The Biological Effect of *Achillea millefolium* Extract on Some Histological Aspects of the Liver and Kidneys of Cholesterol-Induced White Rats

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Abstract

The purpose of the study was to compare the protective effect of the yarrow extract on liver and kidney tissue in hyperlipidemia rats at the concentration of 100, 200, and 400 mg/kg and compare it with the reference drug simvastatin. A total of 42 Swiss male rats were used and were grouped into six categories namely G1 (negative control), G2 (positive control - hyperlipidemia), G3 (simvastatin 2.5 mg/kg), G4 (yarrow 100 mg/kg), G5 (yarrow 200mg/kg) and G6 (yarrow 400 mg/kg). The 1H-NMR was used to analyze the extract and the liver and kidney analyzed by histology through H&E staining. H-NMR analysis showed the existence of terpenoid, phenolic and glycosidic compounds. Group G2 demonstrated severe liver (necrosis, cell destruction, sinusoids, hemorrhage and lymphocytic infiltration) and kidney (glomerulosclerosis, hemorrhage, and lymphocytic infiltration) pathological alteration. Group G3 had improvement having some residual glomerulosclerotic atrophy. Group G4, G5 and G6 exhibited the same partial improvement and progressive glomerulosclerotic atrophy, little hemorrhage, and lymphocytic infiltration of variable extent, as well as foci of mononuclear cells and Kupffer cell proliferation in the liver. Yarrow extract has a partial action on liver and kidney tissues with its active antioxidant and anti-inflammatory constituents.

Keywords: *Achillea millefolium*, Histological, Liver, Kidneys of Cholesterol, White Rats

Introduction

Achillea millefolium L., also known as yarrow, is a significant medicinal plant in the Asteraceae family. It is an ancient medication in traditional medicine that is used to treat liver and digestive discomforts, and inflammation [1]. The presence of bioactive compounds, including flavonoids, terpenoids, and phenolic acids, which have antioxidant and anti-inflammatory effects, is the cause of its therapeutic effects [2]. Recent researches have revealed that ethanolic extract of yarrow is rich in total phenols (232.99 mg/g) and flavonoids (168.25 mg/g) [3]. Cholesterol is a widespread health issue that results in the impaired functioning of the liver and kidney, which leads to oxidative stress and inflammation of the tissues of these vital glands [4]. An in-depth analysis has revealed that different forms of yarrow have hepatoprotective and anti-hyperlipidic properties, which is why they are good options to conduct an experiment [5]. Research has shown that yarrow extracts have protective potential on liver and kidney in different animal models which enhances organ functionality and

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minimizes pathological histological alterations [6]. According to a recent study, a yarrow aqueous extract helps in reducing liver enzymes and liver kidney biomarkers in rats that experience oxidative stress [6]. At the cellular level, a recent review identified the role of yarrow in improving antioxidant defense systems in liver and kidneys by increasing the activity of antioxidant enzymes, including superoxide dismutase, catalase and glutathione and by decreasing levels of malondialdehyde (Sekiou). One cause is high cholesterol [7]. Biological activity of yarrow is strongly associated with the concentration of active compounds. The chemical studies conducted on the extracts by GC-MS have identified a large variety of bioactive compounds in various extracts [8]. Past research has also associated the chemical makeup of yarrow with the protective effect of the chemical against metabolic disorders[9]. Although the therapeutic effects of yarrow are under investigation in several disease models, little is known about its impacts on the liver and kidney in models of hypercholesterolemia [10]. Hence, the purpose of this research is to assess the therapeutic impact of yarrow extract at various concentrations on the liver and kidney tissues in hyperlipidemia rats, and compare it with the reference drug simvastatin.

Material and Methods

Plant Collection and Preparation

Yarrow (*Achillea millefolium*) was collected from the city of Al-Qaim in Anbar Governorate during August 2025. The roots were removed, leaving only the aerial parts (stem, leaves, and flowers). The specimens were cleaned of soil and any adhering impurities, then spread out in a well-ventilated area away from direct sunlight, ensuring they were turned regularly and daily to prevent fungal growth. After confirming the specimens were completely dry, they were ground in an electric grinder, and the powder was stored in special containers until use.

Chemicals

Simvastatin is supplied by the Jordanian International Company and each tablet has 20 mg of active ingredient. Cholesterol was purified, purchased by the American company Sigma-Aldrich.

