

# Hepatoprotective activity of *Artemisia vulgaris* L. against Cisplatin induce hepatotoxicity in mice

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## Abstract

**Background:** *Artemisia vulgaris* L. (*Artemisia V.*) has a great role in treatment of many diseases like gastrointestinal diseases, inflammation and fungal infections. Other important activity is antioxidant which put this plant as a good choice to reduce liver toxicity. In this study, the hepatoprotective effect of Iraqi *Artemisia vulgaris* L. against the liver toxic chemotherapy cisplatin were determined in mice.

**Methods:** Two different doses of Iraqi *Artemisia vulgaris* L. extract were given to mice with and without cisplatin as a liver toxicity inducer. The blood level of liver enzymes ALT, AST, ALP and TSB were measured.

**Results:** The elevation in blood level of liver enzymes reduced significantly in pre-treated mice with *Artemisia* extracted (200 and 400 mg/Kg) and cisplatin, compared with mice group that received cisplatin alone.

**Conclusion:** Hepatoprotective activities of *Artemisia vulgaris* L. extract were confirmed, and 400 mg/kg/day the extract was the most effective dose. This finding provides scientific evidence for the use of safe medicinal herbs such as *A. vulgaris* in the treatment of liver toxicity produced by other medications such as chemotherapy.

**Keywords:** *Artemisia vulgaris*, Cisplatin, Hepatoprotective, hepatotoxicity, herbs.

## 1. INTRODUCTION

Cisplatin is widely used chemotherapeutic drug. It has shown effective effects in treating several human cancers including advanced lung, cervical, esophageal, progressive testicular and ovarian cancers [1].

Liver enzymes elevation has been noted with cisplatin. In addition, transaminase, LDH, and bilirubin rises were also reported with cisplatin. These changes were developed within the first day of cisplatin use and vanish within approximately two weeks [2, 3].

Cisplatin well known to accumulate in hepatocyte and causing liver injury, it's thought that cisplatin induce reactive oxygen species and enhance intrinsic caspases leading to liver cells apoptosis [4]. Many hepatocytes adverse effects reported because of cisplatin administration, it may lead to glutathione (GSH) decline and liver architecture disruption [5].

As oxidative stress is the most key source of the generation of liver toxicity, and free radicals cause direct and indirect liver damage, the use of herbs and plants that carry out antioxidant mechanisms can play a main part in decreasing cisplatin-induced hepatotoxicity [6, 7].

*Artemisia vulgaris* L. (*Artemisia V.*), or mugwort, be classed to compositae family, which has a widely spreading in globally including Asia, northern Africa and Europe.

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This herb has an excessive reputation in the history of traditional medicine, it called the “mother of herbs” in the centenarian [8].

Artemisinin has been commercialized as antimalarial drugs and in addition it has antiviral effect [9]. Recently, *Artemisia* showed to has some activity spectrum against virus SARS-CoV-2 and disease COVID-19 [10].

Scientific studies have proved that *Artemisia* shows antioxidant, reduce lipid level, antispasmodic, analgesic, antifungal and antibacterial effect, reduce blood pressure, and broncholytic activity [11-13].

The existence of essential oils, flavonoids, sesquiterpene lactones, phenolic acids, coumarins, and other groups of metabolites explain its wide spectrum of uses and applications. Little doses of *Artemisia* show no or negligible toxicity. Neurotoxicity noted when large doses like 3 g/kg used [14].

Studies tells that *Artemisia V.* rich source of several heavy metals like (Fe, Mn, Ni, Zn, Cu and Cr) that's promote many reactions and act as a Co-factor for many biological activities if taken by suitable amounts. These elements at all facilitate carbohydrate oxidation, control serum glucose level, normal growth, wound healings and regulate lipid profile [15].

*Artemisia* has other medical uses in gynecology and gastrointestinal diseases [16]. Other researches show it has strong anti-inflammatory, analgesics, bronchodilations and hepatoprotective activities [17, 18].

This study was aimed to examine the Hepatoprotective effect of Iraqi *Artemisia vulgaris* extract against cisplatin liver toxicity in mice.

## 2. MATERIALS AND METHODS

### 2.1 Materials

Chemicals. All chemicals were of analytical grade and purchased from Sigma.

