GMR
The Original

IN VIVO ANTITOXIC ACTIVITY OF SAPONIN ISOLATED FROM *TARAXACUM OFFICINALE* L. AGAINST CCL_4-INDUCED RENAL TOXICITY IN RATS

Mehwish Bibi[1], Dawood Ahmed[2], Nayyab Iftikhar[3], Anuradha Kalansuriya[4], Sehr Syed[5], Bushra Rehman[6], Hina Ali Ahmed[7], Muhammad Siraj[8], Aisha Waheed[9], Waseem Sajjad[10]

[1]Department of Medical Laboratory Technology, Abbottabad University of Science and Technology, Abbottabad, Khyber Pakhtunkhwa, Pakistan:mishikhanmlt@gmail.com
[2]Department of Medical Laboratory Technology, The University of Haripur, Haripur, Khyber Pakhtunkhwa, Pakistan. https://orcid.org/ 0000-0001-7516-0592: biochem_ahmed@yahoo.com
[3]Department of Medical Laboratory Technology, Abbottabad University of Science and Technology, Abbottabad, Khyber Pakhtunkhwa, Pakistan; iftikharnayyab5@gmail.com
[4]Department of Engineering, Technology & Quantity Surveying, INTI International University, Nilai, Negeri Sembilan, Malaysia
Email: i25033404@student.newinti.edu.my: https://orcid.org/0009-0005-9587-8338
[5]Department of Pathology, University College of Medicine and Dentistry, University of Lahore, Lahore, Pakistan.
Sehar.imran@ucd.uol.edu.pk
[6]Institue of pathology and Diagnostic Medicine, Khyber Medical University (KMU) Peshawar, Pakistan; drbushra.ipdm@gmail.com
[7] Department of Zoology, Sardar Bahadur Khan Women University, Quetta, Pakistan; hina29zoo@yahoo.com
[8]Department of Zoology, Abbottabad University of Science and Technology, Abbottabad, Khyber Pakhtunkhwa, Pakistan: aminasardar476@gmail.com
[9]Department of Zoology, Abbottabad University of Science and Technology, Abbottabad, Khyber Pakhtunkhwa, Pakistan; muhammadsirajuop@gmail.com
[10]Department of Microbiology, Abbottabad University of Science and Technology, Abbottabad, Khyber Pakhtunkhwa, Pakistan; waseemsajjad303@gmail.com
*Corresponding Author: Dawood Ahmed; biochem_ahmed@yahoo.com

ABSTRACT
Carbon tetrachloride has been used in many industry as an industrial solvent and drinking water. Its contamination is biggest human health concern. Ithigh concentration has many deleterious effects on human physiology and histology, especially renal and hepatotoxicity. To overcome such pathophysiological and histopathological effects a great supporthas been reported on bioactive compound of natural products for which one of the major source of natural products are plants. One such plant that has phytochemicals and tested as an antioxidant and anti-bacterial agentin many parts of the world is *Taraxacum officinale L.*(dandelion).It is sometime consider as weed presnt in gardens and roadsides however it has been consumed as food and herbal medicine too. This study was designed to find out the antitoxic activity of saponin extracted from *Taraxacum officinale*on renal tissue toxicity induced by carbon tetrachloridein rat model.Plants for this study was collected locally and after taxonomical identification was processed for extraction. Leaf methanolic extracts were exposed to spectrophotometer to estimate the phytochemicals. Later on this extract was processed for separation of phytochemicals (flavonoids, tannins, phlorotannin and saponin) through HPLC. Experimental animals were divided into ten groups having twelve rats in each group: Control group(normal food & water), dose of DMSO, CCl4, rutin CCl4 and from Group V to group IX different fractions of saponin that is rangingfrom 5mg/kg to 25mg/kg body weight dissolved in DMSO were given orally weekly for ten weeks after 48 hours of CCl4 administration. Group X was given only extract of saponin 25 mg/kg body weight mixed in DMSO orally. Different fractions of the saponin were used to check anti-toxic effect of saponin. At end of trial period rats were killed for investigate histological and biochemical parameters. In a group of rats treated with CCl4 versus control, tubular deterioration, tubular dilatation, glomerular degeneration, inflammatory cell infiltration, and capillary blockage were observed, as well as tubular deterioration, tubular dilatation, glomerular degeneration, inflammatory cell infiltration, capillary blockage and cortex and medulla of kidneys were observed.Saponin's protective effect was further demonstrated by decreased histological changes in the kidneys.
Biochemical study also reflects the protective effects of saponin as GFR reduced due to increasedurea & creatinine level in CCl4 treated group. However biochemical parameters significantly reduced tonormal ingroup treated with saponin extract.Our finding suggests that saponin separated from *Taraxacum officinale.* methanolic extract possesses renal protective and antioxidant effects against CCl4-induced renal toxicity.*Taraxacum officinale* could be employed as a protective and supportive treatment for CCL4-induced kidney damage, and the leaves could be useful for drug development in the pharmaceutical industry.