Preparation of the Alcoholic Extract of Yarrow

The alcoholic extract was prepared according to the method of [11]. Fifty grams of the plant powder were soaked in 500 ml of ethanol (70% ethanol + 30% distilled water) in a glass beaker, which was then sealed tightly with silicone. The beaker was placed in a shaking water bath for 24 hours at 110°C. The solution was then filtered through several layers of medical gauze to remove the sediment. The purified solution was taken and placed in an ultrasonic device at 65 Hz for half an hour. The clear product was then placed on a magnetic stirrer until the ethanol and water evaporated, and the product concentrated into a gel. This gel was poured into glass dishes to allow the extract to dry, forming a dry layer that was collected for later use.

Experimental Animals

The 42 male Swiss white rats were aged 6-8 weeks and weighed 180-200 grams when this study was carried out. The animals were procured at the college of veterinary medicine, Tikrit University. The rats were randomly assigned plastic breeding cages and 7 rats each cage. The cages were put in the animal house at the college of Education in Anbar university, Department of Life Sciences. The experimental animals were put under conditions of standard laboratory conditions including a normal lighting cycle (12 hours of light and 12 hours of darkness) and a constant

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temperature of 25degC was maintained during experiment. The animals were given food and water on a daily, round-the-clock basis. The cages were washed and decontaminated every day. The rats took a complete week to settle down to the new laboratory conditions and to be fully free of any diseases or injuries that may bias the results of the study.

Induction of Hyperlipidemia and Experimental Design.

Hyperlipidemia was induced in all but the negative control group at the end of the acclimatization period. The method used in the study by [12] was feeding the animals on a standard diet that was supplemented with 5 percent of purified cholesterol. This diet lasted six weeks in a row so that a considerable rise in lipid and cholesterol levels could be achieved. Once the presence of hyperlipidemia had been established, the animals were randomly separated into six experimental groups of seven rats (n = 7) as follows:

Group 1 (G1): Negative control group - fed the standard diet during the whole experiment.

Group 2 (G2): Positive control group - fed on high-fat diet only (5% cholesterol).

Group 3 (G3): Treatment with a high-fat diet and simvastatin at a dosage of 2.5mg/kg/day.

Group 4 (G4): High-fat diet with yarrow extract (100mg/kg/day).

Group 5 (G5): Control (control) diet + 200mg/kg/day yarrow extract.

Group 6 (G6): Diet high fat content and 400 mg/kg/day yarrow extract.

The dogs received the treatment over 4 weeks in a row, in an oral format.

Dissection and Organ Collection of animals.

The animals were then left without food in the dissection (10- 12 hrs) after treatment (4 weeks). To cut into the internal organs of the animals, a surgical incision was made in the abdominal cavity, beginning at the bottom, and moving up to the heart. The liver and kidneys were removed after which the surrounding fatty and connective tissue were removed by sharp, sterile dissecting instruments. After washing the removed organs with distilled water to get all the remaining blood or impurities off them, they were dried using filter paper. The tissue samples (liver and kidney) were then stored in 10% formalin solution in a 10ml sterile glass vials and allow it to stand till the later histological sectioning and examination were carried out as per the procedure described in [13].

Histological Examination

Histochemical sections were made following the procedure mentioned in [13]. The procedures included repairing the samples with formalin, rinsing them with running water, drying them with the progressively higher levels of ethanol, permeating them with xylene, and embedding them into paraffin wax. Microtome was used to cut-off the samples into 5-um-thick sections, which were stained with hematoxylin and eosin (H&E).

H-NMR Analysis

Proton nuclear magnetic resonance (1H-NM) spectra were recorded for the ethanolic extract of yarrow (Achillea millefolium L.) using a Bruker DRX 400 MHz spectrometer with an HR-MAS probe, under standard operating conditions. All measurements were performed in triplicate for each sample to ensure the reproducibility and accuracy of the results. The spectra were processed using zero-filling and baseline correction techniques. The 1H-NMR spectra were used as input

for statistical chemical analysis using Pirouette software (version 2.0) [14]

Statistical Analysis

The statistical data collected in this study were analyzed using SPSS (version 25). To test for differences between the six experimental groups, one-way ANOVA was used. When statistically significant differences were found between the groups, post-hoc comparisons were conducted using the Least Significant Difference (LSD) test to identify statistically distinct groups. A $p<0.05$ was considered a significant value for differences between groups. All results are presented as the mean ± standard deviation.

