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In Vivo Study on the Role of *Taraxacum Officinale* in Modulating Paracetamol-Induced Hepatotoxicity

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Annotation: Taraxacum officinale is widely used to address various illnesses. This study aimed to explore its key phytochemical components and evaluate the impacts of its methanolic extract on paracetamol-induced hepatotoxicity in mice, in addition to conducting histological analysis of liver tissue. Phytochemical screening revealed that the plant contains varying amounts of flavonoids, tannins, saponins, alkaloids, and polyphenols, relying on the solvent used, with the methanolic extract exhibiting the maximum concentration of total flavonoids (210.2 ± 25.7 mg/g). Biochemical analysis and histological examination of liver sections in the positive control group (mice given paracetamol) showed that paracetamol induces hepatotoxicity, as evidenced by increased blood levels of AST, ALT, ALP, and MDA and reduced catalase levels. Liver sections also revealed zonal acute cellular swelling of hepatocytes, perivascular infiltration of lymphocytes and macrophages, and necrotic hepatocytes. On the other hand, these markers were reduced

in mice that dealt with the plant extract. Hence, it can be stated that Taraxacum officinale demonstrates hepatoprotective activity against paracetamol-induced hepatotoxicity and may be a promising therapeutic option for drug-induced liver damage.

Keywords: Taraxacum officinale; liver markers; hepatotoxicity; paracetamol toxicity; phytochemicals.

1- Introduction

Conventional medical systems are often considering the mind-body-physiology connection as a holistic approach to treating illness. For instance, the Ayurvedic philosophy suggests the use of groups of phytochemical compounds that may target multiple infection sites [1]. Natural products have been integral to healthcare and disease prevention for a long time, as they reduce the high costs of commercial antibiotics and the growing prevalence of multidrug-resistant microorganisms [2]. *Taraxacum officinale* is frequently known as dandelion; it is a perennial plant in the Asteraceae family. This widespread plant can be found in gardens, pastures, agricultural fields, and wastelands throughout Europe, Asia, and North America [3].

The leaves of *Taraxacum officinale* contain high levels of fiber, vitamins, minerals, and essential fatty acids. The plant's medicinal benefits are attributed to the phytochemicals present in various parts of it. Fruits and vegetables, like *T. officinale*, have an important role in human nutrition because of their health-promoting and anti-disease properties. These properties include, but are not limited to, antioxidant, anticancer, and hepatoprotective effects [4]. Acetaminophen (known as paracetamol or APAP) is an antipyretic and mild to moderate pain reliever that is widely available over the counter and considered safe when taken at recommended doses. However, excessive intake of paracetamol is known to have hepatotoxic effects in both humans and animals [5].

The current study examines the hepatoprotective effects of Taraxacum officinale in mice after paracetamol-induced liver toxicity. It analyzes tannins, alkaloids, saponins, flavonoids, and polyphenols to identify the active compounds in T. officinale that contribute to its medicinal properties. It also evaluates biochemical markers using a methanolic extract.

2- Materials and Methods

2-1 Plant material

The Iraqi Center of Herbs identified the plant; in December 2022, the aerial parts of *Taraxacum officinale* were obtained from the local market in Baghdad.

2-2 Preparation of plant extract

2-2-1 Methanolic extraction

20 g of the plant’s aerial parts were extracted using Soxhlet extraction equipment for 6 h at 55°C with 250 ml of 70% methanol. After filtration and drying with a rotary evaporator, the extract was kept at 4°C until needed [6].

2-2-2 Determination of Total Flavonoids

With minor adjustments, total flavonoid content was estimated utilizing the $AlCl_3$ colorimetric method, as detailed by Sakanaka et al. [7].

2-3 Evaluation of the plant's active phytochemicals

The following procedures were followed for the qualitative phytochemical analyses of *Taraxacum officinale* seeds [8].

2-3-1 Detection of Tannins

Each extract was diluted with a 1% lead acetate solution. A gelatinous or white precipitate indicates the presence of tannins.

2-3-2 Detection of Glycosides

2 ml of Benedict's reagent was mixed with one milliliter of each extract, and the mixture was heated to a boil for 5 min before cooling. The formation of a red deposit indicated the presence of polysaccharides.

2-3-3 Detection of alkaloids (Dragangroff test)

Solution A contains 60 mg of bismuth subnitrate dissolved in 0.2 ml of HCl, while Solution B consists of 600 mg of potassium iodide (KI) diluted in 1 ml of distilled water. The mixture of A and B solutions produced an orange-brown tint in the extract, which indicates the existence of alkaloids.