### 2.2 Methods

#### 2.2.1 Preparation of extract

The aerial parts of *Artemisia V.* were purchased from a local herbalist in Baghdad and authenticated by botanical expert. After cleaning, the aerial parts have been grinding gently by an electrical blender, weighed to be ready for the extraction procedure. After that defatted by N-hexane and extracted with 80% ethanol by Soxhlet ((WiseTherm)) and filtered by filter paper, then dried and concentrated by rotary evaporator ((IsoLab)) with 109RPM at 30 °C. The extract weighted and stored in dark place at room temperature. The extract was soluble in normal saline and distilled water.

#### 2.2.2 Phytochemical screening

The presence of alkaloids, polyphenolic compounds, flavonoids, tannins, polysaccharide and saponins had been

identified by performing a preliminary phytochemical screening test of *Artemisia* extract as following [19]:

#### 1 – Detection of Alkaloids (Draganoff test)

Solution A prepared by dissolving of sixty milligram of Bismuth sub nitrate in 0.2 ml HCL. Solution B prepared by dissolving 600 mg potassium iodide in 1 ml distilled water. Solution A and B added to the extract. The production of orange color indicated alkaloid presence.

#### 2 – Detection of Polyphenolic compounds

In this test, some drops of 3 % ferric chloride solution were added to the extract. Black to brown intense color indicates the presence of polyphenolic compounds.

#### 3 – Detection of Flavonoids.

NaOH solution added to the extract solution and left for 1 min. Bright yellow color indicates the presence of flavonoids

#### 4 – Detection of Tannins.

Three drops of lead acetate solution (1%) were mixed with the extract. Thick gelatinous precipitate indicates the presence of tannins.

#### 5 – Detection of Polysaccharide.

A lique of one ml of the extract was mixed with two ml of Benedict reagent, and use boiling bath for 5 min. the production of red deposit color indicates the presence of Polysaccharide.

#### 6 – Detection of Saponins.

This test was performed by well shaking of the extract solution. The result of foam indicates the Saponins presence.

### 2.2.3 Experimental design

Thirty 12-weeks-old Swiss albino male mice (about 25-30gm) were used in this experiment. The mice were housed in a temperature around 25 °C, humidity controlled room (54–78% humidity). Mice were allowed free access to water and standard pellet diet.

The animals were divided into 5 groups, each of 6 as follows:

The first (control group) received 0.01 mL/kg normal saline. Second group received intraperitoneal injection of Cisplatin (Cisplatin, 50mg/100ml, was (cisplatin provided from central pharmacy in Baghdad), 10mg/kg we induce liver injury at day seven. Third group received *Artemisia* extract orally by stomach tube at concentration 400 mg/kg once daily for 10 days. Fourth group received the extract by the same method at concentration 200 mg/kg for 10 days, then injected at day seven with cisplatin 10mg/kg. Fifth group received 400 mg/kg for 10 days, then injected at day seven with cisplatin 10mg/kg.

At day 10, all animal sacrificed and blood sample collected by cardiac puncture, the blood samples lift to coagulate and centrifuged at 3,000 rpm speed for 20 min ready for liver function tests.

### 2.2.4 Statistical Analysis

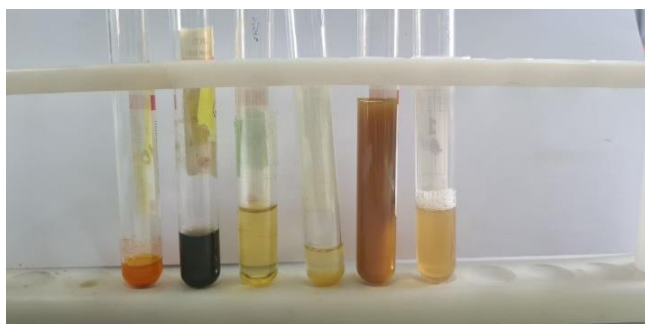
### 2.3 Statistical analysis

All experiments were carried out at least four times. Data are expressed as mean ± standard deviation and SPSS v20 (IBM) was used to carry out a one-way analysis of variance (ANOVA) followed by post-hoc Tukey HSD test. A P-value of less than 0.05 is considered significant.

## 3. RESULTS

### 3.1 Phytochemical screening by chemical tests

The outcomes of preliminary chemical screening tests shown in table 1 and figure 1 indicate the presence of alkaloids, polyphenolic compounds, flavonoids, tannins, polysaccharide and saponins in the extract of aerial parts of Iraqi Artemisia V.