KEYWORDS: Carbon tetrachloride (CCl_4); Renal toxicity; Nephrotoxicity; Taraxacum officinale; Dandelion; Saponin; Methanolic extract; Phytochemicals; Antioxidant activity; Renal protection; Hepatotoxicity; Histopathology; Biochemical parameters; Urea; Creatinine; Glomerular filtration rate (GFR); Kidney damage; Rat model; Natural products; Herbal medicine; HPLC; Phytochemical extraction.

INTRODUCTION

Medicinal plants are an important source of bioactive compounds with potential applications in the prevention and treatment of various diseases. These plants contain a wide range of primary and secondary metabolites, including alkaloids, phenolic compounds, flavonoids, terpenoids, tannins, glycosides, and saponins, many of which possess pharmacological properties and may serve as valuable sources for drug discovery and development (Ahn, 2017; Altemimi et al., 2017). The therapeutic potential of medicinal plants is largely attributed to their diverse phytochemical constituents, which have been associated with antioxidant, antimicrobial, anti-inflammatory, hepatoprotective, and other biological activities (Lichterman, 2004; Leite et al., 2021).

Among plant-derived bioactive compounds, saponins are important secondary metabolites that are structurally characterized as glycosides of steroidal or triterpenoid aglycones. Their amphiphilic nature, resulting from the presence of both hydrophilic sugar moieties and lipophilic aglycones, contributes to their diverse biological activities (Al-Yahya et al., 2013). Saponins have been reported to possess antimicrobial, antioxidant, immunomodulatory, anti-inflammatory, and protective properties, although their biological effects may vary depending on their chemical structure and concentration (Jou et al., 2011; Hu et al., 2020).

Taraxacum officinale L., commonly known as dandelion, belongs to the family Asteraceae and is widely distributed throughout the world, including Pakistan. The plant has traditionally been used as a food source and herbal medicine, and its leaves, flowers, stems, and roots have been associated with various therapeutic applications (Martinez et al., 2015; Quazi Majaz et al., 2011). Traditionally, *T. officinale* has been used for the management of several ailments, including digestive disorders, urinary tract problems, fever, sores, and throat infections. Pharmacological investigations have further demonstrated antioxidant, anti-inflammatory, hepatoprotective, antitumor, and other biological activities of extracts obtained from different parts of the plant (Krishna et al., 2017).

The biological activity of *T. officinale* is attributed to its diverse phytochemical composition. Flavonoids and other phenolic compounds are particularly important because of their ability to scavenge reactive oxygen species (ROS) and reduce oxidative damage. Tannins, terpenoids, and other secondary metabolites may also contribute to the antioxidant and protective effects of the plant (Panche et al., 2016; Pichersky and Raguso, 2018; Fraga-Corral et al., 2021).

Previous studies have demonstrated that extracts of *T. officinale* can protect tissues against chemically induced oxidative injury, including carbon tetrachloride (CCl_4)-induced damage in experimental animals (Gulfraz et al., 2014; Karakuş et al., 2017).

Carbon tetrachloride is a well-established experimental toxicant that induces oxidative stress and tissue damage through its biotransformation into highly reactive free radicals. Following metabolic activation, CCl_4 generates the trichloromethyl radical (CCl_3•), which can subsequently react with oxygen to form the more reactive trichloromethylperoxyl radical (CCl_3O_2•). These reactive metabolites initiate lipid peroxidation and promote oxidative damage to cellular proteins, membranes, nucleic acids, and other cellular components (Dai et al., 2014; Pizzino et al., 2017).

Although the liver is considered the primary target organ of CCl_4 toxicity, exposure can also result in injury to several extrahepatic organs, including the kidneys, heart, lungs, brain, and reproductive tissues. CCl_4-induced oxidative stress is associated with the excessive generation of ROS and impairment of endogenous antioxidant defense systems, including superoxide dismutase, catalase, glutathione, and glutathione-S-transferase (Dai et al., 2014; Hassan et al., 2007). The toxic effects of CCl_4 may therefore result in cellular degeneration, inflammation, lipid peroxidation, and disruption of normal tissue function.

The kidneys are particularly vulnerable to toxic injury because of their high blood flow and their role in the filtration, concentration, and excretion of xenobiotics and their metabolites. The nephron is the functional unit of the kidney and performs filtration, reabsorption, secretion, and excretion. Consequently, toxic substances and their metabolites may accumulate within renal tissues and cause structural and functional damage (Ganie et al., 2011). CCl_4 exposure has been associated with renal dysfunction and histopathological alterations, including tubular degeneration, tubular dilation, glomerular damage, inflammatory cell infiltration, and vascular abnormalities (Ganie et al., 2011).

