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# Investigate the Effects of *Achillea millefolium* Plant Extract as A Hepatoprotection on Carbon Tetrachloride-Induced Liver Toxicity in Female Rats

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## Abstract

***Background:*** The liver is the largest gland that plays a role in the plant metabolism and the removal of toxins from the body. *Achillea millefolium* is familiar herb in traditional medicine. Objective: to investigation the effect of achillea millefolium extract in experiment rats those exposed to hepatic damage by carbon tetrachloride. ***Methods:*** *Achillea millefolium* was collected in the province of Ibb, Yemen, and extracted by maceration method, then physical and phytochemical evaluation were performed for the extract. Finally, evaluation the effect of extract on liver function tests were done on twenty-one female rats were divided into seventh groups, two extract doses 100 mg/kg and 200 mg/kg were used, $CCl_4$ was used for inducer liver toxicity, and liv52 drug used as positive control. ***Results:*** the extract has worthy hepatoprotective activity against $CCl_4$ and the groups treated only *Achillea millefolium* extract as 100mg and 200mg the histological sections no showed any histopathological changes exaggerated from the normal in liver. Also, GPT, GOT, and ALP levels increased with $CCl_4$ treatment but these liver enzyme levels were reduced when treated the rats by concentration 200mg/kg of extract more than by concentration 100mg/kg. ***Conclusion:*** the extract of *Achillea millefolium* has good hepatoprotective activity, may due to presence of flavonoids.

**Keywords**: *Achillea millefolium*, carbon tetrachloride, hepatoprotective and histology.



## INTRODUCTION

Recently, medicinal plants are used as an alternative to drugs around the world and also the herbal medicine has the less effect than the drug and have low cost [1].

Plants have formed the basis of the complicated system of traditional medicine and are constantly providing people with new remedies. Although some of the therapeutic properties attributed to plants have been proven wrong, medicinal plant therapy is based on empirical evidence from hundreds and probably thousands of years of the world's use of herbal medicine to treat disease cost [2]. *Achillea millefolium* is pre-known in traditional herbal medicine also in veterinary medicine [3].

*Achillea millefolium* have over a hundred and twenty species, divided into five sections*: Sectio Millefolium* (ADANS.) W.D.J. KOCH, *Sectio plamica (MILL.)* W.D.J. KOCH, *Sectio achillea*, *Sectio babounya* (DC.) O. HOFFM. and *Sectio arthrolepis* (BOISS) [4].

*Achillea millefolium* used for anti-inflammatory action like rheumatoid and skin inflammation [1], use as antispasmodic [5], also use for antipyretic and analgesic [6].

Liver is the largest organ in the body; it is a highly vascular organ that plays a role in metabolism and ridding of toxic substances [7]. Liver diseases are problems in medicine with high death rate and satisfying [8], like acute liver failure hepatitis [9, 10].

Determination of liver function health by various enzymes such as alkaline phosphatase (ALP), alanine aminotransferase (GPT) and aspartate aminotransferase (GOT). Therefore, this study aims to verify the effect of *Achillea millefolium* hydroalcoholic extract in experimental female rats exposed to carbon tetrachloride damage.

# MATERIALS AND METHODS

**Study area**

This study was performed in Pharmaceutical Laboratories in Al-hikma University, while the animals' trials were carried out in the Department of Biology, Faculty of Applied Sciences –Sana'a University and the liver biological enzymes were measured in Laboratories of Al-Gomhory Government Hospital, Sana'a-Yemen. The histological study for liver and kidney specimens in Al-Thobhani Laboratories**,** Sana'a-Yemen.

**Ethical consideration**

Ethical approval and approval of study protocols by the Research Ethics Committee of Sanaa University in 10/03/2021 (code: 422/2021) and the study followed common ethical principles in phytochemical and experimental pharmacology researches. The Animals included in this study were investigated and housed according to European community guidelines and Guidelines for the Housing of Rats in Scientific Institutions.