2-3-4 Detection of Saponins

The plant extract solutions were extensively agitated as part of the detection procedure. The generation of foam at the top of the extract indicates the existence of saponins.

2-3-5 Detection of Flavonoids

In solution A, 60 mg of bismuth subnitrate is dissolved in 0.2 ml of hydrochloric acid, while Solution B contains 600 mg of potassium iodide (KI) diluted in 1 ml of distilled water. When Solutions A and B were combined with the plant extract, the existence of alkaloids was indicated by the formation of an orange-brown color.

2-3-6 Detection of Polyphenolic Compounds

Each extract solution received a few drops of a 3% ferric chloride ($FeCl_3$) solution. The formation of a brown precipitate following the reaction indicates the presence of polyphenolic compounds.

2-4 *In vivo* assay

Acute poisoning was induced in thirty mature male Swiss albino mice (body weight 22–28 g), which were obtained from the General Company of Veterinary Medicine in Baghdad. The mice were housed in a well-ventilated chamber and fed a specially formulated diet of water and pellets. Each dose was administered intraperitoneally as a single 0.1 ml dose over 15 days. The animals were then euthanized for laboratory testing on day sixteen. Four categories of animals were established based on the following criteria:

Group 1 (-ve control)	Six mice were treated with D.W.
Group 2	Six mice were treated with *Taraxacum officinale* methanolic extract at 300 mg/kg
Group 3	Six mice were treated with paracetamol at 500 mg/kg.
Group 4	Six mice were treated with *Taraxacum officinale* methanolic extract from (1^{st} to 12^{th}) + paracetamol at 500 mg/kg from the 13^{th} to 15^{th} day

2-5 Determinations of Hepatoprotective Effects

The assessment criteria for hepatoprotective determinations included histological analysis of liver tissue and serum levels of AST, ALP, ALT, catalase (Cat), and MDA enzymes. Following cardiac puncture, blood was collected in an Eppendorf tube and allowed to clot at room temperature for 15 min before being centrifuged at 3000 rpm for 10 min to separate the serum [9]. The serum was then used to evaluate liver function enzymes.

2-6 Alanine Amino-Transferase (GOT)

According to [10], the activity of the ALT enzyme in mouse serum was measured using a commercial kit from Randox Company, similar to the process for determining GOT levels. The kit's standard curve was used to calculate GOT activity (units/L).

2-7 Aspartate Amino-Transferase (GPT)

The activity of the GPT enzyme in mouse serum was determined using the evaluation method described in previous work [11]. A commercial kit from Randox Company was employed for this purpose, and the kit's standard curve was used to calculate GPT activity (units/L).

2-8 Alkaline Phosphatase (ALP)

The ALP enzyme in mouse serum was tested utilizing a specialized kit from the Bio Merieux Company. The most widely applied method, as described previously [12], involves hydrolyzing sodium phenyl phosphate to release phenol and produce sodium phosphate. The calorimetric analysis is then employed to determine the amount of phenol generated.

2-9 Malondialdehyde (MDA)

This assay, utilizing a specific kit from the Bio Merieux Company, is based on the reaction between thiobarbituric acid (TBA) and malondialdehyde (MDA), resulting in the formation of an MDA-TBA_2 adduct that strongly absorbs at 532 nm. The most widely used conventional approach is described previously [13].

2-10 Catalase (Cat)

A cuvette containing 1.9 ml of 50 mM phosphate buffer was filled with either 0.1 ml of a standard solution or liver homogenate supernatant. After adding 1.0 ml of freshly prepared 30 mM hydrogen peroxide, the absorbance was measured after 10 and 30 min. The rate of hydrogen peroxide breakdown was assessed spectrophotometrically at 240 nm [14].

2-11 Histopathological Study

Samples were cut into 2 x 2 x 2 mm pieces and pre-fixed in a solution of 2.5% glutaraldehyde diluted in phosphate buffer with a pH of 7.4. After several rinses in the same buffer, the specimens were placed in PBS for 12 h, and histological sections were prepared according to the method described in a previous work [15].

3- Results and Discussion

The extract had the maximum concentration of total flavonoids in *Taraxacum officinale* (210.2±25.7 mg/g). Table 1 displays various phytochemical compounds detected in the methanolic extract of *Taraxacum officinale*.

