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<https://ejos.journals.ekb.eg>

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سعادة أ. د. رئيس تحرير المجلة المصرية للدراسات المتخصصة المحترم  
جامعة عين شمس، كلية التربية النوعية، القاهرة، مصر  
تحية طيبة وبعد،،،

يسر معاميل التأثير والاستشهادات المرجعية للمجلات العلمية العربية (ارسييف - ARCIF)، أحد مبادرات قاعدة بيانات "معرفة" للإنتاج والمحتوى العلمي، إعلامكم بأنه قد أطلق التقرير السنوي التاسع للمجلات لعام 2024.

ويسرنا تهنئكم وإعلامكم بأن المجلة المصرية للدراسات المتخصصة الصادرة عن جامعة عين شمس، كلية التربية النوعية، القاهرة، مصر، قد نجحت في تحقيق معايير اعتماد معاميل "Arcif" المتوافقة مع المعايير العالمية، والتي يبلغ عددها (32) معياراً، وللاطلاع على هذه المعايير يمكنكم الدخول إلى الرابط التالي: <http://e-marefa.net/arcif/criteria>

وكان معاميل "ارسييف Arcif" العام لمجلتكم لسنة 2024 (0.4167).

كما صنفت مجلتكم في تخصص العلوم التربوية من إجمالي عدد المجلات (127) على المستوى العربي ضمن الفئة (Q3) وهي الفئة الوسطى، مع العلم أن متوسط معاميل "ارسييف" لهذا التخصص كان (0.649).

وبإمكانكم الإعلان عن هذه النتيجة سواء على موقعكم الإلكتروني، أو على مواقع التواصل الاجتماعي، وكذلك الإشارة في النسخة الورقية لمجلتكم إلى معاميل "ارسييف Arcif" الخاص بمجلتكم.

ختاماً، نرجو في حال رغبتكم الحصول على شهادة رسمية إلكترونية خاصة بنجاحكم في معاميل "ارسييف"، التواصل معنا مشكورين.

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أ.د. سامي الخزندار  
رئيس مبادرة معاميل التأثير  
"ارسييف Arcif"



+962 6 5548228 -9  
+962 6 55 19 10 7

info@e-marefa.net  
www.e-marefa.net

Amman - Jordan  
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# **Hepatoprotective and Anti-hepatocarcenogenic Effect on Juniper (*Juniperus communis* L.) Leaves, Berries and its Extracts**

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**Prof. Ekbal Mahmud Mohamed <sup>(1)</sup>**

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**Prof. Eshak Mourad El-Hadidy <sup>(2)</sup>**

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**A. Prof. Gehan I. Abdel Wahab <sup>(3)</sup>**

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**Rahma Saber Kamal Elewa <sup>(4)</sup>**

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- (1) Professor Nutrition and Food Sciences Home Economic Dept. Faculty of Specific Education, Ain Shams University
  - (2) Professor food technology research institute Argic. Res. Center National Expert in food safety, Food Quality and safety consultant
  - (3) Assistant Professor Nutrition and Food Science, Home Economic Dept. Faculty of Specific Education, Ain Shams University
  - (4) Home Economics Department, Faculty of Specific Education, Ain Shams University

## Hepatoprotective and Anti-hepatocarcinogenic Effect on Juniper (*Juniperus communis* L.) Leaves, Berries and its Extracts

Prof. Ekbal Mahmod Mohamed Saleh

Prof. Eshak Mourad El-Hadidy

A. Prof. Gehan Ibrahim Abdel Wahab

Rahma Saber Kamal Elewa

### Abstract

Hepatocellular carcinoma (HCC) is the sixth most common cancer, making it a major health concern worldwide. Juniper plant has been reported as hepatic protective and anti-hyperlipidemic activity. Therefore, the aim of this study is evaluate of Juniper dried leaves and fruits (berries) as a natural hepatoprotective and hepatocarcinogenic source. Sixty male albino rats (Sprague Dawley strain) weighed an average between (180±10g). Volatile oils and its fraction, total polyphenols, total flavonoids, carotenoids, vitamin C, saponins, tannins and antioxidants activity were determined in dried juniper leaves and berries.