Oxidative stress plays a central role in CCl_4-induced tissue injury. ROS, including superoxide radicals, hydrogen peroxide, and hydroxyl radicals, are generated during normal cellular metabolism; however, excessive ROS production can overwhelm the antioxidant defense system and lead to oxidative stress. This imbalance can cause lipid peroxidation, protein oxidation, DNA damage, cellular dysfunction, and ultimately tissue injury (Hasanuzzaman et al., 2020; Das and Roychoudhury, 2014). Therefore, natural products containing antioxidant phytochemicals may provide a potential strategy for reducing chemically induced oxidative damage.

Previous studies have reported that *T. officinale* extracts exert protective effects against CCl_4-induced oxidative injury in the liver and kidneys. Ethanolic and other solvent extracts of *T. officinale* have been shown to reduce oxidative stress markers and histopathological alterations in CCl_4-treated experimental animals (Gulfraz et al., 2014; Karakuş et al., 2017). However, limited information is available regarding the specific protective effects of the isolated saponin fraction of *T. officinale* against CCl_4-induced renal toxicity.

Therefore, the present study was designed to investigate the nephroprotective and antioxidant potential of saponin

extracted from *Taraxacum officinale* L. against CCl_4-induced renal toxicity in rats. The study evaluated the effects of different doses of *T. officinale* saponin on renal biochemical parameters, particularly serum urea and creatinine levels, as well as histopathological alterations in kidney tissues. The findings of this study may provide further evidence regarding the potential use of *T. officinale*-derived saponins as natural protective agents against chemically induced renal injury.

MATERIALS AND METHODS

Collection and authentication of plant material

Leaves of *Taraxacum officinale* L. were collected from the surrounding areas of Haripur and Abbottabad, Khyber Pakhtunkhwa, Pakistan, during March 2020. The plant material was taxonomically authenticated by a taxonomist from the Botany Section of Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan, and a voucher specimen was deposited under voucher number 214. The collected plant material was washed thoroughly with tap water followed by distilled water to remove adhering dust and surface contaminants. The leaves were then shade-dried at room temperature for approximately two weeks to minimize the degradation of light- and heat-sensitive phytochemicals (Sagnia et al., 2014).

Preparation of plant material for extraction

The dried leaves were uniformly ground into powder before extraction. Healthy and disease-free plant material was selected for the study. The powdered material was prepared to obtain a relatively uniform particle size to facilitate efficient solvent penetration and extraction of bioactive constituents. Shade drying was used to minimize the potential degradation of thermolabile and photosensitive phytochemicals (Kumar, 2010; Ncube et al., 2008).

Preparation of methanolic extract

For the preparation of the crude methanolic extract, 10 g of powdered *T. officinale* leaves was transferred into a conical flask and mixed with 100 mL of methanol. The flask was plugged with cotton and the mixture was continuously stirred at room temperature for 24 h. Following extraction, the mixture was filtered, and the solvent was evaporated to obtain the crude extract. The resulting extract was stored in airtight containers under refrigerated conditions until further analysis (Aboaba et al., 2006).

Qualitative phytochemical screening

The methanolic extract was subjected to qualitative phytochemical screening for the detection of major secondary metabolites, including saponins, flavonoids, phlorotannins, steroids, glycosides, and terpenoids. The screening was performed using standard phytochemical procedures, with methanol, acetone, chloroform, and distilled water used as extraction solvents where appropriate (Roopashree et al., 2008; Kasolo et al., 2010).

The presence of saponins was assessed by vigorously shaking the plant extract with distilled water and observing the formation of stable foam or emulsion. Flavonoids were detected by the development of a yellow coloration following treatment with ammonia and concentrated sulfuric acid. Phlorotannins were assessed following acid treatment and observation of the characteristic red precipitate. Steroids were detected using acetic anhydride and sulfuric acid, while glycosides were assessed using glacial acetic acid, ferric chloride, and concentrated sulfuric acid. Terpenoids were detected by the formation of a reddish-brown coloration at the interface following treatment with chloroform and concentrated sulfuric acid.

Quantitative determination of phytochemicals

The quantitative estimation of selected phytochemicals in the leaves of *T. officinale* was performed using standard analytical procedures. Total phenolic content was determined using the Folin–Ciocalteu colorimetric method. Briefly, diluted plant extract was mixed with Folin–Ciocalteu reagent and sodium carbonate solution and incubated in the dark. Absorbance was measured spectrophotometrically at 700 nm, and the total phenolic content was calculated using a calibration curve prepared with gallic acid as the standard (Harnafi and Armani, 2008; Mir et al., 2013).

