

Protective Effects of Lady's Mantle (*Alchemilla vulgaris* L.) against Hepatotoxicity in a Rat Model: Insights into Active Compounds and Underlying Mechanisms

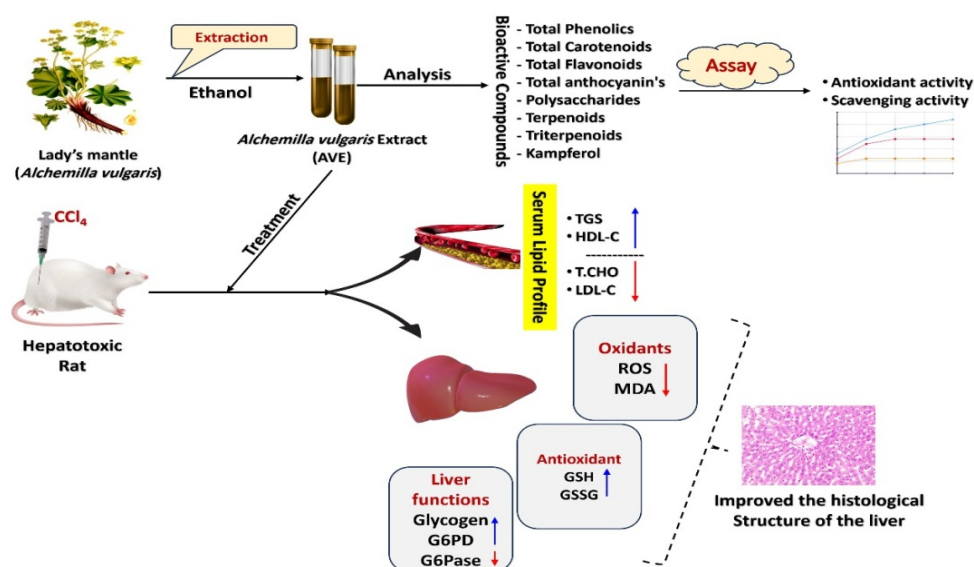
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Abstract: *Alchemilla vulgaris* (Lady's mantle) has shown various therapeutic properties, yet its liver-protective potential remains underexplored. This study aimed to evaluate its hepatoprotective effects in a rat model of CCl₄-induced liver toxicity and to characterize its bioactive compounds. Thirty rats were divided into five groups: a negative control, a CCl₄-induced positive control, and three treatment groups receiving ethanol extract of Lady's mantle (AVE) at doses of 150, 300, and 450 mg/kg body weight/day. Proximate analysis of the aerial parts revealed 39.86% carbohydrates, 30.92% crude fiber, 15.94% protein, 10.83% ash, and 2.45% fat. The ethanol extract was rich in bioactive compounds, including phenolics (621.67 µg GAE/g), terpenoids (411.52 µg/g), anthocyanins (214.66 µg/g), flavonoids (181.45 µg/g), and triterpenoids (129.34 µg/g). AVE exhibited strong antioxidant activity (87.34%), with an IC₅₀ of 14.42 µg/mL. In the biological study, AVE treatment dose-dependently improved body weight gain, food intake, and feed efficiency ratio in hepatotoxic rats. Liver function markers also improved, with glycogen content increasing by 128.64%, G6PD activity by 95.78%, and G6Pase activity decreasing by 53.25% at the highest dose. Serum lipid profiles were corrected, with HDL-c increasing by up to 65.06%, LDL-c decreasing by 32.52%, and total cholesterol dropping by up to 9.42%. Antioxidant defenses were restored as reduced glutathione increased by 89.49%, while oxidative stress markers such as ROS and MDA decreased by up to 53.61% and 26.01%, respectively. Histopathological analysis confirmed reduced liver damage and inflammation in treated groups. In conclusion, *Alchemilla vulgaris* ethanol extract demonstrates potent antioxidant and hepatoprotective properties. Further investigation may support its use as a natural therapeutic agent for liver disorders.



Keywords: Antioxidant activity, scavenging activity, liver functions, serum lipid profile, glutathione fractions, reactive oxygen species, malonaldehyde

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1. Introduction

The liver is a vital organ in all vertebrates and serves as the body's primary metabolic hub. It performs numerous essential functions, including the metabolism of carbohydrates, proteins, and fats. Additionally, the liver synthesizes crucial substances such as glucose, plasma proteins, and urea that are released into the bloodstream. It also produces clotting factors necessary to halt bleeding during trauma or injury and secretes bile into the intestines to facilitate nutrient absorption. Moreover, it stores important substances like glycogen, lipids, and vitamins within parenchymal cells [1,2]. Beyond these metabolic roles, the liver is integral to immune defense and is centrally involved in the detoxification and biotransformation of xenobiotics [3,4,5,6]. Owing to these functions, the liver possesses a substantial functional reserve, which often conceals early clinical manifestations of liver damage. However, as liver disorders progress, particularly that impairing bile flow, damage can rapidly escalate into life-threatening conditions [7].

Liver disorders encompass a broad spectrum, ranging from non-alcoholic fatty liver disease (NAFLD) or the more recently termed metabolic dysfunction-associated steatotic liver disease (MASLD), to viral hepatitis types A through E, alcoholic liver disease, cirrhosis, hepatocellular carcinoma (HCC), and rarer conditions such as Budd–Chiari syndrome and hepatic veno-occlusive disease (SOS) [8]. NAFLD affects approximately one-third of the global population, with a prevalence of around 30% in Western regions and about 10% in Asia. Cirrhosis, which impacts an estimated 4.5–9.5% of the global population, accounts for roughly two million deaths annually—half directly due to cirrhosis and the other half due to complications like viral hepatitis and HCC [9]. It is ranked as the 11th leading cause of death worldwide and is particularly fatal among individuals aged 45 to 64 [10]. Meanwhile, HCC remains the third most common cause of cancer-related mortality, resulting in over 830,000 deaths each year [11].

Systemic therapies such as sorafenib, lenvatinib, regorafenib, cabozantinib, and ramucirumab are employed in managing advanced stages of HCC. However, these chemotherapeutic agents are often associated with challenges including drug resistance, significant liver toxicity, and recurrence rates as high as 70%, particularly in patients with preexisting liver impairment [12]. Moreover, conditioning regimens involving high-dose chemotherapy prior to bone marrow transplantation may lead to SOS, a condition that proves fatal in 10–20% of affected individuals [10].

There is growing scientific support for the hepatoprotective and therapeutic potential of natural substances such as curcumin, silymarin, berberine, resveratrol, phytochemicals, and polyphenols. These compounds exert multiple protective effects, including antioxidant, anti-inflammatory, anti-apoptotic, and anti-fibrotic actions. In certain experimental models, they have also demonstrated the ability to enhance chemotherapy efficacy, counter drug resistance, and alleviate adverse effects [13]. In preclinical and animal studies, many plant-derived extracts have been shown to counteract liver injury induced by agents such as cisplatin or doxorubicin

by boosting antioxidant enzyme activity and reducing inflammatory and hepatotoxic biomarkers like ALT and AST [14].

Recent meta-analyses and randomized controlled trials have confirmed that dietary supplements and lifestyle interventions, including silymarin, curcumin, garlic, *Nigella sativa*, vitamin E, vitamin D, L-carnitine, polyunsaturated fatty acids, and both Mediterranean and high-protein diets—are effective in reducing liver enzyme levels and improving liver health in patients with NAFLD or NASH, while maintaining favorable safety profiles [15]. Additionally, silymarin has shown renal and liver protective benefits in cancer patients undergoing chemotherapy in randomized placebo-controlled studies [16].

Natural and nutritional therapies provide multifaceted advantages: antioxidants counter oxidative damage, anti-inflammatory agents suppress cytokine-driven liver injury, and metabolic modulators improve insulin sensitivity and reduce hepatic fat deposition. Components such as dietary fiber, legumes, omega-3 fatty acids, and adherence to the Mediterranean diet have been associated with a 30–50% reduction in HCC risk [15]. Compared to conventional long-term chemotherapy, these interventions often present with fewer side effects, better patient tolerance, and lower overall cost. More importantly, they address underlying metabolic dysfunctions rather than merely managing the symptoms of liver disease.

Conventional pharmaceuticals are not always effective in treating many pathological conditions, prompting interest in alternative therapies. Among these, plant-based drugs and herbal extracts have gained attention due to their synergistic therapeutic potential and generally low incidence of adverse effects [17,18]. One notable group of medicinal plants with deep roots in traditional healing systems and recognized in various pharmacopeias is the genus (*Alchemilla*). This genus, (*Alchemilla* L.), encompasses more than 300 species of herbaceous perennials typically found in moist, upland meadows across Europe, Western Asia, and North America. Some species also grow in high-altitude areas of South America and Africa [19,20]. Among them, (*Alchemilla vulgaris* L.), commonly referred to as lady's mantle, is the most extensively investigated. Recent taxonomic studies have categorized (*A. vulgaris*) as a complex of 12 closely related apomictic microspecies that frequently hybridize [21]. The European Pharmacopoeia includes this species under the broader classification (*Alchemilla vulgaris* L.) sensu latiore [19].

