

# The Histological Changes on Liver and Spleen of Mice Treated with Alcoholic and Aquatic Extract of *Cupressus sempervirens*

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## Abstract

**Background:** *Cupressus sempervirens* has been widely cultivated as an ornamental tree for millennia away from its native range, mainly throughout the central and western Mediterranean region. It contains some active chemical constituents.

**Objectives:** The aim of the study was to investigate the effect of *Cupressus sempervirens* on the histological structure of liver and spleen.

**Methods:** For this purpose, thirty males Swiss albino mice were divided into five equal groups, the groups of aqueous and alcoholic extract of *cupressus sempervirens* were (350, 150, 0 mg/ml), six mice were used for each group. Then the animals were sacrificed and the histological change on liver and spleen was studied.

**Results:** Histopathology of liver in mice treated with 350 mg/ml alcoholic extract of *Cupressus sempervirens* showed aggregation of inflammation cells mainly neutrophil and lymphocyte, as dilated as congestion in the central vein, and the aquatic extract of 350 mg/ml *Cupressus sempervirens* showed mononuclear cell infiltrate in the portal area together with amyloid deposition in sinusoid and multiple area of liver necrosis, while liver in mice treated with 150 mg/ml alcoholic extract of *Cupressus sempervirens* showed proliferation of kapffer cell and congestion of central vein, while at 150 mg/ml aquatic extract of *Cupressus sempervirens* showed proliferation of kapffer cell with double nucleated. Histopathology of spleen in mice treated with 350 mg/ml alcoholic and aquatic extract of *Cupressus sempervirens* showed severe amyloid deposition in red pulp and around white pulp, while spleen in mice treated with 150 mg/ml alcoholic extract of *Cupressus sempervirens* showed moderate periarterial hyper plasma, and a sectional view of 150 mg/ml aquatic extract of *Cupressus sempervirens* showed depletion of white pulp mainly neutrophil and macrophage.

**Conclusions:** from the result of this study it can be conclude that the high concentration of *Cupressus sempervirens* histopathological effect on liver and spleen of mice that induced with.

**Key Words:** *Cupressus sempervirens*, Alcoholic and Aquatic Extract, liver, spleen, Mice

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## Introduction:

*Cupressus sempervirens* is a tree which grows up to 30 m tall and belongs to the family Cupressaceae. The leaves are 0.5 to 1mm, dark green and obtuse. The male cones are 4 to 8mm, the female 25 to 40mm when ripe with 8 to 14 short and obtusely spiked scales. There are 8 to 20 seeds on each scale. The plant is distributed in Turkey and is cultivated throughout the Mediterranean region <sup>(1)</sup>. *Cupressus* constituent are tannin, flavones, piperitone, and camphore <sup>(2)</sup>.

The plant is rich in flavonoids constituents such as cupressuflavone, amenoflavone, rutin, quercitrin, quercetin, myricitrin <sup>(3)</sup> also it is contained some of phenolic compounds (anthocyanidin, catechines flavones, flavonols and isoflavones) tannins (ellagic acid, gallic acid, phenyl isopropanoids, caffeic acid, coumaric acid, ferulic acid) lignans, catchol, essential oil <sup>(4)</sup>.

The cones and young branches are anthelmintic, antipyretic, anti-rheumatic, astringent, balsamic, vaso- constrictive, anti-inflammatory, and hair tonic. The fruits of the plant were used traditionally for curing diabetes and as antiseptic <sup>(5)</sup>, and it is effective in the treatment of hyperlipidemia <sup>(6)</sup> such as antioxidant <sup>(7)</sup>.

## Material and Method

### Preparation of aquatic extract:

The powder of dry seeds cones of *cupressus sempervirens* (150 gm) was mixed with 500 ml of boiling distilled water by electrical rotator for one hour and then was filtered through four piece sterile gauze. The filtrate product was put in centrifuge 3000 rpm for 15 minutes. The supernatant was placed on glass Petri dishes. Finally, extract was dried under 40 C° in an oven. All concentration is based on the dry weight of extract <sup>(8)</sup>.

