 100 mg/kg b.w. of *T. aestivum* prevented the liver damage as judged by restored enzyme levels (Table 4).

2.6.9.2. Tissue estimations. The effect of ethanolic extract of *T. aestivum* on CCl_4-induced lipid peroxidation in the liver is shown in Table 4. CCl_4 increased the hepatic MDA levels significantly while 100 mg/kg b.w. dose of ethanolic extract of *T. aestivum* significantly decreased the MDA levels when compared to the toxic group animals. Administration of CCl_4 decreased the hepatic GSH levels as seen from Table 4. However, Group 3 animals which were treated with *T. aestivum* along with CCl_4 showed a significant increase in the GSH levels towards normal when compared to the toxic group animals. Liver tissue protein levels (TP) were restored to normal when treated with 100 mg/kg dose of *T. aestivum* (along with CCl_4) compared to toxic group animals which were treated with only CCl_4.

Table 4
Effect of *Triticum aestivum* extract on various biochemical parameters in carbon tetrachloride intoxicated rats.

Treatments	AST (IU/ml)	ALT (IU/ml)	ALP (KA units)	TSP (g/dl)	Bilirubin (g/dl)	Albumin (g/dl)	MDA (nmol/g)	GSH (μg/mg)	TP (mg/ml)
Group 1	28.3 ± 3.38^c	34.8 ± 1.72^c	29.1 ± 0.98^c	15.2 ± 0.91^b	0.75 ± 0.03^c	4.9 ± 0.29^c	0.9 ± 0.19^c	17.5 ± 2.17^c	1.8 ± 0.08^c
Group 2	136.1 ± 5.61	104.5 ± 3.76	48.8 ± 0.45	9.5 ± 0.54	8.5 ± 0.33	10.5 ± 0.28	4.6 ± 0.24	3.4 ± 0.35	2.8 ± 0.19
Group 3	79.8 ± 2.83^c	39.5 ± 5.03^c	30.7 ± 0.69^c	14.3 ± 0.66^b	1.0 ± 0.04^c	6.4 ± 0.04^c	1.6 ± 0.12^c	12.3 ± 0.25^c	1.8 ± 0.10^c
Group 4	116.4 ± 2.90^b	67.7 ± 0.97^c	35.9 ± 1.44^c	10.4 ± 0.96^b	2.5 ± 0.07^c	3.9 ± 0.22^c	3.1 ± 0.18^c	8.2 ± 0.28^c	2.7 ± 0.06

Results are presented as Mean ± S.E.M. of six animals used in each group.
$^a p < 0.05$, $^b p < 0.01$, $^c p < 0.005$ compared with the toxic group (One-way ANOVA followed by Student's t-test).