**Plant collection and extraction**

*Achillea millefolium* leaves were collected from Ibb Mountains, Maayeen valley, Yemen, in December 2020. The sample was washed by distilled water and sterilized by methanol spray then dried within the shade at temperature for 3 days till complete drying. Fresh powder of *Achillea millefolium* leaves (540g) was macerated by hydroalcoholic solvent (70% of ethanol and 30% Distilled water) for three days with intermittent shaking [11], and then the extract was separated by filter aid and filter paper. Subsequently, Rotary Evaporator (BUCHI Rotavapor R-200, Germany) was used to concentrate the extract, which dried by freeze dryer (Labconco., United States).and stored at 4°C in the dark bottles [12].

**Physical and phytochemical evaluation of the extract**

Organoleptic property, pH and solubility of the extract were determined, then phytochemical tests were performed to identify the major constituents of *Achillea millefolium* such as carbohydrate, Glycosides, Saponins, Flavonoids, Triterpenoids and alkaloids [13].

**Experimental on animals**

**Experimental design**

Twenty- one female rats were divided into 7 groups with 3 rats per each group and two doses of the plant extract (a hundred mg/kg, two hundred mg/kg), were used to evaluation the effect of hepatoprotective in rats. $CCL_4$ were used for induced hepatotoxicity in rats. All the rats were weighed in the first day till seven day and the average weight was (201± 0.12).

Group I: were administrated with 0.1ml of distilled water as a control.

Group II: were administrated with 0.2ml of a hundred mg/kg (0.5g of the plant +5ml of D.W) of the extract of the *Achillea millefolium*.

Group III: were administrated with 0.4 ml of two hundred mg/kg (0.5g of the plant+5ml of D.W) of the extract of the *Achillea millefolium*.

Group IV: were administrated with 0.2ml of $CCL_4$ in olive oil (20% of $CCL_4$ +80% of olive oil), then in the second day till seven day were administrated with 0.1ml of D.W.

Group V: were administrated with 0.2ml of $CCL_4$ in olive oil (20% of $CCL_4$ +80% of olive oil), then in the second day till seven day were treated with 0.2ml of a hundred mg/kg of the extract of the *Achillea millefolium.*

Group VI: were administrated with 0.4ml of $CCL_4$ in olive oil (20% of $CCL_4$ +80% of olive oil), then in the second day till seven day were treated with 0.4ml of 200 mg/kg of the extract of the *Achillea millefolium.*

Group VII: were administrated with 0.2ml of $CCL_4$ in olive oil (20% of ccl4 +80% of olive oil), then in the second day till seven day were treated with 0.06 ml of the liv52 drug as positive control.

The rats were injected intraperitoneal for seven days, in the eight-day blood sample from rats' eye was taken by capillary tube and collected in Eppendorf tube. The serum was separated by centrifugation to evaluation the liver function enzymes like alkaline phosphatase (ALP), aspartate aminotransferase (GPT) and alanine amino transferase (GOT) [14]. After that, all the rats were anesthetic by using chloroform then merciful killing, and small pieces from the kidney and liver of each rat were taken after anatomy and stored in formalin 10% for histological examinations [15].

# STATISTICAL ANALYSIS

The IBM SPSS V. 22.0 was used in this study. The study variables were described as a percentage and the mean.

# RESULTS

**Results of Physical and phytochemical evaluation of the extract**

The extract has a powder appearance, greenish black color, and strong characteristic odor, and bitter taste, acidic pH 5.5, slightly soluble in water and soluble in ethanol. The extract also has all the following phytochemical compounds alkaloid, tannins, saponin, glycoside, flavonoid, triterpenoid and phenols.

**Results of liver enzymes analysis**

Effect of hydroalcoholic extract of *Achillea millefolium* is reduce the enzymes of rats like (GPT), (GOT) and (ALP) by the dose of 200mg/kg of extract more than the dose 100mg/kg and increase the dose of extract that lead to give perfect results on the rats' liver enzymes.

**ALP activity**

The results had shown high levels of ALP to 203.3 ±15.3 Unit/L in rats that injected with $CCL_4$ compared with in control (untreated rats) that was 150.7 ± 29 Unit/L. Reducing the ALP in rats that injected by $CCL_4$ in the first day when treated with 100mg/kg of *Achillea millefolium* to 198.3±15Unit/L, with 200 mg/kg of *Achillea millefolium* to 177.3±6 Unit/L and liv52 drug (positive control) to 171.7±12 Unit/L. This suggests that the plant extract in turn had a positive result on the repair of ALP activity in Figure 1.