**Figure 1:** Phytochemical Qualitative Analysis of Artemisia vulgaris extract. From left to right (Alkaloid, polyphenolic compounds, flavonoids, tannins, polysaccharide and saponins).

**Table 1:** The preliminary outcome of phytochemical screening tests for Artemisia vulgaris extract.

Phytochemicals	Results
Alkaloid	Present
Polyphenolic compounds	Present
Flavonoids	Present
Tannins	Present
Polysaccharide	Present
Saponins	Present

### 3.2 Toxicity testing

Oral administration of Artemisia V. extract in doses up to 400 mg/kg showed no symptoms of morbidity or mortality.

Therefore, LD50 is more than 400 mg/kg showing the tested plants extract are safe for use at this dose.

### 3.3 Effect on serum biochemical constituents

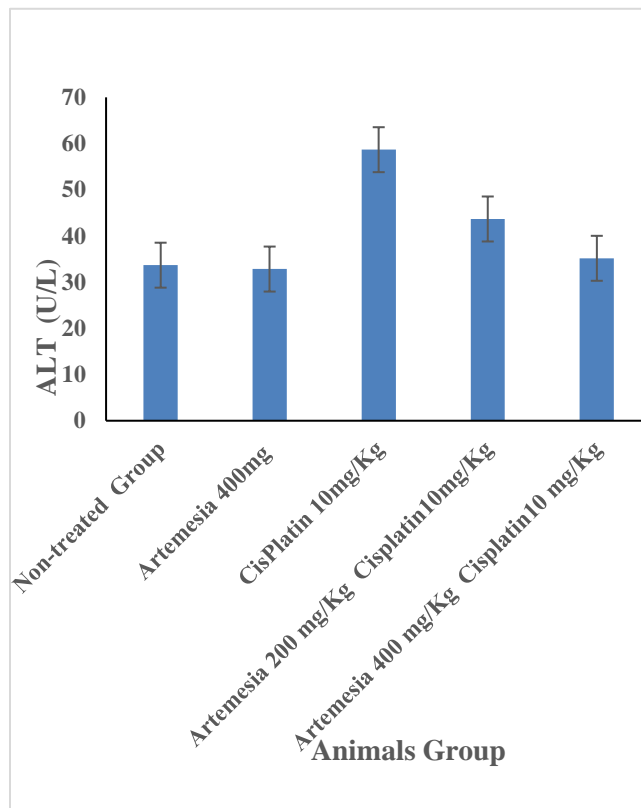
The Cisplatin significantly raised the ALT, ALP and TSB activity as compared to non-treated. Whilst Cisplatin did not significantly elevate AST serum level compared to non-