Lady's mantle holds a prominent place in ethnomedicine around the world. The aerial parts of the plant have traditionally been employed to treat a wide array of conditions, including diabetes, multiple sclerosis, anemia, ulcers, hernias, gynecological and digestive issues, wounds, skin rashes, and inflammatory disorders [22,23]. In Southeastern Europe and the Balkans, (*Alchemilla*) species are particularly valued for managing menstrual, menopausal, and other gynecological concerns, as well as respiratory infections, diarrhea, metabolic diseases such as diabetes, liver and kidney dysfunctions, obesity, skin ailments, and various inflammatory conditions [24,25,26].

The pharmacological potential of this plant extends further to its antimicrobial properties, including antibacterial, antifungal, and antiviral activities [25,26].

Given the growing problem of antimicrobial resistance, natural plant-based compounds offer promising alternatives to synthetic antibiotics, with reduced risk of fostering resistance [27]. In a recent investigation, (*Alchemilla*) infusion demonstrated neuroprotective effects in hypoxia-induced injury models, suggesting its value in central nervous system disorders [28]. Additionally, [29] found that (*Alchemilla vulgaris*) extract inhibits acetylcholinesterase and tyrosinase, enzymes linked to neurodegenerative conditions, reinforcing its potential in preventing diseases like Alzheimer's [28].

Despite these promising properties, certain therapeutic applications of (*Alchemilla vulgaris*) remain underexplored. Notably, the anticancer potential of this plant has only recently gained attention. Vlasisavljević et al. [30] reported its significant cytotoxic effects on estrogen-dependent cancers of the female reproductive system, while Ibrahim et al. [31] showed that a methanolic root extract of (*A. vulgaris*) exhibited strong in vitro cytotoxicity against various cancer cell lines. More recently, studies have revealed that ethanolic extracts from the aerial parts of (*A. vulgaris*) not only offer antioxidant and genoprotective benefits but also have the ability to reduce malignancy in hormone-independent tumor cells. This effect is believed to occur through inhibition of cell proliferation and the induction of both apoptotic and autophagic pathways. Although several earlier studies have explored the impact of lady's mantle (*Alchemilla vulgaris* L.) on liver function, numerous mechanisms and specific aspects of its activity remain insufficiently understood. Accordingly, the current research seeks to evaluate the hepatoprotective potential of lady's mantle (*Alchemilla vulgaris* L.) in an experimental rat model of liver toxicity, aiming to identify its bioactive compounds and clarify the underlying mechanisms responsible for its liver-protective effects

2. Materials and Methods

2.1. Materials

2.1.1. Lady's Mantle Samples

The aerial parts of Lady's mantle (*Alchemilla vulgaris*) were procured from Harraz for Food Industry & Natural Products, Bab Alkhalq, Cairo, Egypt. The staff from the Plant Taxonomy Department at the Faculty of Agriculture, Menoufia University, Shebin El-Kom, Egypt, authenticated the samples.

2.1.2. Chemicals

Standards for bioactive compounds [gallic acid (GA), catechine (CA), α -tocopherol, and Butylated hydroxytoluene (BHT)] and DPPH (2,2-diphenyl-1-picrylhydrazyl) were obtained from Sigma Chemical Co., St. Louis, MO. Casein was purchased from Morgan Chemical Co., Cairo, Egypt. All other chemicals (Except as otherwise stated), reagents, and solvents were of analytical grade and were acquired from El-Ghomhorya Company for Trading Drug, Chemicals and Medical Instruments, Cairo, Egypt. Assay kits for glucose,

glucose-6-phosphate dehydrogenase activity (G6Pase), glucose-6-phosphatase activity (G6PD), and malondialdehyde (MDA) were supplied by BIODIAGNOSTIC, Dokki, Giza, Egypt. Additionally, El-Nasr Pharmaceutical Chemicals provided kits for triglycerides (TGs), total cholesterol (TC), HDL-cholesterol, and LDL-cholesterol.

2.2. Methods

2.2.1. *Alchemilla Vulgaris* Aerial Part Powder Preparation

The aerial parts of *Alchemilla vulgaris* were manually sorted to remove any damaged portions before being dried in a hot air oven (Horizontal Forced Air Drier, Proctor and Schwartz Inc., PA) at 60°C for 2 hours, until the final product's moisture content was approximately 8%. After drying, the plant parts were ground into a fine powder using a high-speed mixer (Moulinex Egypt, Al-Araby Co., Benha, Egypt). This powder was then passed through an 80-mesh sieve, and the material that was retained was collected for subsequent use.

2.2.2. Preparation of *Alchemilla vulgaris* Ethanol Extract (AVE)

To prepare the extract, 20 grams of dried *Alchemilla vulgaris* powder were combined with 180 mL of 80% ethanol and homogenized. The resulting mixture was agitated at 200 rpm for one hour at room temperature in a beaker using an orbital shaker (Unimax 1010, Heidolph Instruments GmbH & Co. KG, Germany). The liquid extract was then separated from the solid residue by filtering it through Whatman No. 1 filter paper. The remaining solid residue underwent two more extractions, and the resulting extracts were combined. A rotary evaporator (Laborata 4000; Heidolph Instruments GmbH & Co. KG, Germany) was used to remove the residual solvent under reduced pressure at 45°C.

2.2.3. Chemical Analysis of *Alchemilla vulgaris* Aerial Part Powder

The proximate chemical composition of the *Alchemilla vulgaris* aerial part powder, including moisture, protein (T.N. $\times$ 6.25, micro - kjeldahl method using semiautomatic apparatus, Velp company, Italy), fat (soxhelt miautomatic apparatus Velp company, Italy, petroleum ether solvent), ash, fiber, and dietary fiber content, was determined using the methods outlined in the AOAC, [32]. Carbohydrate content was calculated by subtraction: Carbohydrates (%) = 100 - (% moisture + % protein + % fat + % Ash + % fiber).

2.2.4. Bioactive Compounds Determination in *Alchemilla vulgaris* Ethanol Extract (AVE)

The total phenolic content in AVE was quantified using the Folin-Ciocalteu reagent, as described by Singleton & Rossi, [33] and Wolfe et al. [34], with results expressed as μ g gallic acid equivalent (GAE) per gram. Total carotenoids in the 80% acetone extract were measured using the method of Litchenthaler, [35] and expressed as μ g rutin equivalent per gram. Total flavonoids were estimated using the colorimetric method of Zhisen et al.

[36], with results expressed as μg resveratrol equivalent per gram. Total anthocyanins were measured spectrophotometrically using the method of Giusti & Wrolstad, [37] and expressed as μg cyanidin-3,5-diglucoside per gram. Polysaccharides were extracted and quantified according to the procedure of Vazirian et al. [38], using starch as a standard and expressing results as mg starch equivalent per gram. Total terpenoids were extracted and measured following the method of Ghorai et al. [39], using linalool as a standard and expressing results as μg linalool equivalent per gram. Total triterpenoids were assessed per Schneider et al. [40], using ursolic acid as a standard, with results expressed in μg ursolic acid equivalent per gram. Tannin content was determined using the method of Van-Burden & Robinson, [41], with catechine as the standard for the calibration curve to estimate tannin levels as mg catechine equivalent per gram. Kaempferol was measured according to the method in Fouda et al. [42] and expressed as μg per gram. Finally, total alkaloids were determined using the method of Zhao & Wang, [43], with atropine as the standard for the calibration curve, from which the alkaloid content was estimated as μg atropine equivalent per gram.

2.2.5. Antioxidant Activity Determination

The antioxidant activity (AA) of AVE and standards (α -tocopherol, and BHT) was determined by the β -carotene bleaching (BCB) assay as described by Marco, [44]. In summary, 1 mL of β -carotene solution (0.2 mg/mL in chloroform) was added to 50 mL flasks containing 0.02 mL of linoleic acid and 0.2 mL of Tween 20. Each mixture was then treated with 0.2 mL of methanol (80%, as a control) or the corresponding *G. lucidum* extracts or standards [α -tocopherol and Butylhydroxy toluene (BHT)]. After the mixtures were evaporated to dryness under vacuum at room temperature, 50 mL of oxygenated distilled water was added and shaken to form a liposome solution. This was then subjected to thermal auto-oxidation at 50°C for 2 hours. The absorbance (Abs) of the solution at 470 nm was measured using a spectrophotometer (UV-160A; Shimadzu Corporation, Kyoto, Japan). All samples were assayed in triplicate. BHT and α -tocopherol at various concentrations in 80% methanol were used as standards. AA was calculated as a percent inhibition relative to the control and was expressed as antioxidant activity (AA) using the equation from Al-Saikhan et al. [45]: $AA = (R_{\text{control}} - R_{\text{sample}}) / R_{\text{control}} \times 100$, where R_{control} and R_{sample} were the bleaching rates of β -carotene in the reactant mixture without and with AVE, respectively.