Total phlorotannin content was determined using a modified Folin–Ciocalteu method, with phloroglucinol used as the reference standard. The diluted extract was mixed with Folin–Ciocalteu reagent and sodium carbonate and incubated under controlled conditions. Absorbance was measured at 730 nm, and the results were expressed relative to the standard calibration curve (Samee et al., 2009; Kamiya et al., 2010).

Total tannin content was determined using a standard colorimetric method, and the absorbance values were compared with a calibration curve prepared using tannic acid as the standard. The total saponin content was determined in the methanolic plant extract using a standard quantitative procedure. Total flavonoid content was estimated based on the formation of a flavonoid–aluminum complex, with quercetin used as the reference standard and absorbance measured at 450 nm.

Thin-layer chromatographic analysis

Thin-layer chromatography (TLC) was performed to characterize the phytochemical constituents of the *T. officinale* leaf extract. Approximately 80 mg of the extract was dissolved in 3 mL of HPLC-grade methanol. Selected reference standards, including vitexin, myricetin, orientin, catechin, hyperoside, quercitrin, rutin, luteolin, and quercetin, were

prepared in methanol. Approximately 8–12 µL of each extract and standard solution was applied to the TLC plate using a capillary tube. The plate was developed in a mobile phase consisting of water, butanol, and propanol at a ratio of 2:2:1. The chromatographic spots were visualized under ultraviolet light at 400 nm. The retention factor (Rf) values were calculated as the ratio of the distance travelled by the sample to the distance travelled by the solvent front (Cieśla and Waksmundzka-Hajnos, 2009).

High-performance liquid chromatographic analysis

High-performance liquid chromatography (HPLC) was used for the comparative analysis of the phytochemical constituents of the *T. officinale* extract. The extract was diluted with methanol and prepared for chromatographic analysis according to the standard procedure. Reference compounds, including orientin, hyperoside, vitexin, catechin, myricetin, and rutin, were analyzed for comparative identification of phytochemical constituents (Cieśla and Waksmundzka-Hajnos, 2009).

Preparation of plant extract for animal experimentation

For the preparation of the extract used in the animal trial, powdered *T. officinale* plant material was soaked in acetone for seven days with intermittent shaking. Approximately 750 g of powdered material was extracted with 2.5 L of acetone. After seven days, the mixture was filtered through fine filter paper, and the filtrate was allowed to evaporate under a fume hood. The resulting viscous extract was stored under refrigerated conditions until use. Before administration, the extract was dissolved in dimethyl sulfoxide (DMSO) according to the required treatment dose.

Experimental animals

A total of 120 healthy male albino rats weighing approximately 200 g were obtained from the National Institute of Health, Islamabad, Pakistan. The animals were acclimatized for two weeks under standard laboratory conditions before the initiation of the experiment. The rats were housed in steel cages, with six animals per cage, and were provided standard feed and water. Only healthy male albino rats were included in the study, whereas unhealthy and female rats were excluded.

Experimental design

The animals were randomly divided into ten groups, with 12 rats in each group. The experimental period lasted 18 weeks. The groups were treated as follows:

Group I (Control): Animals received normal feed and water throughout the experimental period.

Group II (Vehicle control): Animals received DMSO orally and olive oil intraperitoneally according to the experimental protocol.

Group III (CCl_4-treated group): Animals received CCl_4 dissolved in olive oil by intraperitoneal injection to induce toxicity.

Group IV (Positive control): Animals received CCl_4 followed by rutin at a dose of 5 mg/kg body weight, administered in DMSO after 48 h.

Group V: Animals received CCl_4 followed by saponin at a dose of 5 mg/kg body weight in DMSO, administered orally after 48 h.

Group VI: Animals received CCl_4 followed by saponin at a dose of 10 mg/kg body weight in DMSO, administered orally after 48 h.

Group VII: Animals received saponin at a dose of 15 mg/kg body weight dissolved in DMSO.

Group VIII: Animals received CCl_4 followed by saponin at a dose of 20 mg/kg body weight in DMSO, administered orally after 48 h.

Group IX: Animals received CCl_4 followed by saponin at a dose of 25 mg/kg body weight in DMSO, administered orally after 48 h.

Group X: Animals received saponin alone at a dose of 25 mg/kg body weight dissolved in DMSO.

The saponin treatments were administered orally once weekly throughout the experimental period. The treatment groups receiving CCl_4 were administered saponin after a 48-h interval following CCl_4 exposure.

Induction of CCl_4-induced toxicity

CCl_4 was administered intraperitoneally after dilution with olive oil to induce oxidative toxicity in the experimental animals. The administration of CCl_4 was followed by treatment with different doses of *T. officinale*-derived saponin after 48 h. The experimental design was used to evaluate the dose-dependent protective effects of saponin against CCl_4-induced renal injury.