### Preparation alcoholic extract:

Alcoholic extract of *Cupressus sempervirens* was prepared by using ethanol 70% with volume 500 ml which was added to 150gm from powder of plants and then were placed in water bath at 50 C° for 24 hours. The mixture is to be mixed by electrical rotator for one hour and the other steps was completed like to the aqueous extract. <sup>(8)</sup>

### Experimental animals and treatment:

Thirty males Swiss albino mice weighing 30 g were used for the study. The mice were housed under standard conditions with access to food and water. They were divided into five groups, (six mice for each group). These groups were as follows:

- **First group (G1):** received diet and water *ad libitum* and served as **control**.
- **Second group(G2):** injected with 0.2 ml of **350 mg/ml alcoholic extract** of *Cupressus sempervirens*
- **Third group(G3):** injected with 0.2 ml of **350 mg/ml aquatic** extract of *Cupressus sempervirens*
- **Fourth group(G4):** injected with 0.2 ml of **150 mg/ml alcoholic** extract of *Cupressus sempervirens*
- **Fifth group(G5):** injected with 0.2 ml of **150 mg/ml aquatic** extract of *Cupressus sempervirens*

### Chemical analysis

The active chemical constituents in aquatic and alcoholic extract for seeds of cones *cupressus sempervirens* investigation was according to the procedure mentioned in reference <sup>(9)</sup>

### Histological procedure:

After the animals were sacrificed, the mice's liver and spleen were fixed in 10% formalin, dehydrated in ascending series of ethanol, cleared in xylene and embedded in paraffin. Sections were cut out at 6

micrometers thickness and stained with Harris hematoxylin and eosin stain<sup>(10)</sup>.

### Chemical analysis:

Table (1): The active chemical constituents in aquatic and alcoholic extract for seeds of cones *cupressus sempervirens*:

Active compounds	Aquatic extract of <i>Cupressus sempervirens</i>	Alcoholic extract of <i>Cupressus sempervirens</i>
Alkaloids	-	-
Glycoside	+	+
Flavonoids	+	+
Tannin	+	+
Phenol	+	+
Steroids	+	+
Saponin	+	+
Terpenes	+	+
Volatile oil	+	+
Resin	-	+

### Histopathological changes:

A sectional view of 350 mg/ml alcoholic extract of *Cupressus sempervirens* treated mice liver showed aggregation of inflammation cells mainly neutrophil and lymphocyte, as dilated as congestion in the central vein as (fig.2), while the aquatic extract of 350 mg/ml *Cupressus sempervirens* showed mononuclear cell infiltrate in the portal area together with amyloid deposition in sinusoid and multiple area of liver necrosis as (fig.3).

A sectional view of 150 mg/ml alcoholic extract of *Cupressus sempervirens* treated mice liver showed proliferation of kapffer cell and congestion of central vein (fig.4), while a sectional view of 150 mg/ml aquatic extract of *Cupressus sempervirens* treated

### Results:

mice liver showed proliferation of kapffer cell with double nucleated (fig.5).

A sectional view of 350 mg/ml alcoholic extract of *Cupressus sempervirens* treated mice spleen showed severe amyloid deposition in red pulp and around white pulp (fig.7), while a sectional view of 350 mg/ml aquatic extract of *Cupressus sempervirens* treated mice spleen showed amyloid like substance deposition in red pulp and around white pulp (fig.8).

A sectional view of 150 mg/ml alcoholic extract of *Cupressus sempervirens* treated mice spleen showed moderate periarterial hyper plasma (fig.9), while a sectional view of 150 mg/ml aquatic extract of *Cupressus sempervirens* treated mice spleen showed depletion of white pulp mainly neutrophil and macrophage (fig.10)



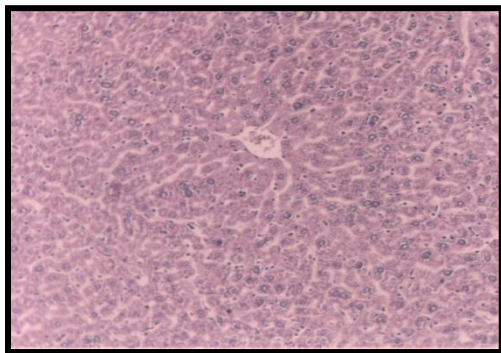


fig.1: a sectional view of control liver showed normal central vein and normal hepatocyte (100X).