2.2.6. DPPH Radical Scavenging Assay

The free radical scavenging ability of AVE was tested using the DPPH radical scavenging assay, as described by Desmarchelier et al. [46]. A solution was prepared by mixing 2.4 mL of 2,2-diphenyl-1-picrylhydrazyl (DPPH) (0.1 mM in methanol) with 1.6 mL of AVE at various concentrations (12.5–150 $\mu\text{g}/\text{mL}$). The reaction mixture was thoroughly vortexed and left in the dark at room temperature for 30 minutes. The absorbance of the mixture was measured spectrophotometrically at 517 nm (UV-160A; Shimadzu Corporation, Kyoto, Japan). BHT

was used as a reference. The percentage of DPPH radical scavenging activity was calculated using the following equation: DPPH radical scavenging activity (%) = $[(A_0 - A_1)/A_0] \times 100$, where A_0 is the absorbance of the control and A_1 is the absorbance of the AVE/BHT. Inhibition (%) was then plotted against concentration, and the IC_{50} value was calculated from the graph.

2.2.7. Biological Experiments

2.2.7.1. Ethical approval

The biological model, specifically the rats used in the study's experimental design, received ethical approval from the Scientific Research Ethics Committee (Animal Care and Use) at the Faculty of Home Economics, Menoufia University, Shebin El-Kom, Egypt (Approval # 10- SREC- 09-2024).

2.2.7.2. Animals

The study used adult male albino rats, with each weighing 160 ± 8 g. They were obtained from the Research Institute of Ophthalmology, Medical Analysis Department, Giza, Egypt.

2.2.7.3. Basal Diet

The basal diet was prepared according to the formula from Reeves et al. [47] and contained the following components: protein (10%), corn oil (10%), vitamin mixture (1%), mineral mixture (4%), choline chloride (0.2%), methionine (0.3%), cellulose (5%), with the remaining portion consisting of corn starch (69.5%). The compositions for both the salt and vitamin mixtures were also based on the same reference.

2.2.7.4. Induction of Hepatotoxicant in Rats

Chronic liver damage was induced in thirty male albino rats by administering an intraperitoneal (IP) injection of carbon tetrachloride (CCl_4) in olive oil, 50% V/V (2 mL/kg bwt), twice a week for two weeks, following the method described by Jayasekhar et al., [48]. Liver intoxication was confirmed by taking a random sample of experimental animals (4 rats) and examining their biochemical liver functions.

2.2.7.5. Experimental Design

All biological experiments were conducted in compliance with the rules of the Institute of Laboratory Animal Resources, Commission on life Sciences, National Research Council, [49]. The study utilized 30 rats, housed individually in wire cages under standard healthy conditions: a room temperature of $24 \pm 3^\circ\text{C}$, relative humidity of $56 \pm 2\%$, and a 12-hour light cycle. For two weeks prior to the experiment, all rats were fed a basal diet (BD) for acclimation. After this period, the rats were divided into main groups. The first group, consisting of six rats, served as a negative control; they were fed BD and received injections of olive oil (5 mL/kg body weight), which acted as the vehicle for the CCl_4 group's treatment. The second main group, composed of 24 rats, was given CCl_4 injections to induce liver damage. This group was then divided into equal subgroups: group 2, which was a positive control and fed BD; and groups 3-5, which were

fed BD supplemented with 150, 300, and 450 mg of AVE per kg of body weight per day, respectively. These concentrations were chosen based on existing toxicity data for *Alchemilla vulgaris* [50].

2.2.7.6. Biological Evaluation

Daily food intake was monitored throughout the 28-day period, and body weight was measured weekly. Body weight gain (BWG,%), food intake (FI), and food efficiency ratio (FER) were assessed using the methods of Chapman et al. [51]. The following formulas were used for these calculations:

BWG (%) = $\frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100$

FER = $\frac{\text{grams of feed consumed (g over 28 days)}}{\text{grams of body weight gained (g over 28 days)}}$

2.2.7.7. Blood Sampling

At the conclusion of the four-week experiment, blood samples were collected from the rats following a 12-hour fasting period. The rats were first anesthetized with ether, and then blood was drawn from the abdominal aorta. The blood was placed in clean, dry centrifuge tubes and allowed to clot at room temperature. It was then centrifuged at 3000 rpm for 10 minutes to isolate the serum [52]. The serum was transferred to clean tubes and stored at -20°C until it was needed for analysis.

2.2.7.8. Organ Sampling

After the rats were euthanized, liver samples were carefully dissected, and any remaining blood was removed. The organs were rinsed with cold saline, dried with filter paper, weighed, and then preserved in a 10% formalin solution for histological examination, as detailed by Drury and Wellington, [52].

2.2.7.9. Preparation of Liver Homogenates

Liver homogenates were prepared using the method described by El-Khawaga et al. [53]. A piece of liver tissue was accurately weighed and homogenized in ice-cold 0.9% saline using a Teflon pestle attached to a homogenizer motor. The homogenate was diluted to a 5% (w/v) solution and then centrifuged at 5000 rpm for 30 minutes at 4°C to remove cellular debris and nuclei. The resulting supernatant was subsequently used for biochemical analysis.

2.2.7.10. Biochemical analysis

a. Liver Functions

The glycogen content in liver homogenates was measured according to the procedure by Damsbo et al. [54]. The activity of hepatic glucose-6-phosphate dehydrogenase (G6PD) was evaluated as per the method of Chan et al. [55], and the activity of hepatic glucose-6-phosphatase (G6Pase) was determined using the method described by Rossetti et al. [56].

b. Serum Lipid Profile

The levels of triglycerides (TGs), total cholesterol (TC), HDL-cholesterol, and LDL-cholesterol in the serum were determined using methods from Ahmadi et al. [57], Fossati & Prenape, [58], Lopes-Virella et al. [59], and Richmond & Richmond, [60], respectively.

c. Glutathione Fractions

Reduced (GSH) and oxidized (GSSG) glutathione levels in serum samples were quantified colorimetrically, following the procedure by Elman et al. [61].

d. Reactive Oxygen Species (ROS) and Malonaldehyde (MDA) Content

MDA levels were measured as described by Buege and Aust, [62]. A half milliliter of plasma was combined with 1.0 ml of thiobarbituric acid (TBA) reagent (15% TCA, 0.375% TBA, and 0.01% butylated hydroxytoluene in 0.25 N HCl). Then, 25 microliters of 0.1 M FeSO₄·7H₂O were added, and the mixture was heated in boiling water for 20 minutes. The samples were then centrifuged at 1000 xg for 10 minutes, and the absorbance was measured at 535 nm against a reagent blank. The absorbance of the samples was compared to a standard curve of known MDA concentrations. ROS was detected as described by Jambunathan, [63] by measuring the colorimetrically quantifiable blue formazan produced by ROS reducing Nitroblue Tetrazolium (NBT).

2.2.7.11. Histopathological Examination

Liver specimens were collected immediately after the rats were sacrificed and were immersed in 10% neutral buffered formalin. The fixed tissues were then trimmed, dehydrated using ascending grades of alcohol, cleared with xylene, embedded in paraffin, and sectioned to a thickness of 4-6 µm. These sections were stained with hematoxylin and eosin and examined microscopically [64].

2.3. Statistical Analysis

All data were statistically analyzed using a one-way ANOVA with a computerized Costat program. The results were presented as means ± standard deviation (SD). Differences between treatments were considered statistically significant if $P \leq 0.05$ [65].

3. Results and Discussion

3.1. Proximate Composition of Lady's Mantle

The proximate chemical composition of lady's mantle (*Alchemilla vulgaris*) aerial parts powder, as presented in Table (1), reveals that on a dry weight (DW) basis, the plant is primarily made up of carbohydrates (39.86%), followed by crude fiber (30.92%), total protein (15.94%), ash (10.83%), and a small proportion of crude fat (2.45%), with a high dry matter content of 91.68% reflecting low moisture levels, an important factor for product preservation and storage stability. This composition, particularly the dominance of carbohydrates and fiber, is consistent with typical plant-based substances where these elements play key roles in energy provision and structural integrity. The notable amount of crude fiber highlights its potential contribution to dietary fiber intake, while the protein content, being relatively high for a leafy herb, suggests its value as a supplementary protein source beyond traditional medicinal applications. The low fat content conforms with the characteristics of most herbs, which typically store energy in carbohydrate form rather than as lipids, and the ash content represents the mineral

constituents of the plant. Overall, the nutritional profile of *Alchemilla vulgaris* shows a strong resemblance to other edible green leafy plants, making it a promising source of nutrients such as protein and fiber, with low fat levels [66,67,68,69,70,71]. Variability in composition may result from differing environmental factors, genetic traits, and processing techniques. The elevated levels of carbohydrates and fiber are also in agreement with findings from other studies on *Alchemilla* species, such as that of Jakimiuk and Tomczyk [72], who emphasized the plant's potential role in functional food products and digestive health supplements. The significant fiber content (30.92%) enhances satiety and may aid in blood glucose regulation. Similarly, the protein value of 15.94% aligns with the ranges seen in other leafy vegetables and medicinal plants, with Bouba et al. [73] reporting similar findings in wild edible species from Cameroon, further supporting *Alchemilla vulgaris*'s role as a moderate plant-based protein source. The minimal fat content (2.45%) confirms existing research that most herbs have low lipid storage, with studies by El-Hadidy et al. [74], El-Nassag et al. [68], and Elhassaneen et al. [69] reinforcing this observation. The 10.83% ash level reflects the total mineral load, consistent with studies evaluating mineral content in leafy herbs and influenced by factors like soil composition and growing practices [69,75,76,77]. In conclusion, the higher-than-average fiber and protein levels, combined with low fat and substantial mineral content, position *Alchemilla vulgaris* as a beneficial functional food, with applications in supporting digestive function, muscle maintenance, and healthy weight management, particularly for those seeking plant-based dietary alternatives, aligning with the growing global interest in plant-derived nutrition.