**Keywords:** Juniper, Dried Leaves, Dried Berries, Extracts, Hepatoprotective, Albino Rats, hepatic cell cancer (HPG2).

### ملخص:

**العنوان:** التأثير الوقائي و المضاد لسرطان الكبد لأوراق و ثمار و مستخلصات العرعر  
**المؤلفون:** إقبال محمود صالح ، إسحق مراد الحديدي ، جيهان إبراهيم عبد الوهاب، رحمة صابر كمال عليوه

يعد سرطان الخلايا الكبدية (HCC) سادس أكثر أنواع السرطان شيوعاً، مما يجعله مصدر قلق صحي كبير في جميع أنحاء العالم. يعد نبات العرعر ذو نشاط وقائي للكبد ومضاد لفرط دهون الدم. ولذلك فإن الهدف من هذه الدراسة هو تقييم أوراق و ثمار العرعر التوتية المجففة كمصدر طبيعي للوقاية من التهاب الكبد ومسببات سرطان الكبد. تم الحصول على ستون فأراً ألبينو ذكراً (سلالة Sprague Dawley) بمتوسط أوزان يتراوح بين (180±10 جرام). تم تحديد المحتوى من الزيت الطيار وجزئياته والفينولات الكلية والفلافونويدات الكلية والكاروتينات وفيتامين ج والثانينات والصابونينات ومضادات الأكسدة في أوراق و ثمار العرعر المجفف  
**الكلمات الدالة:** العرعر، الأوراق المجففة، الثمار التوتية المجففة، المستخلصات، وقاية الكبد، الفئران الألبينو، سرطان الخلايا الكبدية

## 1. Introduction

Hepatocellular carcinoma (HCC) is the sixth most common cancer, making it a major health concern worldwide. Prevalent treatments for HCC include surgery, chemotherapy, radiation therapy, and targeted therapy (*Dhanasekaran et al., 2012*). Chemotherapy is usually used to promote the patients' survival. However, chemodrugs are often associated with toxicity and strong side effects that impair therapeutic efficacy, resulting in high death rates (*Ozer Etik et al., 2017*). Consequently, the discovery of novel antitumor agents with better therapeutic efficacy and less toxicity has become an attractive avenue for preventing and treating HCC. Natural products from plants may be a safer choice because use alone or a combination of medicinal plants and conventional therapies has a beneficial effect on survival, immune regulation, and quality of life (*Yin et al., 2013*).

Juniper plant has been reported as diuretic, having anti-inflammatory properties antifungal activity, analgesic activity, hepatic protective activity and diabetic and anti-hyperlipidemia activity antimicrobial activity antioxidant activity anti hypercholesterolemia activity, anticataleptic activity, and neurons protective activity in Parkinson's disease (*Bais 2014 and Rolta, et al., 2020*).

The genus *Juniperus* is an important component of arid and semi-arid ecosystems throughout the northern hemisphere *Adams (2008)*. Previously, from the genus *Juniperus* some terpenoids have been isolated, neolignans and flavonoids. The species of *Juniperus* is considered as an important medicinal plant largely used in traditional medicine. The anti-inflammatory activity of some diterpenoids of leaves *juniperus Mansouri et al (2010)* and several studies about the essential oil of eaves *juniperus* have been published *Adams (2008)*. All oils of *Juniperus phoenicea* of five localities from eastern Algeria have a high content of  $\alpha$ -pinene,  $\Delta^3$ -carene, limonene, terpinolene and the  $\alpha$ -terpinyl acetate (*Messaoud et al., 2013*). The phenolic and the flavonoids compounds are groups of secondary metabolites with broad range

of biological properties such as: antioxidant, antibacterial, anti-atherosclerosis, cardiovascular protection and improvement of the endothelial function, it has been reported that antioxidant activity of the phenolic compounds is mainly due to their redox properties which allow them to act as reducing agents, hydrogen donors play an important role by adsorbing and neutralizing reactive free radicals, and chelating ferric ions which catalyses lipid peroxidation, and regarded as promising therapeutic agent for free radical-linked pathologies (*Nyangono et al., 2012*).

Fruits of *Juniperus communis L.* contain flavonoids, glycoside, bitter compounds (juniperine), resin (10%), invert sugar (15-30%), catechin (3-5%), organic acids, essential oil (0.5% in fresh and 2.5% in dry fruit), terpenic acids, and leucoanthocyanidin (*Koc, 2002; Sengül et al., 2008 and Avan, 2010*).

*Miceli et al., (2009)* determined total polyphenols by Folin-Ciocalteu method. Results showed that the berries of *Juniperus communis* were  $59.17 \pm 1.65$  mg GAE/g extract. Flavonoid and bioflavonoid content was  $25947 \pm 0.86$  and  $4346 \pm 3.95$   $\mu\text{g/g}$  extract.

The total phenol content of ethanol extract, hexane fraction, ethyl acetate fraction, and aqueous fraction were found to be 238.78, 189.65, 315.33, and 205.33 mg/GAE/g extract/fraction, respectively (*Ved et al., 2017*).

The juniper berry volatile oil is largely comprised of monoterpene hydrocarbons such as  $\beta$ -pinene (5.0%),  $\alpha$ -pinene (51.4%), sabinene (5.8%), myrcene (8.3%), and limonene (5.1%) (*Hoferl, 2014*). The seeds and fruits of the plant contain d- $\alpha$ -pinene, camphene, pectins, glycolic acid, malic acid, formic acid, acetic acid, cyclohexitol, terpene, proteins, fermentable sugars, wax, gum, ascorbic acid, dihydrojunene,  $\beta$ -pinene, hydrocarbon-junene, cadinene, and camphor (*Chandra et al., 2007*).