RESULTS AND DISCUSSION

Proton NMR spectroscopy analysis of the ethanolic extract of yarrow revealed distinct spectral signals in three main chemical shift ranges. In the low chemical shift region (δ 0.5–3.0 ppm), a multi-signal band was observed, attributed to aliphatic protons of terpene compounds and sesquiterpene lactones. In the intermediate region (δ 3.0–5.5 ppm), spectral peaks were observed, reflecting the presence of protons bound to carbohydrates and glycosides. In the aromatic region (δ 6.0–9.0 ppm), In the millionth percentile, distinctive signals were recorded confirming the presence of polyphenolic and flavonoid compounds. The overall spectral pattern reflects the extract's considerable chemical diversity and richness in bioactive compounds .

Extract analysis using H-NMR technique

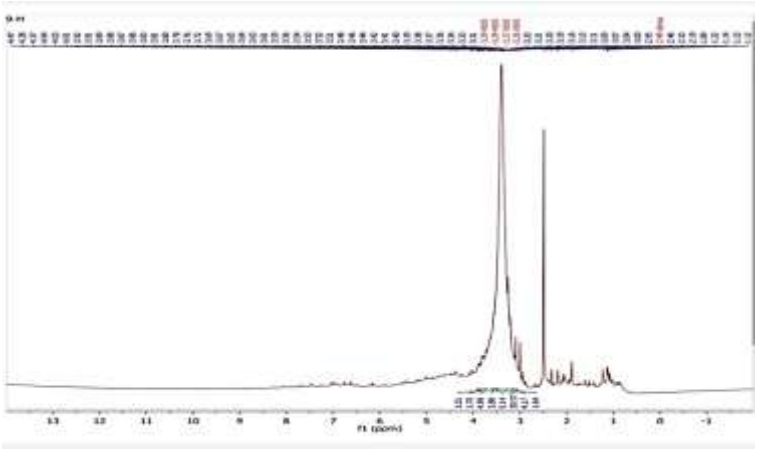

Figure 1. Proton nuclear magnetic resonance spectrum (^{1}H-NMR) of the alcoholic extract of *Achillea millefolium.*

indicated a normal renal tissue architecture, that is, intact Bowman Capsule, normal glomeruli with open capillaries, and normal cortical renal tubules without deposition. Such findings

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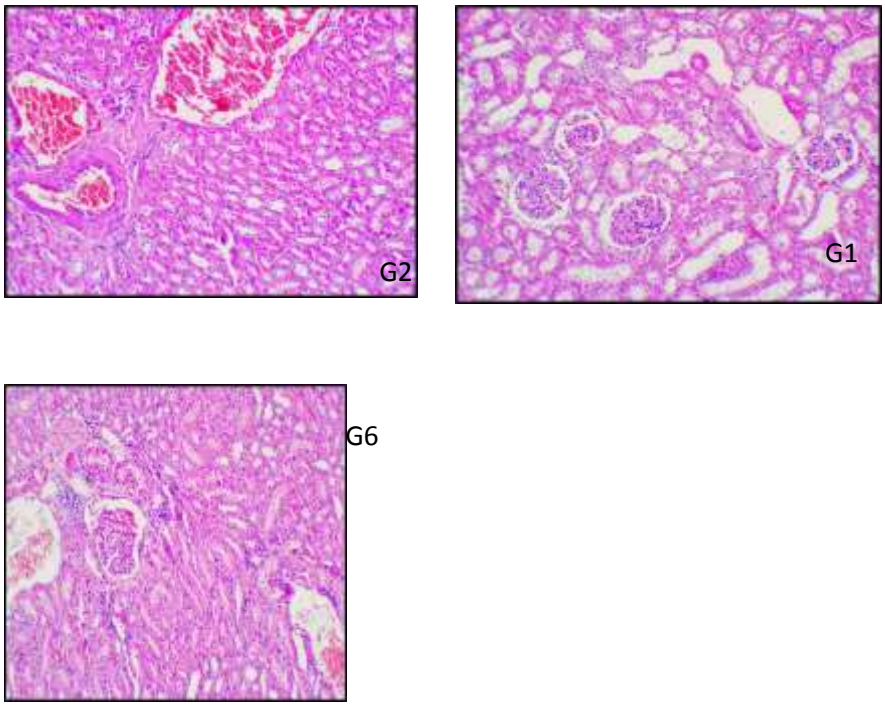
indicate the histological integrity of the kidney in the normal conditions and provide a fundamental point of reference with which the other groups can be compared.