Table 1. Proximate chemical composition (g/100 DW) of Lady's mantle (*Alchemilla vulgaris*) aerial parts powder

Factor	Mean $\pm$ SD
Dry matter	91.68 $\pm$ 0.29
Total protein	15.94 $\pm$ 0.96
Crude fat	2.45 $\pm$ 0.23
Crude fiber	30.92 $\pm$ 1.17
Ash	10.83 $\pm$ 0.59
Carbohydrates (by difference)	39.86 $\pm$ 2.19

Values represent the mean $\pm$ SD (n=3). DW, dry weigh

3.2. Bioactive Compounds in Lady's Mantle Ethanol Extract

The provided data (Table 2) highlights the concentration of several bioactive compounds in the ethanol extract of *Alchemilla vulgaris* (AVE), emphasizing its potential as a multifunctional phytochemical-rich plant. The extract exhibits a notable abundance of total phenolics (621.67 μ g GAE/g), followed by significant amounts of terpenoids (411.52 μ g linalol/g), anthocyanins (214.66 μ g cyanidin-3,5-diglucoside/g), polysaccharides (199.06 mg starch/g), flavonoids (181.45 μ g rutin/g), carotenoids (141.31 μ g resveratrol/g), triterpenoids (129.34 μ g ursolic acid/g), and kaempferol (88.43 μ g/g). This composition suggests a broad spectrum of biological activities, including

antioxidant, anti-inflammatory, and antimicrobial [78,79]. These findings align with other studies, such as El-Hadidy et al. [74], who reported high polyphenolic (395.65 mg/100g) and flavonoid (183.10 mg/100g) content in dried lion's foot leaves, and support AVE's comparison with other nutrient-dense green leafy plants and herbs [68,69,70,71,80]. The phytochemical profile of *A. vulgaris* is well-supported by literature, though variability in compound concentrations may arise due to differences in extraction techniques, geographical origin, and environmental influences [81]. Total phenolics and flavonoids are particularly prominent in *A. vulgaris*, contributing significantly to its antioxidant properties; Jakimiuk et al. [82] similarly observed high phenolic and flavonoid levels in the aerial parts of the plant and attributed its antioxidant activity to these components. The presence of kaempferol (88.43 μ g/g), a flavonoid linked to various health benefits, further supports its therapeutic relevance [72,79]. Likewise, the extract's rich content of terpenoids and triterpenoids, notably linalol and ursolic acid, corresponds with previous findings on *Alchemilla* species, confirming their antimicrobial, anti-inflammatory, and anticancer properties [83,84]. Furthermore, anthocyanins and carotenoids, with their strong antioxidant activities, are commonly found in sun-exposed plants and play essential roles in mitigating oxidative stress [85]. The notable polysaccharide content (199.06 mg starch/g) adds another dimension to the plant's bioactive profile, as these compounds are recognized for their immunomodulatory, anti-inflammatory, and prebiotic properties, indicating possible benefits for gut health and immune regulation [86]. Collectively, these findings underscore *Alchemilla vulgaris* as a promising candidate for functional food and therapeutic applications due to its diverse and potent phytochemical composition.

Table 2. Some bioactive compounds content of Lady's mantle (*Alchemilla vulgaris*) ethanol extract (AVE)

Compounds	Mean $\pm$ SD
Total phenolics (μ g gallic acid equivalent, GAE. g ⁻¹)	621.67 $\pm$ 10.03
Total carotenoids (μ g rutin equivalent, . g ⁻¹)	141.31 $\pm$ 8.21
Total flavonoids (μ g resveratrol equivalent. g ⁻¹)	181.45 $\pm$ 9.65
Total anthocyanin's (μ g cyanidin-3,5-diglucoside. g-1)	214.66 $\pm$ 10.96
Polysaccharides (mg starch. g ⁻¹)	199.06 $\pm$ 11.44
Terpenoids (μ g linalol. g ⁻¹)	411.52 $\pm$ 20.17
Triterpenoids (μ g ursolic acid.g ⁻¹)	129.34 $\pm$ 8.08
Kampferol (μ g.g ⁻¹)	88.43 $\pm$ 7.14

Values represent the mean $\pm$ SD (n=3).

3.3. Antioxidant Activity of Lady's Mantle Ethanol Extract

The data presented in Table 3 clearly illustrates the potent antioxidant activity (AA) of the ethanol extract of lady's mantle (*Alchemilla vulgaris*), referred to as AVE, which shows an impressive AA of 87.34%, signifying its strong capacity to neutralize free radicals. This activity is benchmarked against standard antioxidants, namely butylated hydroxytoluene (BHT) and α -tocopherol. At a concentration of 50 mg/ml, the AVE demonstrates an AA of 97.93% in comparison to BHT's 89.19%, and at 100

mg/ml, AVE still maintains a notable AA of 91.12% relative to BHT's 95.85%. When compared with α -tocopherol at 50 mg/ml, the AVE displays an AA of 90.85%, while α -tocopherol registers at 96.14%. These values highlight that AVE's antioxidant capacity is not only substantial but, in certain instances, surpasses both synthetic (BHT) and natural (α -tocopherol) standards. Such findings are consistent with prior scientific literature and even surpass previously reported values, underscoring the richness of AVE's phytochemical composition, especially in phenolics, flavonoids, and other bioactive constituents [79]. This high antioxidant performance has significant implications, particularly given that BHT is a commonly used synthetic antioxidant in the food industry and α -tocopherol represents the natural form of Vitamin E. Supporting literature, including a review by Jakimiuk and Tomczyk, [72], confirms that *Alchemilla* extracts often demonstrate antioxidant activity comparable to or greater than synthetic benchmarks.

Table 3. Antioxidant activity (AA, %) of Lady's mantle (*Alchemilla vulgaris*) ethanol extract (AVE)

Factor	<i>Alchemilla vulgaris</i> ethanol extract (AVE)
Antioxidant activity (AA, %)	87.34 ^b
AA [% of BHT (50 mg/ml)]	97.93
AA [% of BHT (100 mg/ml)]	91.12
AA [% of a-tocopherol (50 mg/ml)]	90.85
Standards:	
BHT (50 mg/ml)	89.19 ^b
BHT (100 mg/ml)	95.85 ^a
a-tocopherol (50 mg/ml)	96.14 ^a

Values represent the mean $\pm$ SD (n=3). Means in the same raw with the different superscript letters were significantly ($p \leq 0.05$).

The result that AVE reaches up to 97.93% of the AA of BHT strongly supports its potential as a natural alternative to synthetic antioxidants. This elevated antioxidant activity is likely the result of a synergistic interaction between the diverse bioactive compounds present in the extract. Previous studies have reported a strong, statistically significant correlation ($p \leq 0.01$) between the presence of such compounds—especially phenolics and flavonoids—and the antioxidant activity of *Alchemilla vulgaris* extracts across different plant parts [87,88,89,90,91,92]. Additional constituents such as terpenoids and carotenoids may also contribute to this cumulative effect [93]. This synergy reinforces the notion that whole plant extracts can often yield greater efficacy than isolated individual compounds. However, it is crucial to consider that the antioxidant capacity of plant extracts may vary depending on several variables, such as the geographic source of the plant material, the extraction solvent used, and the analytical methodology employed. The ethanol-based extraction method applied in this study appears particularly efficient in isolating antioxidant-active components. Though other solvents like methanol or water may produce slightly different antioxidant readings, the prevailing trend across the literature

consistently points to the strong antioxidant properties of *Alchemilla vulgaris* [94].