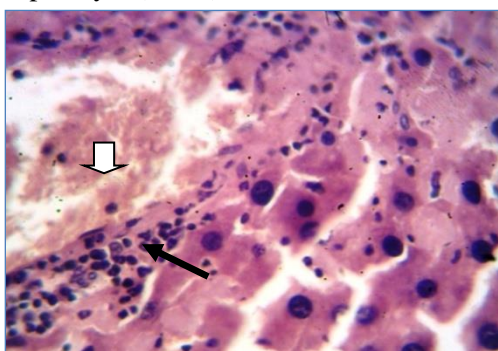


fig.2: A sectional view of 350 mg/ml alcoholic extract of *Cupressus sempervirens* treated mice liver showed aggregation of inflammation cells mainly neutrophil and lymphocyte (◀) as dilated as congestion in the central vein (◻) (400X).

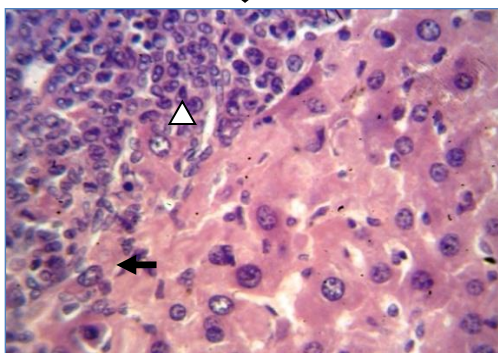


Fig.3: A sectional view of 350 mg/ml aquatic extract of *Cupressus sempervirens* treated mice liver showed Mononuclear cell infiltrate in the portal area (△) together with amyloid deposition in sinusoid and multiple area of liver necrosis (↔), (350X).

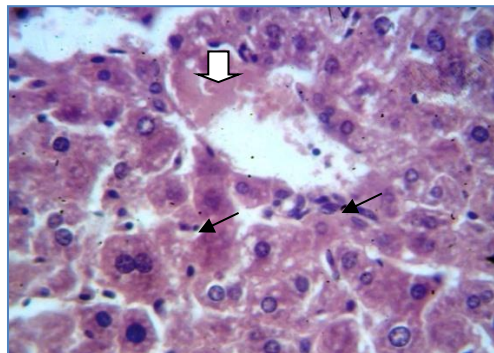


Fig.4: A sectional view of 150 mg/ml alcoholic extract of *Cupressus sempervirens* treated mice liver showed proliferation of kapffer cell (◀), congestion of central vein (◻), (400X).

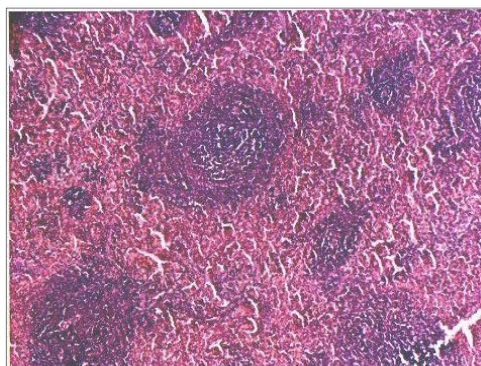


Fig.6: a sectional view of control spleen showed normal white pulp and normal red pulp (100X).

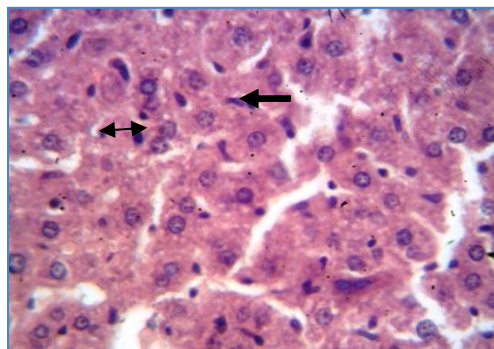


Fig.5: A sectional view of 150 mg/ml aquatic extract of *Cupressus sempervirens* treated mice liver showed Proliferation of kapffer cell (◀) with double nucleated (↔), (400X).

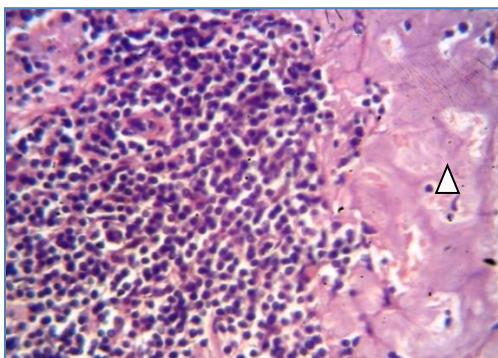


Fig.7: A sectional view of 350 mg/ml alcoholic extract of *Cupressus sempervirens* treated mice spleen showed sever amyloid deposition in red pulp ( Δ )and around white pulp,(400X).