Table 1: Examination of the main bioactive phytochemical compounds of *Taraxacum officinale* methanolic extract

Test name	Methanolic extract
Tannins	++ve
Glycoside	++ve
Alkaloids	+ve
Saponins	++ve

Flavonoids	++ve
Polyphenols	++ve

3-1 Determination of Liver function enzymes (L.F.E.)

ALP concentrations also significantly increased in the drug-treated group (91 U/L) compared to the negative control (79 U/L), with no significant differences in enzyme levels between the plant and interaction groups (84 U/L and 85 U/L, respectively). For the MDA enzyme, the concentration rose in the drug group (9.5 U/L) compared to the negative control (5.6 U/L), while it decreased in the plant and interaction groups (6.2 U/L and 7.3 U/L, respectively). The concentration of the Cat enzyme increased in the interaction group (0.915 U/L) compared to the negative control group (0.320 U/L). Still, it decreased in the drug group (0.255 U/L), with the plant group showing a concentration of 0.652 U/L, as shown in Table 2.

Table 2: Effect of *Taraxacum officinale* methanolic extract on (L.F.E.) (aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, Malondialdehyde, and Catalase in sera of albino male mice.

Groups	AST (unit/l)	ALT (unit/l)	ALP (unit/l)	MDA (unit/l)	Cat (unit/l)
Group 1: (-ve control)	45	40	79	5.6	0.320
Group 2: *Taraxacum officinale*	42	45	84	6.2	0.652
Group 3: *Paracetamol*	56	62	91	9.5	0.255
Group 4: *Taraxacum officinale* + paracetamol	48	49	85	7.3	0.915

3-2 Liver histopathological study

As illustrated in Figure 1, the histology of the mouse liver in the negative control group displays a normal histological structure, featuring a central vein surrounded by threads of hepatocyte cells arranged in a radial pattern.

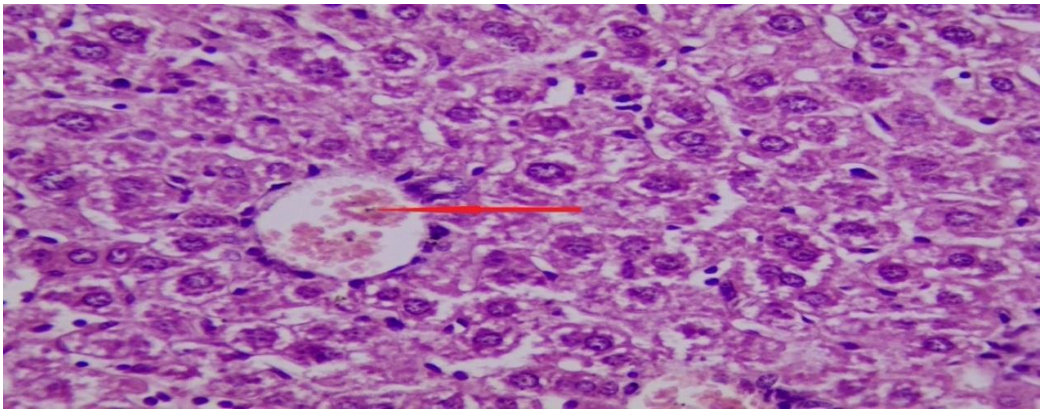

Figure 1: Liver tissue section from a negative control mouse treated with distilled water, showing normal histological structure with a central vein surrounded by radially arranged hepatocyte cells. Stained with H&E, magnification 40x.

The histopathological examination of the liver treated with plant extract, presented in Figure 2, showed mild congestion and dilation of the central vein and vascular elements within the portal triad, with a normal arrangement of hepatic cords. In contrast, liver sections from the drug-treated group (Figure 3) displayed significant zonal acute cellular swelling in the hepatocyte cords, along with perivascular infiltration of mononuclear leukocytes, primarily lymphocytes and macrophages, and some areas of necrosis. The liver treated with both plant extract and drug was illustrated in Figure 4 and exhibited mild congestion of the central vein, small necrotic foci, and mild zonal vacuolar degeneration of hepatocytes. The liver is a vital organ in the human body,

performing numerous essential activities and regulating various physiological processes. Due to its unique anatomical position and functions, the liver is vulnerable to different forms of damage. However, it also has a remarkable capacity for self-repair, allowing it to recover from a range of injuries [16].