3.4. DPPH radical scavenging activity of Lady's mantle ethanol extract

The data presented in Figure 1 and Table 4 highlight the half-maximal inhibitory concentration (IC₅₀) values of lady's mantle (*Alchemilla vulgaris*) ethanol extract (AVE) and a standard antioxidant, butylated hydroxytoluene (BHT), as determined through the DPPH radical scavenging assay, where IC₅₀ refers to the concentration required to neutralize 50% of DPPH radicals, with lower values indicating stronger antioxidant capacity. According to the results, AVE has an IC₅₀ of 14.42 μ g/mL, whereas BHT exhibits a significantly lower IC₅₀ of 8.52 μ g/mL, implying greater antioxidant efficacy for BHT under the test conditions, and this difference is statistically significant ($p \leq 0.05$), indicating that the observed variation is not due to random chance. Nevertheless, despite having a higher IC₅₀ than BHT, the AVE value remains relatively low, suggesting a potent antioxidant effect, particularly notable considering that AVE is a complex mixture of bioactive phytochemicals, in contrast to BHT, a synthetic compound formulated specifically for its antioxidant properties. This finding aligns with prior research, including that of Vlaisavljević [31], where *Alchemilla* extracts showed higher IC₅₀ values than synthetic antioxidants but still retained substantial antioxidant capacity. When compared with IC₅₀ values from other plant-based extracts, as reported in the study by Khalighi-Sigaroodi [95], which ranged from 10 to over 200 μ g/mL, the AVE's IC₅₀ of 14.42 μ g/mL places it among the more potent natural antioxidants. The potent antioxidant activity observed in AVE can be attributed to its high content of phenolic and flavonoid compounds, which have been shown to strongly correlate with low IC₅₀ values [93,96,97,98,99,100], and the synergistic effects of these compounds likely contribute more to the antioxidant effect than any single constituent. Antioxidants such as those found in AVE are essential in protecting cells from oxidative stress, a biological condition that contributes to the pathogenesis of numerous chronic illnesses, including cancer, cardiovascular diseases, diabetes, obesity, rheumatoid arthritis, and neurodegenerative disorders like Alzheimer's and Parkinson's disease [90,101,102,103]. Given this, the phytochemical profile and antioxidant efficacy of AVE make it a promising candidate for functional food development, a finding supported by various studies that highlight the high antioxidant potential of plant materials and agricultural by-products due to their rich bioactive compound content [7,70,76, 104,105,106,107,108,109,110,111,112]. In summary, the ethanol extract of *Alchemilla vulgaris* exhibits substantial antioxidant activity and could serve as a natural alternative to synthetic antioxidants like BHT, and even stands in favorable comparison with recognized natural antioxidants such as α -tocopherol, reinforcing its potential application in health-promoting food and pharmaceutical formulations.

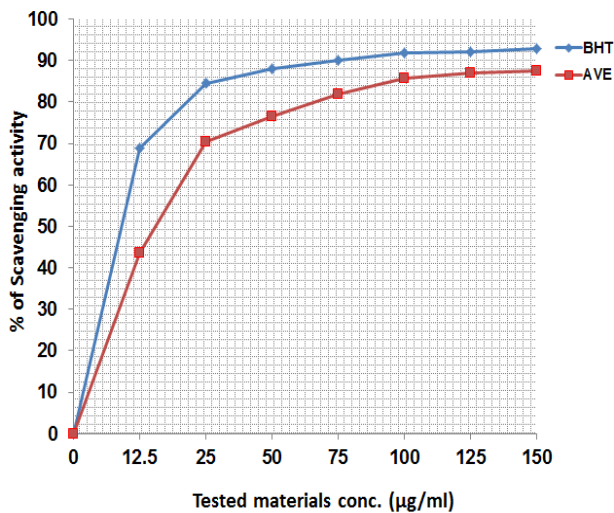


Figure 1. DPPH radical scavenging activity (%) of Lady's mantle (*Alchemilla vulgaris*) ethanol extract (AVE) and standard (Butylated hydroxytoluene, BHT)

Each value represents the mean value of three replicates.

Table 4. IC₅₀ (DPPH) of Lady's mantle (*Alchemilla vulgaris*) ethanol extract (AVE) and standard (Butylated hydroxytoluene, BHT)

Name of sample	BHT	AVE
IC ₅₀ (µg/mL)	8.52± 0.11 ^b	14.42±0.40 ^a

Each value represents the mean value of three replicates ±SD. Values with different superscript letters in the same row are significantly different at $p \leq 0.05$.

3.5. Effect of Lady's Mantle Ethanol Extract on BWG, FI and FER in Rats with Hepatotoxicity

The data presented in Table 5 demonstrates the impact of a four-week treatment with *Alchemilla vulgaris* ethanol extract (AVE) on body weight gain (BWG), food intake (FI), and food efficiency ratio (FER) in rats subjected to carbon tetrachloride (CCl₄)-induced hepatotoxicity. In the "Normal control" group, which included healthy rats, BWG was 0.990%, FI was 13.01 g/day/rat, and FER was 0.079, while the "Model control" group, which received CCl₄, exhibited substantial negative alterations with a 42.12% decrease in BWG, a 33.27% reduction in FI, and a 30.14% decline in FER. These changes validate the hepatotoxic effect of CCl₄, as evidenced by the marked deterioration in the rats' nutritional status and metabolic health. The groups treated with increasing doses of AVE (T1: 150 mg/kg, T2: 300 mg/kg, T3: 450 mg/kg) showed a dose-dependent reversal of these adverse effects. Specifically, T1 showed modest improvements in BWG (7.68%), FI (8.51%), and FER (10.79%), while T2 displayed more robust recovery with BWG increasing by 21.47%, FI by 19.11%, and FER by 21.69%. The highest dose in T3 led to the most significant improvements, with BWG increasing by 53.40%, FI by 33.85%, and FER by 28.95%, clearly indicating that *Alchemilla vulgaris* extract can counteract the physiological disruptions caused by CCl₄. These results reinforce the hepatoprotective efficacy of *Alchemilla vulgaris*, aligning with previous research, including findings by Al-Qarawi et al. [113], which indicated that the flavonoids in *A. vulgaris* L. enhance

metabolic rate, digestive enzyme activity, and general metabolic stimulation. The amelioration of CCl₄-induced declines in BWG, FI, and FER further suggests the involvement of the extract's bioactive constituents, likely phenolics, flavonoids, and terpenoids, in restoring liver function and metabolism. This is consistent with the study by El-Hadidy et al. [74], which highlighted the hepatoprotective effect of *Alchemilla vulgaris* extract against CCl₄-induced damage, attributing it to its antioxidant capacity. The potent antioxidant activity, supported by a low IC₅₀ value, underscores the extract's ability to neutralize oxidative stress, prevent lipid peroxidation, and mitigate liver cell damage. Furthermore, the anti-inflammatory properties of the phytochemicals help suppress hepatic inflammation, a major aspect of hepatotoxic pathology, as supported by Platzer et al. [114], who reviewed the anti-inflammatory and antioxidant functions of *Alchemilla* species. When compared with other natural hepatoprotective agents such as milk thistle (*Silybum marianum*), which is known for its silymarin content, *Alchemilla vulgaris* demonstrates similar therapeutic potential. A study by Elhassaneen et al. [115] on milk thistle also confirmed its efficacy in alleviating CCl₄-induced liver injury, thereby highlighting the value of plant-based treatments in liver disease. Overall, the evidence positions *Alchemilla vulgaris* extract as a promising and effective natural remedy for liver protection and recovery.

3.6. Effect of Lady's Mantle Ethanol Extract in Live Functions in Hepatotoxic Rats

The data in Table 6 explores the hepatoprotective effects of a four-week treatment with *Alchemilla vulgaris* ethanol extract (AVE) on rats with CCl₄-induced liver damage by examining critical parameters such as liver glycogen content, glucose-6-phosphate dehydrogenase (G6PD) activity, and glucose-6-phosphatase (G6Pase) activity. The "Normal control" group, indicative of healthy liver function, presented high glycogen content (12.78 mg/g) and elevated G6PD activity (14.92 U/g), with a comparatively low G6Pase activity (4.28 µmol/min/g), while the "Model control" group subjected to hepatotoxic insult showed drastic liver dysfunction, including a 68.86% reduction in glycogen, a 61.87% decline in G6PD activity, and a 200.84% surge in G6Pase activity. These biochemical disruptions signify impaired energy storage and metabolic balance due to liver cell damage. However, AVE treatment in increasing doses (T1, T2, T3) elicited a marked dose-dependent improvement. The highest dose group (T3) exhibited a 128.64% increase in glycogen content compared to the model control, nearing normal levels. Similarly, G6PD activity was significantly restored, with the T3 group achieving a 95.78% increase, suggesting the extract's efficacy in supporting hepatic metabolic pathways. Furthermore, G6Pase activity, which had surged in response to hepatotoxicity, was significantly curtailed—by 53.25% in the T3 group—demonstrating a reversal of pathological changes. Collectively, these findings underscore the potential of AVE to alleviate liver dysfunction induced by CCl₄ exposure. Mechanistically, the extract's ability to replenish glycogen reserves and reduce G6Pase activity

indicates protection of hepatocytes and restoration of glucose metabolism pathways, which are typically impaired by oxidative stress caused by toxins like CCl₄ [74]. Comparable results were observed by Elhassaneen et al. [115] using milk thistle, further substantiating the current findings. The recovery of G6PD activity is especially significant since G6PD is essential in the pentose phosphate pathway for NADPH production, a vital component of the cell's antioxidant system [116]. Its restoration by AVE implies both direct antioxidant action

and support for endogenous antioxidant defense mechanisms, a process likely mediated by the extract's rich phenolic and flavonoid content [117]. The consistent dose-dependent response across all parameters—glycogen content, G6PD, and G6Pase activities—not only reinforces the therapeutic potential of *Alchemilla vulgaris* but also aligns with established pharmacological patterns seen in botanical treatments, where increased extract concentrations yield stronger protective outcomes [5,70,92,96].