Biochemica analysis of renal function

At the end of the experimental period, blood samples were collected for biochemical analysis. The samples were centrifuged at 3000 rpm for 5 min at room temperature to separate the serum. Serum urea and creatinine levels were determined using commercially available diagnostic kits and an automated biochemical analyzer (Cobas Integra 800; Roche/Hitachi, Basel, Switzerland) according to the manufacturer's instructions (Cray et al., 2009).

Collection and processing of kidney tissues
Following completion of the experimental period, the animals were anesthetized and euthanized. Blood samples were collected by cardiac puncture for biochemical analysis. The kidneys were carefully removed, weighed individually, and washed with ice-cold saline. Tissue samples were subsequently fixed for histopathological examination.

Histopathological examination
Kidney tissues were fixed in 4% paraformaldehyde and processed using standard histological procedures. Following fixation, the tissues were dehydrated through ascending concentrations of ethanol and embedded in paraffin wax. Paraffin blocks were sectioned using a microtome to obtain sections approximately 3–4 μm thick. The sections were stained with hematoxylin and eosin for routine morphological examination, while Masson's trichrome staining was used for the evaluation of connective tissue. Histological sections were examined under a light microscope for pathological alterations, including tubular degeneration, tubular dilation, glomerular degeneration, inflammatory cell infiltration, vascular abnormalities, and other structural changes associated with CCl_4-induced renal injury (Suzuki et al., 2015; Gyurászová et al., 2020

RESULTS AND DISCUSSIONS
The plants were collected from several locations in Haripur district between July and September 2020 and sent to the University of Haripur's microbiology department, where they were double washed. Plants were initially washed with regular tap water to remove dust and other contaminants before being treated with distilled water to remove various bacteria present on the plant surface.
Different parts of plants i.e. stem, roots, leaves and flowers of *T. officinale*were shade dried during period of 15-20 days, after that plants were grinded by mechanical grinder and powder was filled in the porous thimble present inside the extraction chamber of soxhlets extractor behr Labor- Technik.Germany-2013. Solvent used for extraction were taken into the round bottom flask and then heating mantle as a source of heat flask was heated by provided temperature according to the boiling point of the solvent used. When solvent heated it evaporated to the condenser where it was cooled down and fall drop by drop into the thimble filled with plant powder and filtered out from thimble and dropped back into round bottom flask by siphon arm when the extraction chamber was filled. The cyclic process was continued until final product was obtained. Extract was collected in china dish from the flask with the help of sterile wire loop. Further for complete drying extracts were processed in freeze drier at temperature of - 60 to -65^{o}C for 24 hours.The goal of this study was to evaluate the effect of saponin on kidney damage caused by CCl4 in rats using histological and biochemical measures.

PROXIMATE Analysis of *T. officinale*
Table 1 shows the findings of proximate analysis of T. officinale leaves. All plant samples were found to have large amounts of total protein, crude oil, ash, and crude fiber content.

Table 1: Proximate analysis (%) of leaves extract of Taraxacum officinale.

Sample	**Dry matter %**	**Moisture %**	**Crude protein %**	**Crude oil %**	**Crude fiber %**	**Ash %**
Traxacum Officinale	**85± 1.2**	**89± 0.6**	**8.7±0.9**	**1.9± 0.1**	**34.6±0.4**	**11±0.5**

Samples were analyzed to find mean±SE

Qualitative estimation of phytochemical *Taraxacum Officinale L*
The preliminary screening of phytochemicals from leaf samples of T. Officinalis revealed the presence of methanolic extract, Acetone extract, chloroform extract, distilled water extract. Results indicate that proportion of saponin, flavonoids terpenoid, steroid and phlorotanninfound in the extract was relatively higher than the other phytochemicals (Table 1). Our results are in line with other studies in which they showed low level of these phytochemicals(Palipoch, 2013).

Table 2: Qualitative estimation of phytochemical (%) from T. officinale leave

Sample	**Methanolic Extract**	**Acetone Extract**	**Chloroform Extract**	**Dist. Water Extract**
Saponins	+	+	++	+
Flavonoid	+	+++	+++	+++

Steroid	++	+	++	+++
Phlorotannin	+++	+++	+++	+++
Terpenoid	-	++	+	+

+++, High Concentrated; ++, Moderate Concentrate; +, Low Concentrate; - Absent.