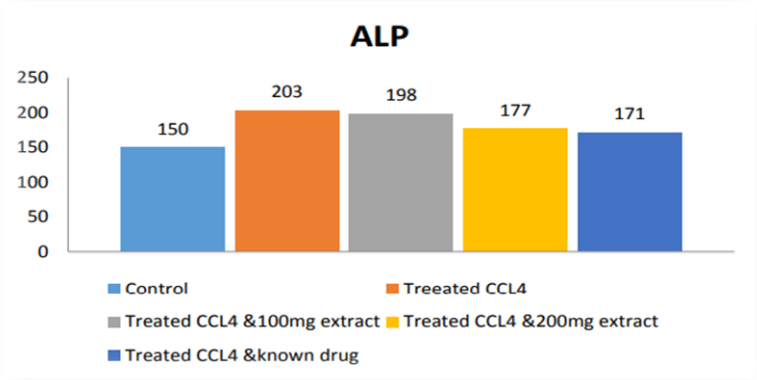


**Fig-1: ALP activity appears in $CCL_4$-treated rats compared to control rats (untreated rats) based on treatment with *Achillea millefolium* extract 100, 200 mg/kg and drug liv52 (positive control).**

**GPT activity**

The results had shown high levels of GPT to 90.7±4 Unit/L in rats that injected with $CCL_4$ compared with in control (untreated rats) that was 41±3.6 Unit/ L.

Reducing the GPT levels in rats that injected by $CCL_4$ in the first day when treated with 100mg/kg of *Achillea millefolium* to 80.7±4 Unit/L, with 200 mg/kg of *Achillea millefolium* to 50.7±1.5 Unit/L and liv52 drug to 40.7±1.5 Unit/L. This suggests that the plant extract in turn had a positive result on the repair of GPT activity in Figure 2.

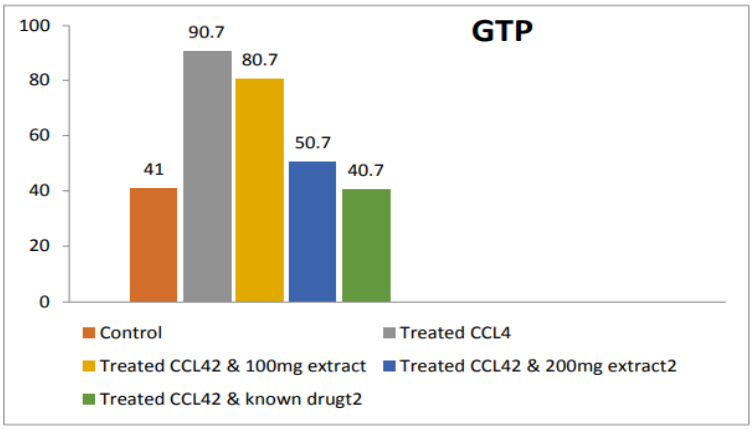


**Fig-2: Appear GPT activity in treated rats with $CCL_4$ comparing with control rats (untreated rats) according to treatment with the extract 100, 200 mg/kg of *Achillea millefolium* and liv52 drug (positive control).**

**GOT activity**

The results had shown high levels of GOT to 99.7±1.5.Unit/L in rats that injected with $CCL_4$ compared with in control (untreated rats) that was 40.7±1.5 Unit/L. Reducing the GOT levels in rats that injected by $CCL_4$ in the first day when treated with 100mg/kg of *Achillea millefolium* to 97.7±0.4 Unit/L, with 200 mg/kg of *Achillea millefolium* to 59.7±1.5 Unit/L and liv52 drug to 44.7±1.5 Unit/L. This suggests that the plant extract in turn had a positive result on the repair of GOT activity in Figure 3.