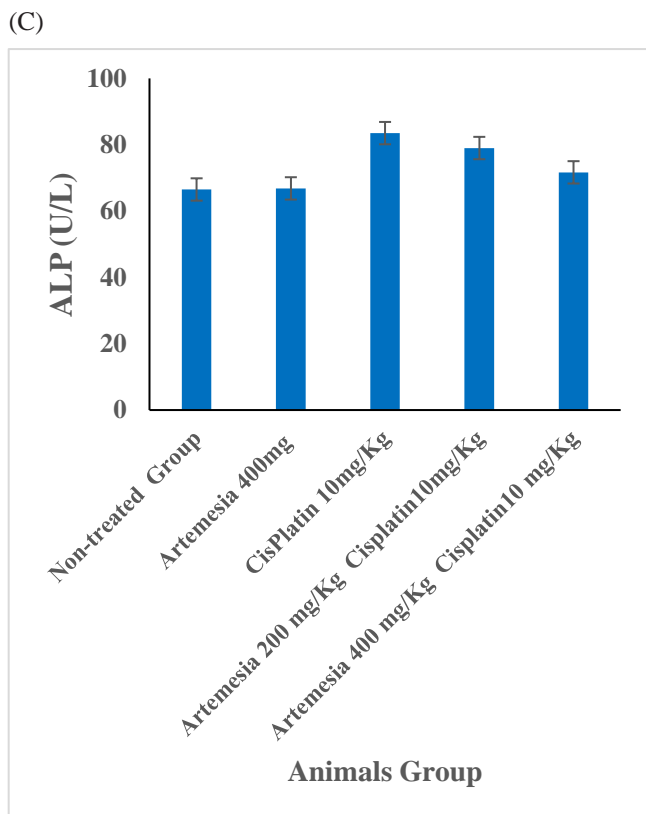
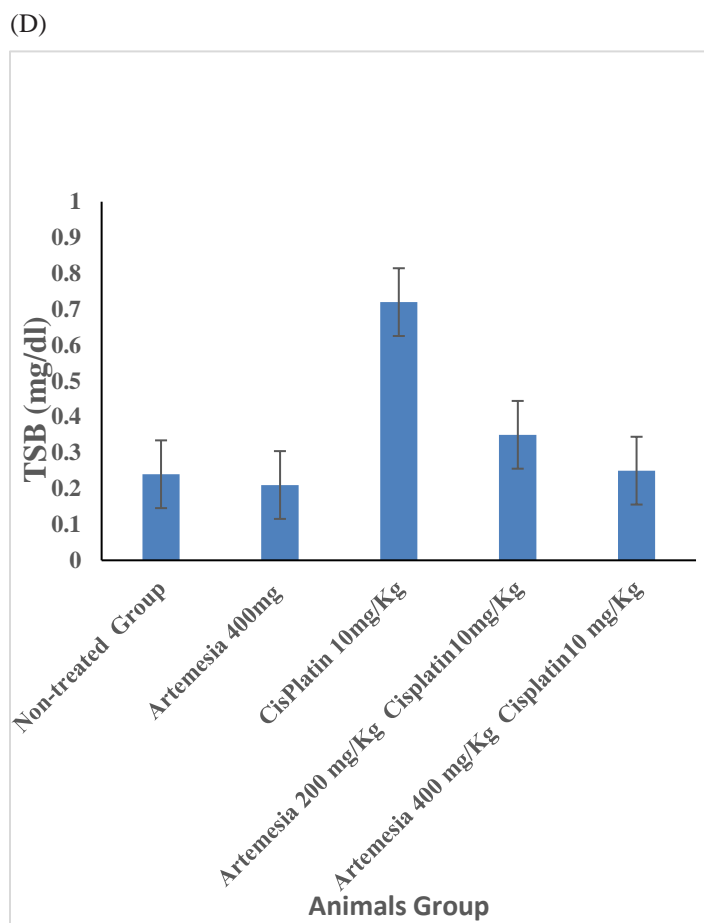
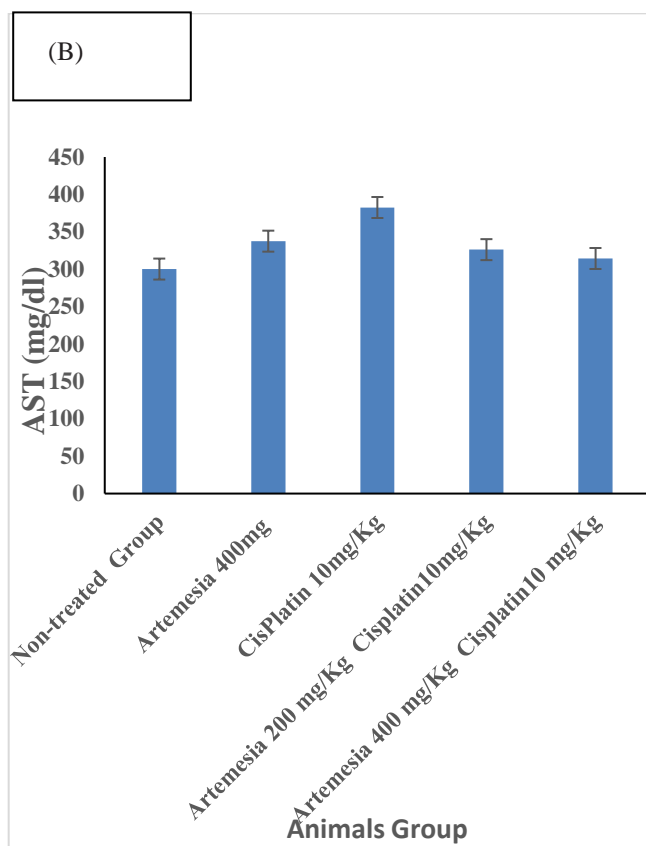
treated control animals. There were no significant differences in liver enzymes level between non-treated animals group and Artemesia 400 mg/Kg treated group (Figure 2).

Artemesia extract in small dose (200 mg/ Kg) significantly ( $p < 0.05$ ) reduced the serum level of ALT, ALP and TSB. More significant ( $p < 0.001$ ) effects were observed when mice were treated with 400 mg/ Kg of Artemesia extract compared to Cisplatin Group (Figure 2).