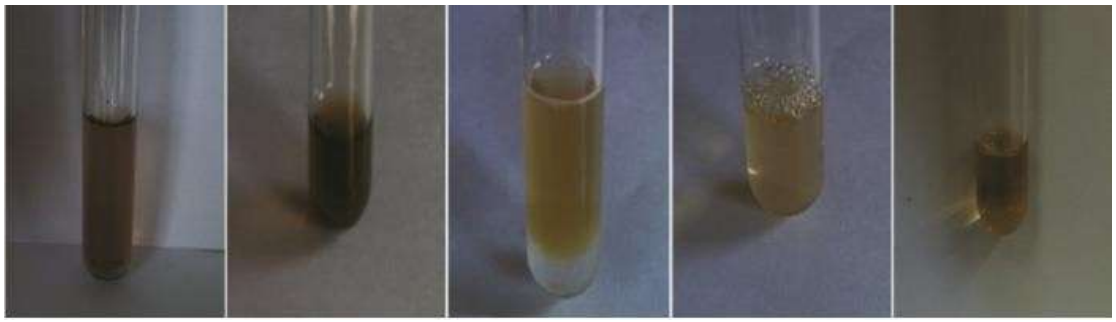

Figure 2: screening of phytochemicals in Methanolic extract which shows results for terepnoid, steroid, flavonoid, saponin &pholorotanin from left to right respectively.

4.3 Quantitative Analysis of phytochemical *Taraxacum officinile* L Leaf extract of *T. officinale* in methanol and chloroform shows higher saponin content while moderate content of saponin were detected in leaf extract of *T. officinale* in ethyl-acetate and hexane and in stem extracts of *T. officinale* in methanol and chloroform, also very low saponin content was observe in the seeds extracts of *T. officinale* in all the four solvents. Roots extract of *T. officinale* in all the four solvents shows no saponin content. The leaf extracts of in methanol, chloroform and ethyl-acetate, stem extracts in hexane and ethyl-acetate shows very high content of saponin while the leaf extract in hexane,
stem extracts in methanol and chloroform shows moderate content of saponin. Flower extracts of all the four solvents and root extract of methanol and chloroform having very low saponin content**.** The leaf extracts extractsshows moderate saponin content. While the root extracts in methanol, chloroform and ethyl-acetate shows low saponin content. Shows presence of saponin in leaf of *T. officinale* in methanolConstituents bestow high medicinal activities (including antioxidant and antiallergic). Coumaric acid derivatives and saponin were recognized from dandelion flower (2006) (Schütz et al., 2006).

Table 3: Quantitative estimation of phytochemical (%) from *T. officinale* leave

Plants	**Flavonoids**	**Phenols**	**Saponin**	**Tannin**	**Phlorotannin**
T. Officinale	11.2±0.5	10.3±0.2	0.3±0.1	0.7±0.1	0.4±0.1

Table 4: Protective role of different fraction of *T. officinale* on the body weight

Group	**Treatments**	**Body mass before treatment (g)**	**Body mass after treatment (g)**	**Increased body Mass (%)**
1	Control	267.60 ± 3.94	374.90 ± 2.86	16.69.72 ± 1.84
2	DMSO + Olive oil	268.90 ± 2.73	372.20 ± 2.54	16.25 ± 1.66
3	3 mL/kg CCl4	271.70 ± 2.26	338.00 ± 2.50	11.00 ± 1.35
4	5 mg/kg Rutin+ CCl4	267.40 ± 3.41	374.00 ± 2.70	16.39 ± 2.31

5	5 mg/kg saponins+ CCl4	266.80 ± 2.45	347.70 + 2.86	13.21 ± 1.34
6	10mg/kg saponins+ Cl4	264.20 ± 1.92	354.10 ± 3.60	14.10 ± 1.84
7	15 mg/kg saponins+ CCl4	265.10 ± 3.45	348.65 + 2.56	13.35 ± 1.19
8	20mg/kg saponins+ CCl4	266.50 ± 2.72	374.50 ± 2.80	16.65 ± 1.71
9	25 mg/kg saponins+ CCl4	265.85 ± 2.51	376.53 ± 2.62	17.10 ± 1.57
10	Saponins alone	264.80 ± 2.1	355.10 ± 3.40	14.17 ± 1.52

According to Amim*et al.,* (2013) carbon tetrachloride is a colorless and evaporated compound that can enter into the body through inhalation, digestion and penetration of skin. It is used in in vivo researches due to its toxic effect. He stated that toxic effect of ccl4 canharm body tissues and reduced body weight by developing of free radicals. Our study also reflects the similarity that CCl4 group displayed significant decline in weight of ccl4 treated rats due to tumor formation. Adewole*et al., (*2007) conducted a study in the rats in which they induced toxicity using ccl4 and administered melatonin to the rats. They examined reduction in body weight of experimental animals, however they determined an increase in body weight in the groups directedwith melatonin. From here they stated that this effect was resulted from the melatonin which has an antioxidant feature. As according to our study the body weight showed significant increase during the treatment of different fractions of saponin against ccl4 which is the proof of antitoxic effect of saponin extracted from *T.Officinale(*Amin *et al.,* 2013).