Traditional treatments for chronic liver diseases, such as fibrosis, cirrhosis, steatosis, and chronic hepatitis, often fall short due to the side effects of various medications and chemicals. To address this limitation, recent research has focused on medicines derived from medicinal plants. Rich in flavonoids and polyphenolic compounds, these plant-based treatments have been extensively evaluated for hepatoprotective effects against drug- and chemical-induced hepatotoxicity in both in vivo and in vitro studies [17]. Several plant-derived compounds, including silibinin, berberine, dandelion, curcumin, silymarin, and resveratrol, have shown promising hepatoprotective properties [18]. Among these, dandelion (Taraxacum officinale) is a notable traditional medicinal herb used to treat jaundice, liver conditions, gallbladder issues, and other hepatic disorders [19].

Dandelion has long been valued as a liver tonic in Chinese, Indian, and Russian folk medicine. In Traditional Chinese Medicine, dandelion is used alongside other treatments for hepatitis [18]. Historically, *Taraxacum officinale* root and herb have been used to address various ailments, particularly those related to liver and gallbladder health [20]. Phytochemical studies reveal that terpenoids and sterols, primarily taraxacin and taraxacerin, are distributed similarly across the roots, leaves, and flowers of the plant. Other terpene and sterol compounds include beta-amyrin, taraxasterol, and taraxerol, as well as free sterols like sitosterin, stigmasterin, and phytosterin, which are structurally related to bile [21]. The beneficial effects of dandelion extract are attributed to these physiologically active components.

Dandelion extract has shown promising results in experimental models of chemical- and drug-induced hepatic fibrosis. For instance, a water-ethanolic extract of dandelion root (DWE) has been demonstrated to ameliorate CCl_4-induced hepatic fibrosis in mice. Key hepatotoxicity indicators, including liver enzymes aspartate and alanine transaminases (AST and ALT), superoxide dismutase, hydroxyproline, and α-smooth muscle actin (α-SMA) protein expression, showed significant recovery following administration of 600 mg/kg of DWE for ten days in CCl_4-induced hepatic fibrotic mice [22].

A substantial body of research underscores the traditional and alternative medical use of dandelion worldwide. Ongoing clinical trials are investigating the potential of *Taraxacum officinale* (dandelion) in patients with chronic liver conditions. Current evidence on its mechanisms of action is promising, with initial findings supporting both its efficacy and safety.

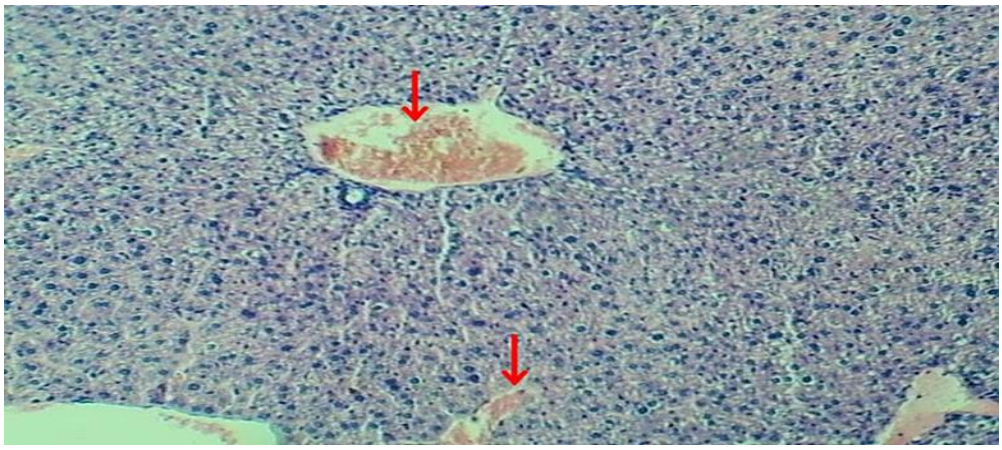

Figure 2: Section of liver tissue from the plant-treated group showing mild congestion and vein dilation (indicated by arrows). Stained with H&E, magnification 100x.