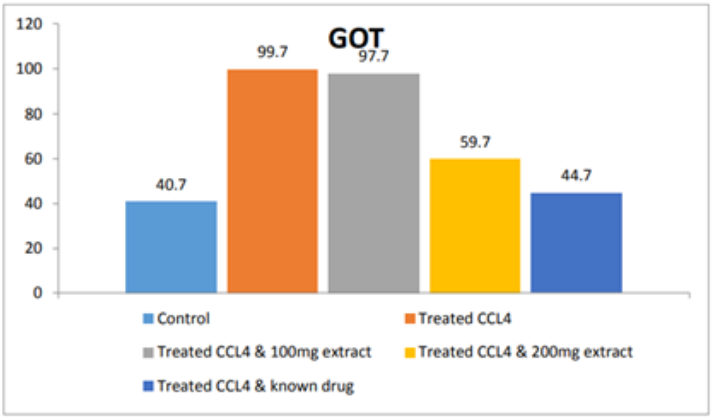


**Fig-3: Appear GOT activity in treated rats with $CCL_4$ comparing with control rats (untreated rats) according to treatment with the extract 100, 200 mg/kg of *Achillea millefolium* and liv52 drug (positive control).**

**Results the histological studies on sample of liver rats**

Regarding to the liver, the histological changes during hepatocellular lesions as well as the protective effects of *Achillea millefolium* extract were first identified from the histological analysis of the liver section. In the untreated group, liver histology showed a healthy and normal distribution of hepatocytes with clearly visible nuclei, portal triad and central vein (Fig. 4. A). However, after $CCL_4$ treatment, the central vein was congested and some of these central veins showed little damage to their endothelial layer in the $CCL_4$-treated group compared to the untreated group. (Fig 4. B). Additionally, other pathological changes were observed in the $CCL_4$-treated group. These changes include the finding of Kupffer cells having ingested brown material, inflammatory cell infiltration, hepatic vein congestion, unhealthy cells, and the presence of bulging cells and some degenerative cells. (Fig. 5) However, the liver sections from the group treated with *Achillea millefolium* extract 100mg and 200mg and $CCL_4$ shows regular arrangement of hepatocytes with and the integrity of the hepatic cells was closed to normal and displayed a little dilated central vein. On the other hand, the groups treated group treated only *Achillea millefolium* extract as 100mg and 200mg the histological sections not showed any histopathological changes exaggerated from the normal in liver.