Table 5: Biochemical parameters of renal system (RFTs)

Group	Treatments	Urea (µM /mL)	Total Creatinine (mg /dl)	Creatinine clearance test (mL /min)
1	Control	39.01±1.16	38.1±0.87	1.87±0.02
2	DMSO + Olive oil	45.60±1.04	35.1±1.80	1.84±0.03
3	3 mL/kg CCl4	80.32±1.23	81.0±2.02	0.93±0.07
4	5 mg/kg Rutin+ CCl4	43.01±1.71	52.5±2.01	1.63±0.04
5	5 mg/kg saponins+ CCl4	62.43±1.83	56.0±1.76	1.41±0.04
6	10mg/kg saponins+ CCl4	55.26±1.34	52.5±1.02	1.5±0.02
7	15 mg/kg saponins+ CCl4	53.29±1.4	46.5±1.06	1.64±0.05
8	20mg/kg saponins+ CCl4	47.19±1.05	44.0±1.03	1.82±0.06
9	25 mg/kg saponins+ CCl4	45.12±1.03	41.7±2.42	2.129±0.05
10	25mg Saponins alone	42.19±1.01	44.5±1.24	2.21±0.01

Results of biochemical analysis showed the urea & creatinine level was increased in ccl4 treated group while these level becomes normal in group animals treated

withsaponin.According to study liver and kidney toxicity using CCl4 due to which serum ALP, ALT, AST, levels of urea, uric acid and creatinine, increased expressivelyand due to which they stated that this was due to liver and kidney histopathology and the impairment of their functions(Syed et al., 2014).

4.4 Effect of Saponins on histological changes of Renal tissue

Table 6: Effect of Saponins on histological changes of renal tissue

Group	Treatment	Dilatation of Tubules	Epithelial Necrosis's	Capillary Congestion	Edema in Interstitial Tissue
1	Control	–	–	–	–
2	Vehicle	–	–	–	–
3	3 mL/kg CCl4	++	++	++	++
4	5mg/kg Rutin+ CCl4	–	–	–	–
5	20mg/kg saponin+ CCl4	+	+	–	+
6	25mg/kg saponin+ CCl4	–	–	–	–
7	25 mg/ kg saponin alone	–	–	–	–
8	20mg/kg saponins+ CCl4	--	+	--	--
9	25 mg/kg saponins+ CCl4	+	+	+	+
10	Saponins alone	+	+	+	+

(-) shows no change in histology; (+) show moderate change (++) show highly effected histology.

Figure7: Effect of saponin on histology of glomerular tubule&bowman capsule.