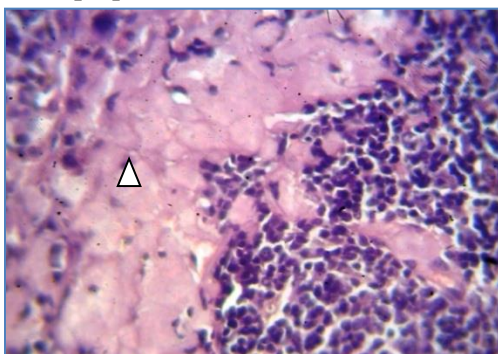


Fig.8 A sectional view of 350 mg/ml aquatic extract of *Cupressus sempervirens* treated mice spleen showed amyloid like substance deposition in red pulp and around white pulp ( Δ ), (400X).

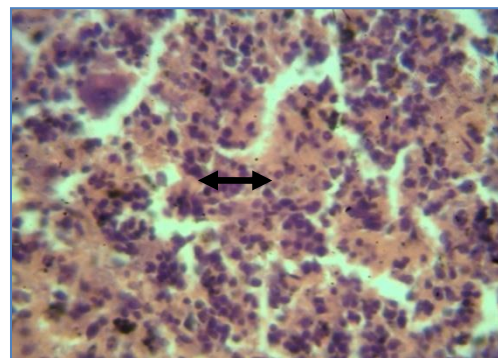


Fig.9: A sectional view of 150 mg/ml alcoholic extract of *Cupressus sempervirens* treated mice spleen showed moderate periarterial hyper plasma( ↔ ) (400X).

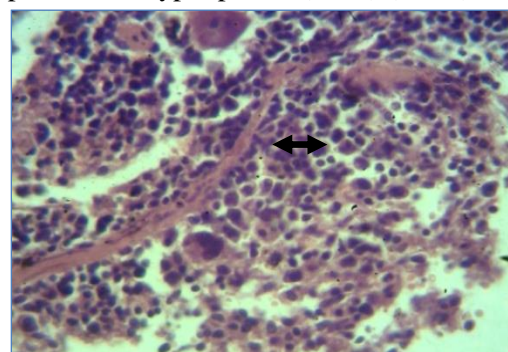


Fig.10: A sectional view of 150 mg/ml aquatic extract of *Cupressus sempervirens* treated mice spleen showed depletion of white pulp mainly neutrophil and macrophage(↔) (400X).

## Dissscusion:

Plants have been used to treat various ailments since the advent of human history, because the herbals have been usually considered to be safe and nontoxic compared to synthetic compounds. So, there are abundant studies about plant pharmacological properties<sup>(11, 12)</sup>.

The liver performs a number of functions some of which are plasma protein synthesis, production of bile and detoxification of most substances and spleen performs three functions related to blood cell: removal by macrophages of ruptured, defective blood cells and platelets, storage platelets, production of blood cells<sup>(13)</sup>.



Results revealed serious damage in the hepatic structure and spleen by using high concentration. The observation did not coincide with the researches who observed that oral administration of *C. sempervirens* cone extract with low Concentration (8mg/150gm) produces protective effect on rats intoxicated with lead <sup>(14)</sup>. The administration of *C. sempervirens* extract and its flavonoids (quercetin and rutin) prevented liver tissue injury approach to normal pattern. This is parallel to that who observed that the hepatic cells, central vein and portal triad are almost normal in *C. sempervirens* methanol extract in CCL4 intoxicated group <sup>(15)</sup>. Mitchell and cotran (2003) <sup>(16)</sup> showed that damaging in the cells depends on dose amount of the material toxic giving. The plant contains tannin, phenol, flavon, if it is used with high concentration; it will be formed toxic and occurred necrosis on the liver <sup>(17)</sup> and in addition to white pulp in the spleen because it consists of lymphatic tissue <sup>(18)</sup>.

A myloidosis occurs deposition in red pulp and around white pulp in spleen as result to disturbance in protein metabolism and causative factors in amyloidosis which are still unknown <sup>(19)</sup> but may be used the plant with high concentration. In summary, it was demonstrated that *C. sempervirens* extract could produce disturbance effect and toxic on mice by using high concentration.

In summary, it was demonstrated that *C. sempervirens* extract could produce protective effect on mice.

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