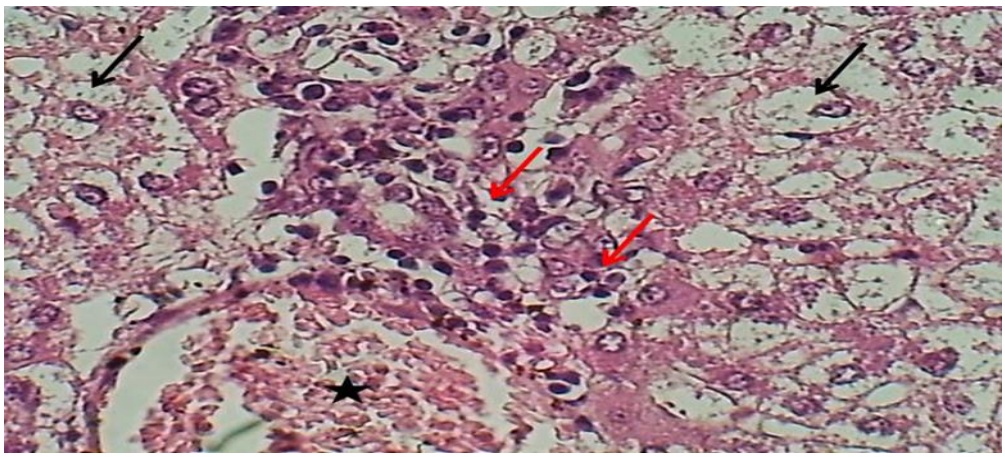

Figure 3: Liver section from a mouse treated with paracetamol, showing zonal acute cellular swelling of hepatocytes (black arrow), along with perivascular infiltration of lymphocytes and macrophages, and necrotic hepatocytes (red arrow). Stained with H&E, magnification 400x.

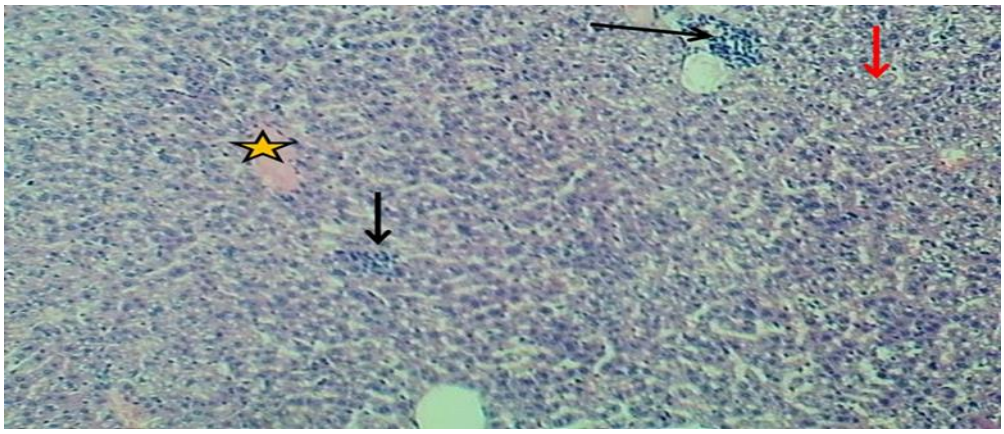

Figure 4: Liver section from a mouse treated with both paracetamol and plant extract, showing mild congestion of the central vein (asterisk), small necrotic foci (black arrows), and mild zonal vacuolar degeneration of hepatocytes. Stained with H&E, magnification 100x.

4- Conclusion

The results of this study highlight the significant hepatoprotective potential of *Taraxacum officinale*, particularly in cases of drug-induced hepatic damage. Through its rich phytochemical profile—including terpenoids, sterols, and polyphenolic compounds—dandelion demonstrates promising effects in stabilizing liver enzymes, reducing inflammation, and promoting hepatic tissue recovery. Experimental models of chemically-induced hepatic fibrosis have shown that dandelion extract can mitigate liver injury by normalizing markers of hepatotoxicity such as AST, ALT, and oxidative stress enzymes, as well as inhibiting fibrosis-associated proteins like α-SMA.

With traditional uses of dandelion in Chinese, Indian, and Russian medicine serving as a foundation, this study supports the broader potential of *Taraxacum officinale* as a viable hepatoprotective agent. The continuing clinical trials was essential in validating the therapeutic role of dandelion extract in chronic liver diseases. If substantiated, dandelion could provide an affordable, accessible, and natural adjunct or alternative to conventional liver treatments, offering a promising strategy for managing liver disorders with fewer adverse effects. Further research should continue to focus on refining the dosage, formulation, and understanding of its molecular mechanisms to optimize its use in clinical settings.

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None.

Conflict of Interest

There is no known conflict associated with this work.

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