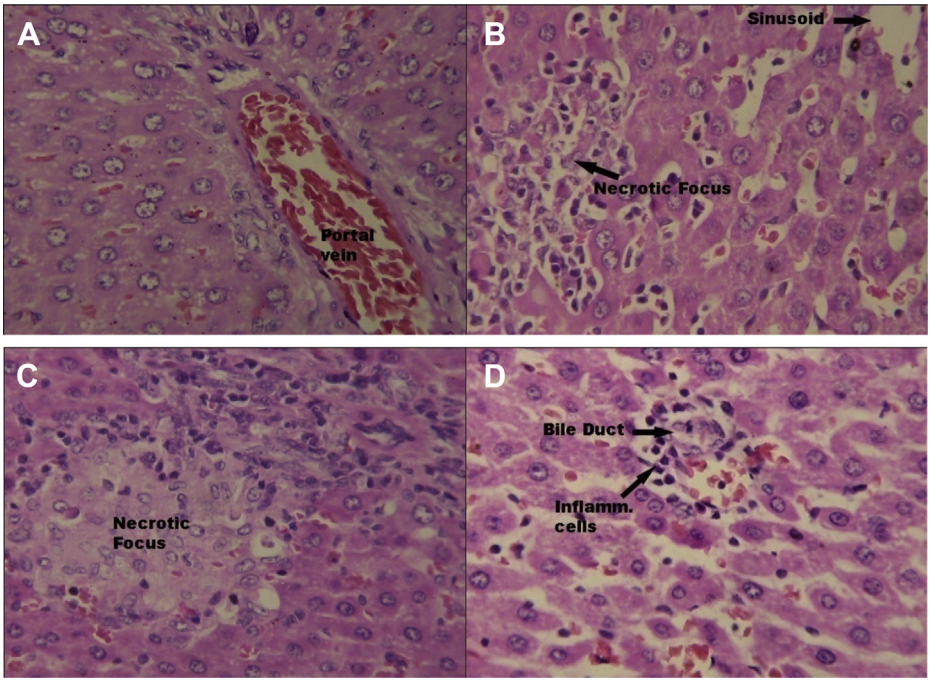

Fig. 2. A: High power photomicrograph of liver from control group animal showing a normal portal tract. A large portal vein is also seen. (H&E x 400). **B**: High power photomicrograph of liver from animal treated with CCl_4 only showing sinusoidal dilatation and a focus of necrosis with inflammatory cell infiltration and haemorrhage. (H&E x 400). **C**: High power photomicrograph of liver from animal treated with CCl_4 and Silymarin showing the necrotic focus in the periportal area. (H&E x 400). **D**: High power photomicrograph of liver from animal treated with CCl_4 and *Triticum aestivum* showing inflammatory cell infiltration and haemorrhage around a bile duct. (H&E x 400).

2.6.9.3. Histopathological results. Histopathological examination of the liver slides of rats of normal control showed normal parenchyma and normal portal tract. Livers of the rats administered only CCl_4 (toxic control) showed moderate inflammation of the portal triad; fatty change and necrosis of the periportal zone and they also showed severe necrosis, sinusoidal dilatation, inflammation, haemorrhage and vascular congestion of the centrizonal area. Rat livers treated with CCl_4 along with Silymarin (100 mg/kg/day) showed almost normal appearance of liver parenchyma. However, a necrotic focus was seen in the periportal area. Animals that had received CCl_4 along with extract of *T. aestivum* (100 mg/kg/day) showed a little fatty change in periportal zone. Centrizonal area showed mild sinusoidal dilatation, moderate inflammation and mild haemorrhage (Fig. 2).

2.7. Discussion

Pharmacognostic standardization including physico-chemical evaluation is meant for identification, authentication, and detection of adulteration and also compilation of quality control of crude drugs. Hot aqueous extract was found to be having highest extractive value. The analysis of ash values suggested the presence of inorganic substances in considerably normal amounts. Heavy metal analysis and microbial load for the drug was in normal values as set down by WHO. The moisture content of the seeds is not too high, thus it could discourage bacteria, fungi or yeast growth, as the general requirement for moisture content in crude drugs is not more than 14% w/w. When physical and chemical methods are inadequate, the plant material may be identified from their adulterants on the basis of fluorescence characteristics. Behaviours of the powdered drug with different chemical reagents and preliminary phytochemical analysis are helpful for detection of various phytoconstituents. In the last two decades HPTLC method has emerged as an important tool for the qualitative and quantitative phytochemical analysis of herbal drugs and formulations. In the present study, CCl_4 given in the dose range of 1 ml/kg b.w. (along with olive oil 1:1) produced a significant rise in AST, ALT, ALP, serum bilirubin, albumin MDA, tissue protein levels and a significant fall in the total protein and GSH levels on exposure to CCl_4, indicating considerable hepatocellular injury (Table 4). The ethanolic extract of *T. aestivum* effectively attenuated the increased levels of AST, ALT, ALP, total bilirubin, albumin and MDA; effectively increased the total protein and GSH levels produced by CCl_4 and caused subsequent recovery towards normalization comparable to the control and the standard group animals.

Conflicts of interest

All authors have none to declare.

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