Table 5. Effect of four weeks treatment with Lady's mantle (*Alchemilla vulgaris*) ethanol extract (AVE) on BWG, FI and FER in rats with hepatotoxicity induced by CCl₄

Groups	BWG (%)		FI (g/day/rat)		FER	
	Mean $\pm$ SD	% of change	Mean $\pm$ SD	% of change	Mean $\pm$ SD	% of change
Normal control	0.990 $\pm$ 0.056 ^a	0.00	13.01 $\pm$ 0.39 ^a	0.00	0.079 $\pm$ 0.006 ^a	0.00
Model control (Hepatotoxic)	0.573 $\pm$ 0.039 ^c	-42.12	8.68 $\pm$ 0.43 ^c	-33.27	0.055 $\pm$ 0.007 ^c	-30.14
T1	0.617 $\pm$ 0.068 ^{bc}	7.68	9.42 $\pm$ 0.40 ^{bc}	8.51	0.061 $\pm$ 0.004 ^{bc}	10.79
T2	0.696 $\pm$ 0.041 ^b	21.47	10.34 $\pm$ 0.55 ^b	19.11	0.067 $\pm$ 0.003 ^{ab}	21.69
T3	0.879 $\pm$ 0.074 ^a	53.40	11.62 $\pm$ 0.58 ^{ab}	33.85	0.071 $\pm$ 0.004 ^a	28.95

Each value represents the mean $\pm$ SD (n=6) Means under the same column with different superscript letters exhibited significant at $P \leq 0.05$. AVE, *Alchemilla vulgaris* ethanol extract, normal control, healthy rats group; Model control, hepatotoxic rats group by CCl₄; T1, T2 and T3, model group treated with 150, 300 and 450 mg.kg⁻¹ body weight per a day of AVE. The percentage of change (%), with comparisons made between the hepatotoxic (Model) group and the normal group, as well as between the groups treated with AVE and the hepatotoxic (Model) group.

Table 6. Effect of four weeks treatment with Lady's mantle (*Alchemilla vulgaris*) ethanol extract (AVE) on live functions of hepatotoxic rats induced by CCl₄

Groups	Glycogen content (mg/g wet tissue)		Glucose-6-phosphate dehydrogenase activity (G6PD, U/g wet tissue)		Glucose-6-phosphatase activity (G6Pase, μ mole/min/g wet tissue)	
	Mean $\pm$ SD	% of change	Mean $\pm$ SD	% of change	Mean $\pm$ SD	% of change
Normal control	12.78 $\pm$ 0.81 ^a	0.00	14.92 $\pm$ 0.88 ^a	0.00	4.28 $\pm$ 0.26 ^d	0.00
Model control (Hepatotoxicity)	3.98 $\pm$ 0.52 ^d	-68.86	5.69 $\pm$ 0.92 ^d	-61.87	12.88 $\pm$ 1.04 ^a	200.84
T1	5.41 $\pm$ 0.40 ^c	35.93	7.98 $\pm$ 0.61 ^{cd}	40.25	9.78 $\pm$ 0.81 ^b	-24.04
T2	6.96 $\pm$ 0.39 ^c	74.87	10.04 $\pm$ 0.49 ^b	76.45	7.81 $\pm$ 0.54 ^{bc}	-39.34
T3	9.10 $\pm$ 0.73 ^b	128.64	11.14 $\pm$ 0.70 ^b	95.78	6.02 $\pm$ 0.23 ^c	-53.25

Each value represents the mean $\pm$ SD (n=6) Means under the same column with different superscript letters exhibited significant at $P \leq 0.05$. AVE, *Alchemilla vulgaris* ethanol extract, normal control, healthy rats group; Model control, hepatotoxic rats group by CCl₄; T1, T2 and T3, model group treated with 150, 300 and 450 mg.kg⁻¹ body weight per a day of AVE. The percentage of change (%), with comparisons made between the hepatotoxic (Model) group and the normal group, as well as between the groups treated with AVE and the hepatotoxic (Model) group.

3.7. Effects of Lady's Mantle Extract on Serum Lipid Profile in Hepatotoxic Rats

The data presented in Table 7 highlights the impact of a four-week treatment using *Alchemilla vulgaris* ethanol extract (AVE) on the serum lipid profile of rats with CCl₄-induced hepatotoxicity, including comparisons between a normal control group, a model control group (hepatotoxic), and three treatment groups (T1, T2, T3) receiving increasing doses of AVE (150, 300, and 450 mg/kg body weight/day, respectively). The model control group exhibited significant dyslipidemia, evidenced by a marked reduction in high-density lipoprotein cholesterol (HDL-c) by -45.03%, and a sharp rise in low-density lipoprotein cholesterol (LDL-c) by +77.17%, along with a modest increase in total cholesterol (TCho) by +11.90% and a slight decrease in triglycerides (TGs) by -13.61%, reflecting a lipid imbalance indicative of increased cardiovascular risk and impaired liver function. AVE treatment produced a dose-dependent corrective effect on the lipid abnormalities, where TCho was progressively

reduced by -3.34%, -4.86%, and -9.42% in T1, T2, and T3 groups respectively; HDL-c levels were substantially improved by +34.94% (T1), +57.83% (T2), and +65.06% (T3); and LDL-c concentrations declined notably by -12.27%, -17.79%, and -32.52% across the same groups. These results affirm AVE's efficacy in reversing dyslipidemia, with the highest dose showing the strongest therapeutic effect. This dose-dependent lipid correction supports existing literature on the hypolipidemic and hepatoprotective actions of plant-based extracts, particularly those rich in flavonoids, phenolic acids, and tannins known to influence lipid metabolism [118]. Mechanistically, such phytochemicals may decrease intestinal cholesterol absorption and enhance its biliary excretion [119], while also promoting reverse cholesterol transport, thereby increasing HDL-c levels and facilitating cholesterol removal from peripheral tissues [118]. These processes likely underpin the lipid-normalizing effect observed in this study. In line with this, Singdam et al. [120] demonstrated similar improvements in lipid profiles using another plant extract, suggesting that AVE's lipid-modulating properties are shared among phytochemicals

with antioxidant and anti-inflammatory activity. Additionally, AVE's hepatoprotective role may enhance hepatic lipid regulation indirectly by restoring liver function, allowing it to resume normal lipid synthesis and metabolism. The observed decline in TGs in the model control group, while contrary to the usual hypertriglyceridemia seen in liver disease, aligns with findings from hepatotoxicity models where impaired liver capacity suppresses TG synthesis due to dysfunctional lipoprotein production [76,77,121,122,123,124]. The gradual increase in TGs toward normal levels in the AVE-treated groups (T1–T3) thus likely reflects hepatic recovery rather than an adverse lipid response. Overall, the collective data underscore the therapeutic promise of *Alchemilla vulgaris* extract in mitigating dyslipidemia associated with liver injury, as demonstrated by improvements in key lipid markers, particularly HDL-c elevation and LDL-c reduction, supporting its value in plant-based strategies for metabolic and hepatic health.