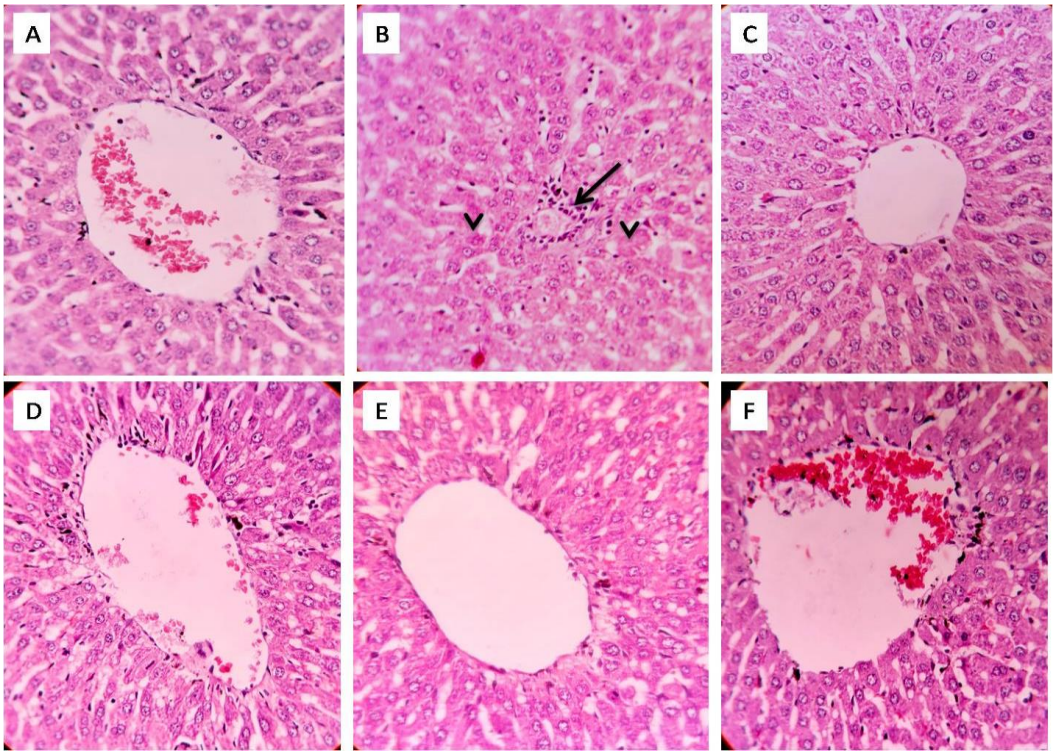


**Fig-4: A- Untreated control group, B- $CCL_4$, C- 100 mg, D- 200 mg, E- 100 mg with $CCL_4$, F- 200mg with $CCL_4$ (Large arrow indicate of inflammatory cells infiltrate the arrow head indicted if degenerative hepatic cells.**

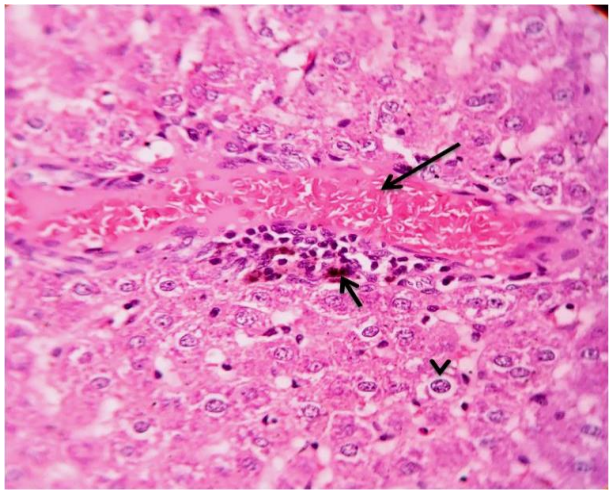

**Fig-5: $CCL_4$ treated (large arrow indicates of central vein occlusion with blood, while the small arrow indicates with inflammatory cells infiltrate, the arrow head indicate of ballooning hepatic cells**