However, there was no significant difference in AST level in mice-treated with 200 mg and 400 mg per Kilogram of Artemesia ( $326.16 \pm 60.38$  and  $314.33 \pm 66.26$ , respectively) compared to Cisplatin Group ( $382.50 \pm 60.38$ ) (Figure 2). Find Table 2 for more details.

(A)





**Figure 2:** Effect of Artemesia extract on serum enzymes level in mice with Cisplatin –induced hepatotoxicity.

Mice were treated as follow as mentioned previously. Panels are representative as follow: (A) ALT enzyme, (B) is AST enzyme, (C) ALP enzyme and (d) TSB.

Data were analyzed using one-way analysis of variance (ANOVA) followed by post-hoc Tukey HSD test and are presented as the mean ± SD of 6 animals, \*\*p<0.001, \*p<0.05.

**Table (2)** The effects of Artemesia Vulgaris on serum enzyme levels in mice.

Enzymes	Non-treated Group	Artemesia 400 mg/kg Group	Cisplatin Group 10 mg/kg	Artemesia 200 mg/kg – Cisplatin 10mg/kg	Artemesia 400 mg/kg – Cisplatin 10mg/kg
ALT	**33.67 ± 7.45	32.83 ± 5.74	58.67 ± 7.66	*43.67 ± 12.88	**35.16 ± 6.17
AST	300.16 ± 67.27	337.50 ± 44.58	382.50 ± 60.38	326.16 ± 60.38	314.33 ± 66.26
ALP	**66.5 ± 5.36	66.83 ± 4.40	83.50 ± 6.15	*79.00 ± 4.82	**71.67 ± 6.45
TSB	**0.24 ± 0.05	0.21 ± 0.07	0.72 ± 0.32	*0.35 ± 0.11	**0.25 ± 0.10

#### 4. Discussion

Cisplatin is a broadly used chemotherapeutic agent in the treatment of several cancer types, and has a toxic effect on numerous organs, especially on liver.

Cisplatin creates reactive oxygen species like single oxygen, hydrogen peroxide, hydroxyl radicals, and superoxide ions [5]. Elevation in reactive oxygen species might reduce the antioxidant enzymes activity, reduce the natural body antioxidant defense mechanism, and that lead to lipid peroxidation [20]. Cisplatin is r absorbed by the human liver quickly and thus, large doses of the drug could produce liver toxicity [21].

Various substances have been tested to diminish the toxicity that produced by cisplatin, but unfortunately none have been demonstrated to be effective for clinically therapeutic use [22]. Therefore, in this study Artemesia Vulgaris had been examined to reduce the liver toxicity of cisplatin in the mice model.

The preliminary chemical screening tests approved the presence of alkaloids, polyphenolic compounds, flavonoids, tannins, polysaccharide and saponins in the extract of aerial parts of Iraqi Artemesia vulgaris. This is in agreement with studies reporting the presence of the above compounds [23].

In this study, cisplatin administered in mice increased ALT, ALP and TSB activity levels, indicating liver damage. This results are consistent with other studies which showed the elevation of these enzyme after cisplatin treatment [24].

Treatment with Artemesia extract in control non-treated group with doses up to 400 mg/kg shows no symptoms of toxicity in mice.

In the current study we successfully demonstrate that the pre-treatment of Artemesia extract decreased the serum level of ALT, ALP and TSB in dose dependent manner, 400 mg /Kg dose reduced the enzymes level more than the 200 mg/kg dose. This finding is supported by the studies of Amat et al. that showed the Hepatoprotective effect of Artemesia absinthium L against chemically induced liver injury [25]. This results also consistent with another study which approved the protective effect of Artemesia absinthium L in rat that suffered from liver toxicity produced by diclofenac [26]. However, no other research approved the Artemesia

Vulgaris effect against the chemotherapy drug cisplatin. Therefore, this is the first study to provide evidence of Hepatoprotective activity of specifically Iraqi Artemesia V. extract against cisplatin liver toxicity in mice.

#### 5. CONCLUSION

Ethanollic extract of Iraqi Artemesia V. show marked potential for hepatoprotection against cisplatin induce liver injury in mice, and the preliminary tests show variant phytochemicals that may contribute to its efficacy.

#### 6. CONFLICT OF INTEREST

The authors declare no conflict of interest

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