Figure (3): A histological section of the kidney of the positive control group (G2) having hyperlipidemia under H&E staining. Histological samples of the kidney in the untreated control group with hyperlipidemia (G2) depicted definite pathological alterations i.e., atrophy of the glomerular bundle, presence of interstitial hemorrhages and periglomerular lymphocytic infiltration densities. These alterations validate the destruction of the functional unit of the kidney

improvement to the G5 group, with normal Bowman's capsule and intact glomeruli, and continued glomerular atrophy, limited hemorrhage, and similar degree of lymphocytic infiltration. This indicates that the therapeutic effect reached saturation at a concentration of 200 mg/kg.



Figures 2. Histological sections of the kidney

Liver

Histological section of the liver of the negative control group (G1) using H&E staining. Histological examination of the liver in the negative control group showed a normal structure consisting of an intact central vein, regular hepatocytes with central nuclei, and normal-sized, non-congested blood sinuses. These results represent a baseline for the normal structure of liver tissue. Histological section of the liver of positive control group (G2) hyperlipidemia. The untreated hyperlipidemia group (G2) had a severe pathological alteration in the histological section of the liver, evident in large regions of necrosis, the destruction of hepatocytes with disruption of normal platelet organization, dilated sinuses, hemorrhages, and infiltration by lymphocytes in large amounts. These alterations are an indication of an acute inflammatory process and gross tissue destruction.

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Figure (7): histological section of liver of group receiving 400 mg/kg (G6) yarrow extract stained with using H and E staining. The 400 mg/kg (G6) yarrow extract treated group demonstrated comparable amelioration with the other treatment groups, with normal

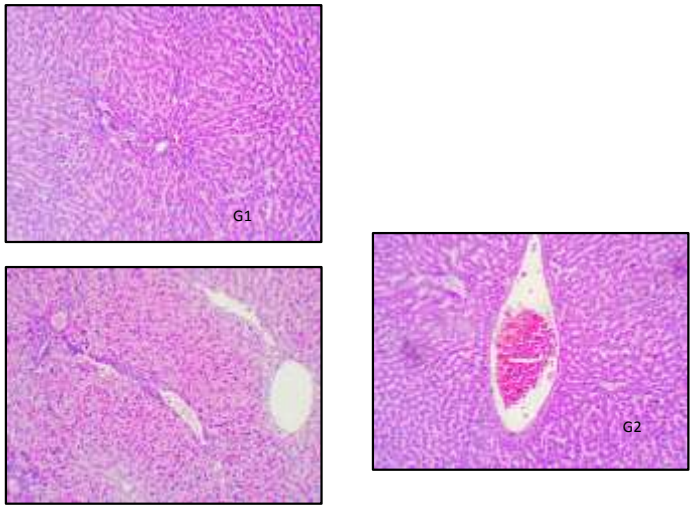

Figure 3. Histological sections of the liver.

As shown in Figure (2), the negative control group (G1) showed normal kidney histological structure, which is consistent with studies that confirmed tissue integrity under normal conditions (Baran et al., 2025). The histological sections in Figure (3) show severe pathological changes in the untreated hyperlipidemia group (G2), represented by glomerular atrophy, hemorrhage, and lymphocytic infiltration, which confirms the role of hyperlipidemia in stimulating oxidative stress and inflammation of renal tissues [7, 15]. Renal damage in hyperlipidemia is associated with the accumulation of oxidized lipids and the activation of inflammatory pathways leading to podocyte damage. A study showed that oxidized cholesterol promotes activation of the Gq pathway via the AT1-LOX1 receptor complex, leading to oxidative stress, increased inflammatory response, and damage to endothelial cells and podocytes [16]. As shown in Figure 4, the simvastatin treatment group (G3) showed significant improvement with near-complete resolution of bleeding and lymphocytic infiltration, attributed to simvastatin's ability to lower total cholesterol, triglycerides, and low-density lipoprotein levels. A study has shown that simvastatin at a dose of 2.5 mg/kg/day reduces TNF-α and MDA levels in kidney tissue, thus contributing to kidney protection against damage caused by hyperlipidemia [17]. A more recent study also demonstrated that simvastatin alleviates kidney injury by inhibiting the NF-κB pathway, reducing inflammatory cytokines, and enhancing antioxidant defenses by increasing SOD and CAT [17]. The continued limited atrophy in some glomeruli may be due to the limited regenerative capacity of rheumatoid cells (GPx) [7]. The histological sections in Figures (5, 6, and 7) show partial improvement in the groups treated with yarrow extract (G4-G5 and G6), although glomerular atrophy, limited hemorrhage,