3.8. Effect of Treatment with Lady's Mantle Ethanol Extract on Hepatic Glutathione Fractions in Hepatotoxic Rats

The data in Table 8 explores the impact of a four-week treatment with *Alchemilla vulgaris* ethanol extract (AVE) on hepatic glutathione fractions in rats subjected to CCl₄-induced hepatotoxicity, using a design that includes a normal control group, a model control group (hepatotoxic), and three treatment groups (T1, T2, T3) administered increasing doses of AVE (150, 300, and 450 mg/kg body weight/day). In the model control group, a severe depletion of the liver's antioxidant defense system is evident, particularly a 58.04% reduction in reduced glutathione (GSH) levels compared to the normal group, signaling significant oxidative stress since GSH is essential for neutralizing reactive oxygen species and maintaining hepatic redox balance. Although oxidized glutathione (GSSG) also decreases by 18.58%, the dominant loss in GSH reflects the exhaustion of the liver's protective mechanisms due to CCl₄ metabolism. AVE treatment shows a dose-dependent restoration of

glutathione status, with GSH levels increasing by 27.80% (T1), 46.78% (T2), and 89.49% (T3) compared to the model group, nearly restoring GSH to normal levels at the highest dose, thereby demonstrating the extract's strong antioxidant potential. GSSG levels also improve incrementally—rising 4.90% (T1), 7.27% (T2), and 10.85% (T3)—indicating that the dynamic equilibrium between GSH and GSSG is being reestablished, a sign of restored oxidative balance and hepatic resilience. These findings support the role of AVE in countering CCl₄-induced oxidative damage, likely by replenishing antioxidant reserves. This effect is consistent with a large body of literature documenting the hepatoprotective and antioxidant activities of phytochemical-rich plant extracts, particularly in models where CCl₄ triggers free radical generation and subsequent GSH depletion [5,125]. Previous studies have reported similar protective effects from other plant-based treatments rich in flavonoids, tannins, and phenolics, which are known to scavenge free radicals and support GSH homeostasis. For instance, Elhassaneen et al. [104], Mansour [126], Tahoon [127], and Elhassaneen et al. [77] demonstrated that polyphenol-rich extracts can enhance antioxidant enzyme activity and prevent GSH loss under oxidative conditions, likely through direct scavenging effects and by upregulating enzymes like glutathione reductase that regenerate GSH from GSSG [123]. Additional evidence comes from research by Mollazadeh and Hosseinzadeh [128], who showed that *Nigella sativa* ethanol extract also restored hepatic GSH levels and reduced oxidative stress following CCl₄ exposure, reinforcing the relevance of plant-derived antioxidants in liver protection. The dose-response observed in the present study further substantiates the conclusion that AVE's efficacy is closely tied to the concentration of its bioactive components. In summary, the results provide compelling evidence that AVE has potent antioxidant and hepatoprotective effects, with the capacity to restore the liver's endogenous glutathione-based defense system in a dose-dependent manner, supporting its potential application as a natural therapeutic agent against oxidative liver injury.

Table 7. Effect of four weeks treatment with Effect of four weeks treatment with Lady's mantle (*Alchemilla vulgaris*) ethanol extract (AVE) on serum lipid profile (mmol/L) in rats with induced by CCl₄

Groups	TGs		TCho		HDL-c		LDL-c	
	Mean ±SD	% of change	Mean ±SD	% of change	Mean ±SD	% of change	Mean ±SD	% of change
Normal control	1.47 ± 0.09 ^a	0.00	2.94 ± 0.12 ^c	0.00	1.51 ± 0.08 ^a	0.00	0.92 ± 0.04 ^c	0.00
Model control (Hepatotoxic)	1.27 ± 0.05 ^b	-13.61	3.29 ± 0.54 ^a	11.90	0.83 ± 0.10 ^d	-45.03	1.63 ± 0.11 ^a	77.17
T1	1.31 ± 0.03 ^{ab}	3.15	3.18 ± 0.41 ^a	-3.34	1.12 ± 0.07 ^c	34.94	1.43 ± 0.07 ^{ab}	-12.27
T2	1.38 ± 0.02 ^a	8.58	3.13 ± 0.45 ^{ab}	-4.86	1.31 ± 0.05 ^b	57.83	1.34 ± 0.04 ^b	-17.79
T3	1.42 ± 0.07 ^a	11.81	2.98 ± 0.21 ^{bc}	-9.42	1.37 ± 0.03 ^{ab}	65.06	1.10 ± 0.06 ^{bc}	-32.52

Each value represents the mean ± SD (n=6) Means under the same column with different superscript letters exhibited significant at $P \leq 0.05$. AVE, *Alchemilla vulgaris* ethanol extract, normal control, healthy rats group; Model control, hepatotoxic rats group by CCl₄; T1, T2 and T3, model group treated with 150, 300 and 450 mg.kg⁻¹ body weight per a day of AVE. The percentage of change (%), with comparisons made between the hepatotoxic (Model) group and the normal group, as well as between the groups treated with AVE and the hepatotoxic (Model) group. TGs, triglycerides, TCho, total cholesterol, HDL-c, high density lipoprotein cholesterol, LDL-c, low density lipoprotein cholesterol.

Table 8. Effect of four weeks treatment with Lady's mantle (*Alchemilla vulgaris*) ethanol extract (AVE) on hepatic glutathione fractions of hepatotoxic rats induced by CCl₄

Groups	Reduced glutathione conc. (GSH, $\mu\text{mol/mg}$ tissue protein)		Oxidized glutathione conc. (GSSG, $\mu\text{mol/mg}$ tissue protein)	
	Mean $\pm$ SD	% of change	Mean $\pm$ SD	% of change
Normal control	7.03 $\pm$ 0.25 ^a	0.00	0.701 $\pm$ 0.033 ^a	0.00
Model control (Hepatotoxic)	2.95 $\pm$ 0.48 ^c	-58.04	0.571 $\pm$ 0.061 ^b	-18.58
T1	3.77 $\pm$ 0.31 ^{bc}	27.80	0.599 $\pm$ 0.044 ^b	4.90
T2	4.33 $\pm$ 0.29 ^b	46.78	0.613 $\pm$ 0.048 ^{ab}	7.27
T3	5.59 $\pm$ 0.53 ^{ab}	89.49	0.633 $\pm$ 0.031 ^a	10.85

Each value represents the mean $\pm$ SD (n=6) Means under the same column with different superscript letters exhibited significant at $P \leq 0.05$. AVE, *Alchemilla vulgaris* ethanol extract, normal control, healthy rats group; Model control, hepatotoxic rats group by CCl₄; T1, T2 and T3, model group treated with 150, 300 and 450 mg.kg⁻¹ body weight per a day of AVE. The percentage of change (%), with comparisons made between the hepatotoxic (Model) group and the normal group, as well as between the groups treated with AVE and the hepatotoxic (Model) group.

Table 9. Effect of four weeks treatment with Lady's mantle (*Alchemilla vulgaris*) ethanol extract (AVE) on hepatic glutathione fractions of hepatotoxic rats induced by CCl₄

Groups	Reactive oxygen species (ROS, U/ml tissue)		Malondialdehyde concentration (MDA, nmol/g)	
	Mean $\pm$ SD	% of change	Mean $\pm$ SD	% of change
Normal control	0.61 $\pm$ 0.04 ^d	0.00	637.38 $\pm$ 11.56 ^d	0.00
Model control (Hepatotoxic)	1.94 $\pm$ 0.12 ^a	218.03	967.98 $\pm$ 15.78 ^a	51.87
T1	1.52 $\pm$ 0.08 ^b	-21.65	873.61 $\pm$ 8.34 ^b	-9.75
T2	1.19 $\pm$ 0.06 ^{bc}	-38.66	821.94 $\pm$ 10.89 ^b	-15.09
T3	0.90 $\pm$ 0.09 ^c	-53.61	716.20 $\pm$ 13.71 ^c	-26.01

Each value represents the mean $\pm$ SD (n=6) Means under the same column with different superscript letters exhibited significant at $P \leq 0.05$. AVE, *Alchemilla vulgaris* ethanol extract, normal control, healthy rats group; Model control, hepatotoxic rats group by CCl₄; T1, T2 and T3, model group treated with 150, 300 and 450 mg.kg⁻¹ body weight per a day of AVE. The percentage of change (%), with comparisons made between the hepatotoxic (Model) group and the normal group, as well as between the groups treated with AVE and the hepatotoxic (Model) group.

3.9. Effect of Treatment with Lady's Mantle Ethanol Extract on Hepatic Glutathione Fractions in Hepatotoxic Rats

Table 9 presents the effects of a four-week treatment with Lady's mantle (*Alchemilla vulgaris*) ethanol extract (AVE) on hepatic oxidative stress markers in rats with CCl₄-induced hepatotoxicity, involving a normal control group, a model control group (hepatotoxic), and three treatment groups (T1, T2, T3) administered increasing AVE doses (150, 300, and 450 mg/kg body weight/day). The model control group, reflecting CCl₄-induced liver damage, shows severe oxidative stress marked by a dramatic 218.03% increase in Reactive Oxygen Species (ROS) and a significant 51.87% rise in malondialdehyde (MDA) levels compared to the normal group, where ROS, known for their high reactivity, contribute to cellular damage, and MDA, a key lipid peroxidation byproduct, serves as an essential oxidative stress biomarker (Su et al., 2014). Treatment with AVE reveals a dose-dependent antioxidative response, with ROS levels reduced by -21.65% (T1), -38.66% (T2), and -53.61% (T3), and MDA concentrations decreased by -9.75%, -15.09%, and -26.01% respectively, compared to the model control, indicating that the highest dose (T3) substantially restores these markers near normal levels and underscores AVE's potent antioxidant action against CCl₄-induced hepatic damage. This inverse relationship between AVE dose and oxidative stress markers suggests that the therapeutic efficacy is directly related to the concentration of bioactive constituents in the extract. These findings align with existing literature on plant-derived antioxidant and hepatoprotective agents, particularly within the well-

established CCl₄ model that consistently exhibits increased ROS and MDA levels as markers of induced oxidative liver damage [25,104].