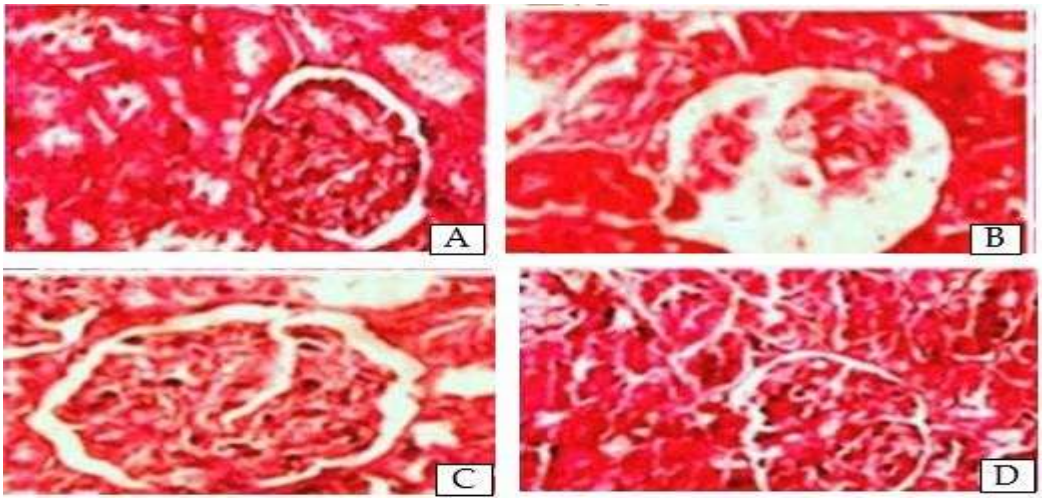

Figure 7: (A)Control; (B)CCl4 (C) Rutin D) Saponin treated

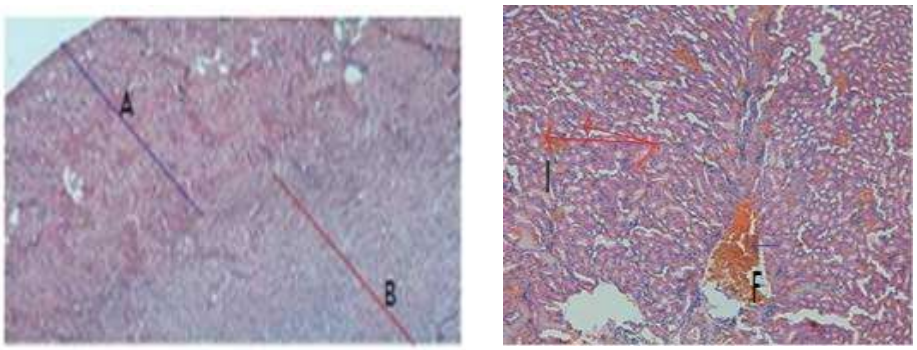

Figure 8:Effect of CCl4 on histology of Renal tissues.

Normal histology of kidneydescribed by blue line marked A which indicates the renal cortex and red line marked B shows the renal medulla while histopathology of kidney described by red arrow marked I showed mild interstitial congestion and blue arrow marked F showed focal interstitial hemorrhage which indicate mild tissue damage. A study reported that by treating experimental rats with CCl4, level of Na, K, Ca, and kidney tissue MDA levels intensely increased, tubular occlusion occurred andepithelium disseminated on and tubules lumen were observed because

REFERENCES

1. **Aboaba, O.O., Smith, S.I., Olude, F.O. (2006).** Antibacterial effect of edible plant extract on *Escherichia coli* O157:H7. *Pakistan Journal of Nutrition*, 5(4), 325–327.

2. **Altemimi, A., Lakhssassi, N., Baharlouei, A., Watson, D.G., Lightfoot, D.A. (2017).**
of structural and functional impairment of liver and kidney. They examined that these changestreated with honey and saponin were decreased, that showed antioxidant properties.As shown in our results, tubular degradation, tubular dilatation, glomerular degeneration, inflammatory cell infiltration, and capillary blockage occur in rats treated with CCl4 compared to controls.While in comparison to CCl4, saponin and positive control drug recover the normal histology of renal tissues(Al-Yahya et al., 2013)**.** Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts. *Plants*, **6**(4), 42. https://doi.org/10.3390/plants6040 042.

3. **Das, K., Roychoudhury, A. (2014).** Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants. *Frontiers in Environmental Science*, **2**,53.

4. https://doi.org/10.3389/fenvs.2014. 00053.

5. **Fraga-Corral, M., Otero, P., Cassani, L., et al. (2021).** Traditional applications of tannin rich extracts supported by scientific data: Chemical composition, bioavailability and bioaccessibility. *Foods*, **10**(2), 251.
https://doi.org/10.3390/foods10020 251.

6. **Ganie, S.A., Haq, E., Hamid, A., Qurishi, Y., Mahmood, M., Zargar, M.I., Masood, A., Zargar, B.A., and Ahmad, B. (2011).** Carbon tetrachloride induced kidney and lung tissue damages and antioxidant activities of the aqueous rhizome extract of *Podophyllum hexandrum*. *BMC Complementary and Alternative Medicine*, **11**, 17. https://doi.org/10.1186/1472-6882-11-17.

7. **Gulfraz, M., Ahmad, D., Ahmad, M.S., Qureshi, R., Mahmood, R.T., Jabeen, N., and Abbasi, K.S. (2014).** Effect of leaf extracts of *Taraxacum officinale* on CCl_4- induced hepatotoxicity in rats, in vivo study. *Pakistan Journal of Pharmaceutical Sciences*, **27**(4),
825–829.

8. **Hasanuzzaman, M., Bhuyan, M.H.M.B., Zulfiqar, F., Raza, A.,** Mohsin, S.M., Mahmud, J.A., Fujita, M., and Fotopoulos, V. (2020). Reactive oxygen species and antioxidant defense in plants under abiotic stress: Revisiting the crucial role of a universal defense regulator. *Antioxidants*, 9(8), 681.
https://doi.org/10.3390/antiox9080 681.

9. **Panche, A.N., Diwan, A.D., and Chandra, S.R. (2016).** Flavonoids: An overview. *Journal of Nutritional Science*, **5**, e47. https://doi.org/10.1017/jns.2016.41.

10. **Pichersky, E., and Raguso, R.A. (2018).** Why do they make so many essential oils? *Nature Plants*, **4**, 243–249.

11. **Pizzino, G., Irrera, N., Cucinotta, M., Pallio, G., Mannino, F., Arcoraci, V., Squadrito, F., Altavilla, D., and Bitto, A. (2017).** Oxidative stress: Harms and benefits for human health. *Oxidative Medicine and Cellular Longevity*, **2017**, Article ID 8416763. https://doi.org/10.1155/2017/84167 63.