and varying lymphocytic infiltration persist. This improvement is attributed to the active compounds in yarrow. One study demonstrated that an ethanolic extract of yarrow at a dose of 250 mg/kg reduced creatinine, urea, uric acid, MDA, and TNF-α levels, while increasing SOD, CAT, GPX, and GSH levels in a cisplatin-induced nephrotoxicity model [18]. Another study showed that yarrow extract at doses of 100 and 200 mg/kg improved kidney function and reduced histological changes in a doxorubicin-induced nephrotoxicity model by inhibiting oxidative stress and inflammation [6]. A study confirmed that a 300 mg/kg dose of yarrow extract protects against acetaminophen-induced acute kidney injury through its antioxidant effect (Mirzaei et al., 2024). The disappearance of lymphatic infiltration in G4 with its reappearance in G5 and G6 may be due to individual differences in response to treatment [19]. These effects are associated with the presence of phenolic and flavonoid compounds in the extract. A study showed that the methanolic extract of yarrow contains a high percentage of total phenols and flavonoids, along with high levels of chlorogenic acid, caffeic acid, and salicylic acid [19]. The convergence of the results for G5 and G6 indicates that the therapeutic effect reaches saturation at a concentration of 200 mg/kg, consistent with studies that showed similar efficacy for doses of 200 and 400 mg/kg [6], as illustrated in Figure (8). The negative control group (G1) showed normal liver structure, consistent with studies confirming the integrity of liver tissue under normal conditions [20]. Histological sections in Figure 9 show severe pathological changes in the untreated hyperlipidemia group (G2), including extensive necrosis and destruction of hepatocytes, dilated sinuses, hemorrhage, and extensive lymphocytic infiltration. This damage is associated with fat accumulation and the stimulation of oxidative stress, as obesity and dyslipidemia lead to ectopic fat deposition, which promotes inflammation and structural damage in hepatocytes [21]. Furthermore, excess fat load stimulates the production of reactive oxygen species and oxidative stress, which damage cell membranes [22], as shown in Figure 10. The simvastatin-treated group (G3) showed significant improvement, with few focal clusters of mononuclear cells and limited Kupffer cell proliferation. This effect is attributed to simvastatin's ability to inhibit HMG-CoA reductase, thereby reducing cholesterol synthesis and promoting the removal of low-density lipoprotein (LDL) from the blood. Studies have also shown that simvastatin reverses changes in inflammatory markers and improves histological structure in animal models fed a high-cholesterol diet [23]. Histological sections are shown in Figures 11, 12, and 13 Significant improvement was observed in the groups treated with the yarrow extract (G4-G5-G6), with continued focal clusters of mononuclear cells and Kupffer cell proliferation. This improvement is attributed to the active compounds in yarrow, as a study showed that the aqueous alcoholic extract of yarrow at doses of 25 and 100 mg/kg led to a significant decrease in the levels of liver enzymes ALT and AST, triglycerides, total cholesterol, and low-density lipoprotein in diabetic rats (Rezaei et al., 2020. These effects are attributed to yarrow's high content of flavonoids (39.45 ± 1.84 µg/mg) and phenolic compounds, This showed 72% antioxidant activity at a concentration of 900 µg/ml [3]. A comprehensive review confirmed that yarrow possesses lipid-lowering properties, promotes collagen proliferation regulation, inhibits inflammatory responses, and exhibits significant antioxidant activity [24]. Furthermore, the phenolic compounds in yarrow contribute to hepatocyte protection by enhancing antioxidant enzyme activity and reducing oxidative stress [25]. Kupffer

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cell proliferation indicates continued liver cleansing activity and the removal of damaged cells. A study showed that yarrow extract reduces levels of inflammatory cytokines such as IL-8, IL-18, TNF-α, and COX-2, which explains the improvement in tissue structure and the reduction in lymphatic infiltration in the treated groups (Montaser et al., 2019). Furthermore, the antioxidant properties of yarrow contribute to protecting liver cells from oxidative stress and promoting tissue recovery [25].

Conclusion

The extract of yarrow (*Achillea millefolium*) in its three concentrations shows a partial therapeutic effect on the liver and kidney tissues of rats with hyperlipidemia, and this is attributed to its content of terpene and phenolic compounds with antioxidant and anti-inflammatory activity.

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