The observed protective effect of AVE is plausibly attributed to its rich phytochemical composition—namely flavonoids, tannins, and phenolic acids—compounds renowned for their radical scavenging and lipid peroxidation-inhibiting abilities [129]. Supporting evidence includes similar studies such as that by Mollazadeh and Hosseinzadeh [128], where *Avena sativa* extract also significantly reduced MDA levels in a comparable hepatotoxicity model, reinforcing the current findings. The dose-related ROS and MDA reductions highlight that AVE's antioxidative effect is concentration-dependent, a typical pattern in phytotherapeutic research. Moreover, the MDA decline reflects improved protection of hepatic cellular membranes, as AVE's radical-scavenging action helps prevent lipid peroxidation and preserves membrane integrity, which is critical for normal liver function [129,130]. Consequently, this study not only validates AVE's antioxidant capability but also strongly supports its therapeutic potential in preventing and managing oxidative liver injury.

3.10. Effect of Treatment with Lady's Mantle Ethanol Extract on the Liver Histopathology in Hepatotoxic Rats

The provided data describes the histopathological findings in the livers of five groups of rats following different treatments (Figure 1). Group 1 (control), the liver tissue appears normal, with a healthy histoarchitecture, as expected for a control group. Group 2 (CCl₄-induced

hepatotoxicity), this group shows significant liver damage, including steatosis (fatty changes in hepatocytes), focal hepatocellular necrosis (localized cell death), and inflammatory cell infiltration in both the liver tissue and the portal areas. These findings are typical of severe liver injury induced by CCl₄, a well-known hepatotoxin. Group 3, 4, and 5 (treated groups): these groups, presumably treated with different doses or forms of Lady's mantle extract, show less severe damage compared to Group 2. The primary finding across these groups is Kupffer cell activation and, in Group 3, leucocytic exocytosis (white blood cells migrating out of blood vessels). The absence of necrosis and extensive inflammation suggests that the *Alchemilla vulgaris* ethanol extract (AVE) provided a protective effect against CCl₄-induced liver damage, reducing the severe signs of injury seen in Group 2. The presence of Kupffer cell activation, however, indicates that there's still an ongoing response to the CCl₄ insult, but it's likely a less destructive, more controlled inflammatory process.

The results are consistent with a growing body of research demonstrating the hepatoprotective effects of various plant extracts, including those from the *Rosaceae* family, which includes Lady's mantle. The CCl₄-induced hepatotoxicity model is a standard method for inducing acute liver injury in research, and the resulting pathological features, like steatosis and necrosis, are well-documented [74, 96,131,132]. The observations from the control and CCl₄-only groups in this study align perfectly with this established model. The key finding is the significant improvement in liver histopathology in the treated groups compared to the CCl₄-only group. The presence of Kupffer cell activation without extensive necrosis in the treated groups is a particularly important observation. This suggests that AVE may modulate the inflammatory response rather than completely preventing it. Kupffer cells are the resident macrophages of the liver and are crucial in the initial inflammatory response to toxins like CCl₄. Their activation, in the absence of severe tissue destruction, could be interpreted as a positive sign, part of the liver's natural defense and repair mechanism, possibly facilitated by the compounds in the extract. Several studies have shown similar effects with other herbal extracts. For instance, research on natural products has demonstrated their ability to reduce CCl₄-induced liver injury by preventing lipid peroxidation and modulating inflammatory pathways [133]. The phenolic compounds and flavonoids present in *Alchemilla vulgaris* are likely responsible for its observed protective effects, acting as antioxidants to neutralize the CCl₄-induced free radicals and as anti-inflammatory agents to mitigate the destructive inflammatory cascade [74]. The results of this study add to this body of evidence, specifically for Lady's mantle, by providing histological proof of its protective action. The dose-response relationship among groups 3, 4, and 5, though not explicitly detailed, would further clarify the efficacy and mechanism of the extract. The results are also comparable to a study by El-Hadidy et al. [74] which highlighted the antioxidant properties of *Alchemilla vulgaris* extracts. They found that the extract significantly reduced oxidative stress, a primary cause of CCl₄-induced damage.

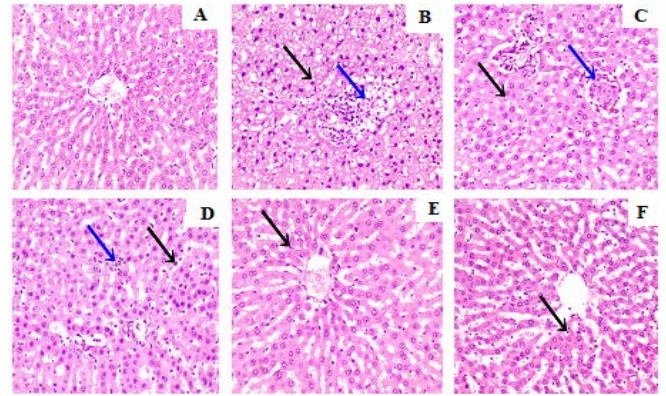


Figure 1. Effect of treatment with Lady's mantle (*Alchemilla vulgaris*) ethanol extract (AVE) on the liver histopathology of rats with CCl₄-induced hepatotoxicity

Photo A. Photomicrograph of liver of rat from group 1 showing normal histoarchitecture of hepatic tissue, **Photo B.** Photomicrograph of liver of rat from group 2 showing steatosis of hepatocytes (black arrow) and focal hepatocellular necrosis associated with inflammatory cells infiltration (blue arrow), **Photo C.** Photomicrograph of liver of rat from group 2 showing steatosis of hepatocytes (black arrow) and focal hepatocellular necrosis associated with inflammatory cells infiltration (blue arrow), **Photo D.** Photomicrograph of liver of rat from group 3 showing Kupffer cells activation (black arrow) and few leucocytic exocytosis (blue arrow), **Photo E.** Photomicrograph of liver of rat from group 4 showing Kupffer cells activation (black arrow), and **Photo F.** Photomicrograph of liver of rat from group 5 showing Kupffer cells activation (black arrow) (H & E X 200).

Conclusion

A standard and widely-accepted animal model for studying liver toxicity involves the use of carbon tetrachloride (CCl₄). The aerial portions of Lady's mantle (*Alchemilla vulgaris*) have demonstrated their utility as a functional food. This is due to their high concentration of bioactive compounds possessing antioxidant qualities, which can help protect against or lessen the liver damage caused by chemical agents such as CCl₄. The protective action of *Alchemilla vulgaris* against CCl₄-induced toxicity likely operates through several key pathways. These include: 1) enhancing liver function, which supports the organ's ability to process and detoxify substances, 2) improving the body's antioxidant defenses, thereby preventing lipid peroxidation, a process where free radicals damage cell membranes, 3) improving lipid metabolism, which helps regulate how the liver handles fats, and 4) inducing positive changes in the microscopic structure of liver tissue, as observed through histopathology. These results suggest that *Alchemilla vulgaris* has the potential to function as a hepatoprotective agent to combat liver injury. Future research should concentrate on how to incorporate Lady's mantle as a natural food additive in a variety of everyday items, including drinks, meals, and other food products.

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Conflicting Interests

The authors acknowledge that certain information has been omitted from this article to facilitate its publication.

Authors' Contribution

Yousif Elhassaneen was instrumental in the study's design and execution, contributing to protocol development, supervising experiments, and collecting conceptual data. He also validated the results and statistical analyses and assisted with manuscript writing and revision. Alaa Mosa performed the experimental work, collected, organized, and analyzed the results, and compiled foundational information. He was the primary author of the initial manuscript draft. Mai Gharib helped prepare the study protocol and oversee the practical experiments. Her work also included gathering conceptual insights, confirming the accuracy of the results, and drafting the manuscript.

Abbreviations

AA, antioxidant activity, Abs, absorbance, AVE, *Alchemilla vulgaris* ethanol extract, BCB, β -carotene bleaching (BHT, butylated hydroxytoluene, BWG, body weight gain, CCl₄, carbon tetrachloride, DPPH, 2,2-diphenyl-1-picrylhydrazyl, FI, feed intake, dimethyl sulfoxide, G6PD, glucose-6-phosphatase, FER, feed efficiency ratio, G6Pase, glucose-6-phosphate dehydrogenase, GSH, reduced glutathione, GSSG, oxidized glutathione, HDL-c, High density lipoprotein-cholesterol, IC₅₀, half-maximal inhibitory concentration LDL-c, low density lipoprotein-cholesterol, MDA, malondialdehyde, MLP, mulberry leaves powder, ROS, reactive oxygen species, SD, standard deviation, TGs, triglycerides.

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