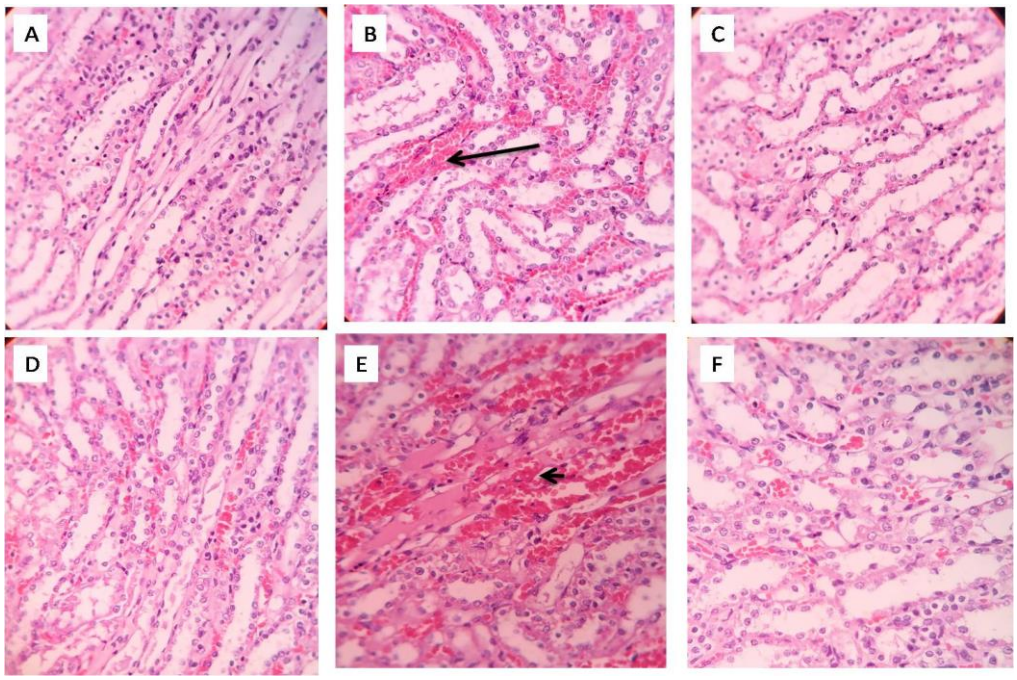


**Fig-6: A- Normal control group, B- $CCL_4$, C- 100 mg D- 100mg +$CCL_4$, E- 200 mg+ $CCl_4$, F- 200 mg (large and small arrow indicate of hemorrhage)**

The kidney tissues section of different groups evaluated at the level of cortex and medullar regions. The normal group showed normal histology of Bowman's capsule with well defines proximal and distal convoluted tubules. The medulla also showed normal tubule structure without any histopathological changes. On the other hand, the $CCL_4$ treated group histological tissues sections showed mild hemorrhage at the cortex area and moderate to severe hemorrhage at the in-medulla area (Fig 6 B). Furthermore, some of hyaline deposits and casts were noted in between at the cortex area and down in the medulla in $CCL_4$ group treated tissues section. The toxicity effect of $CCL_4$ is attenuated on kidney hemorrhage attenuated by the 100mg *Achillea millefolium* extract, while the 200mg may not.

## DISCUSSION

It is well established that the liver is the primary target for detoxification and that the absorption of certain drugs results in substantial hepatic arrest due to the production of pro-oxidant reactive oxygen species (ROS, which in turn results in the activation of a cellular defect which effect on certain biomolecules such as DNA and proteins [16]. The principal cause for the usage of $CCL_4$ is to result in this harm, as carbon tetrachloride induces hepatotoxicity in rats ensuing in excessive necrosis and harm to the structural integrity of the liver, which produces an extraordinary growth in stages liver enzymes [17]. This compound is characterized by its ability to produce CCL3, which is thought to be a free radical that alkylates cellular protein and leads to liver damage manifested in

cirrhosis and necrosis [18]. In this study, a significant increase in GPT, GOT and ALP levels after treatment with CC4 was found, therefore such an increase should be avoided; it is necessary to inhibit the production of reactive metabolites [19]. This study reveals that ethanolic extract of yarrow *millefolium* possesses a desirable effect on $CCL_4$-treated rats while there was no defective effect on normal rats. This was clear in the reduction in liver enzyme levels in rats treated with the plant extract after their treatment with $CCL_4$. Previous studies had shown that the active compound found in *Achillea millefolium* species is primarily considered a potent antioxidant compound; flavonoids [20-22]. The results coincided with investigations that rats infected with $CCL_4$ and treated with 200 mg/ml showed the best protective effect against carbon tetrachloride-induced liver damage. Therefore, the possible hepatoprotective mechanisms of *Achillea millefolium* extract may be due to the prevention of the lipid oxidation process, inhibiting the activity of cytochrome p450, stabilizing the hepatocellular membrane and improving protein synthesis [23]. Preliminary phytochemical studies indicated the presence of flavonoids in *Achillea millefolium*. Flavonoids consumed in large amounts in the diet are known to protect the liver [24]. Therefore, the anti-hepatic toxicity of *Achillea millefolium* may be due to the presence of flavonoids.

**Authors' Contributions**

Abdulkarim K. Alzomor: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Writing - Original Draft, Writing - Review & Editing, Visualization, Supervision; Nada H. Al-Absi: Investigation, Resources, Writing - Original Draft; Helmy S. Al-Salahi : Investigation, Resources, Writing - Original Draft; Abubaker F. Al-hssany: Investigation, Resources, Writing - Original Draft

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**Conflict of Interest**

The author is the supervisor of this research in the department, he received no funding for this research but the university and the other laboratories that are mentioned in the acknowledgment helped him by supplying some materials, the equipment and the animal rats and the suitable places to carry out this project.

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