*Junipers communis* contained monoterpenes, sesquiteerpenes, essential and volatile oils, wide range of



phenolic compounds and many other biochemical constituents. It exerted many pharmacological effects included antimicrobial, antiparasitic, antifertility, antioxidant, cytotoxic, hepatoprotective, vessels and trachea protective effects in passive smoking, gastrointestinal, antidiabetic, antihyperlipidemic, anti-inflammatory, analgesic, diuretic, antiurolithiatic, anti-Parkinsonian, memory enhancing, tyrosinase suppressive activity and many other effects. This review was designed to highlight the chemical constituents and pharmacological effects of *Juniperus communis* (Al-Snafi, 2018).

Chemical composition of the essential oil of *J. Phoenicea* is dominated by the presence of a major product,  $\alpha$ -pinene with an average (48.08%), terpinolene (13%) and  $\Delta^3$ -carene (12.4%) (Mansouri et al., 2011), which is an antimicrobial compound having wide spectra of antimicrobial effects against enter bacteria. Similar findings have been reported by other investigators (Messaoud et al., 2013). The results of the current study using the *Juniper phoenicea* correlated with the findings of other investigators (Barrero et al., 2006).

Cirrhosis is an important cause of morbidity and mortality among patients with chronic liver disease. Cirrhosis can lead to hepatocellular carcinoma (HCC) and hepatic decompensation, including ascites, hepatic encephalopathy and variceal bleeding (Huang et al., 2022) and is a leading cause of death worldwide — it was associated with 2.4% of global deaths in 2019 (WHO, 2023).

Chronic liver disease (CLD) refers to a long-term pathological process of continuous destruction of liver parenchyma and its gradual substitution with fibrous tissue, which eventually brings about CL related with a deadly result. Risk factors of CLD incorporate viral (Hepatitis B and C), non-alcoholic steatohepatitis (NASH), stoutness, diabetes mellitus, and the utilization of natural and dietary enhancements, immune system hepatitis, and Wilson infection (Kooffreh et al., 2017).

The aim of this study is evaluation of Juniper dried leaves and fruits (berries) and its ethanolic extracts as a natural hepatoprotective and anti-hepatocarcinogenic source.

## Materials and Methods:

Juniper leaves and berries were obtained from Horticultural Institute, Agricultural Research Center Giza, Egypt. Sixty male albino rats (Sprague Dawley strain) were obtained from Agricultural Research Center Giza, Egypt weighed an average between (180±10g). Carbon tetrachloride (CCl<sub>4</sub>), casein, vitamins, minerals, cellulose and choline chloride were purchased from El-Gomhoreya Company, Cairo Egypt. Corn and starch were obtained from the local market. The kits of serum aspartate amino transaminase (AST), alanine amino transaminase (ALP) total cholesterol, triglycerides, total lipids, high density lipoprotein (HDL-C), Superoxide dismutase (SOD), Glutathione reductase (GSH) and Malonaldehyde (MDA) were purchased from Bio-diagnostic Company in Egypt.

*Schopfer (1989)* method was used to estimate the total carotenoid content as β-carotene. Additionally, volatile oils were determined by International Standard Organization method (*ISO, 2011*). Total polyphenol contents was measured using Folin–Ciocalteu method described by *Singleton et al., (1999)*. Gallic acid was used as standard and samples were read in triplicate at 730 nm by a spectrophotometer. Total flavonoids was determined according to the methods of *Mohdaly et al., (2012)*. Ascorbic acid was determined spectrophotometrically according to the method of *Klein and Perry (1982)*. Saponins content estimated gravimetrically according to *Hiai et al., (1976)*. Total tannins was determined colorimetrically as described by *A.O.A.C. (2000)*. The antioxidant activity of leaves and berries was determined by 2,2 diphenyl-picrylhydrazyl (DPPH) method described by *Brand-Williams et al., (1995)*.

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### **Dried leaves and berries volatile oil fractions**

The GC analysis of the essential oil samples performed using gas chromatography- mass spectrometry instrument stands at the Department of Medicinal and Aromatic Plants Research, Horticultural Institute, Agricultural Research Center, Egypt with the following specifications: The chromatograph apparatus was fitted with capillary column BPX-5, 5%phenyle (equiv.) polysilphenylene-siloxane 30m X 0.25 mm ID X 0.25µm film. Temperature program ramp increase with a rate of 10°C/ min from 70 to 200°C. Flow rates of gases were nitrogen at 1 ml/min, hydrogen at 30 ml/min and 330 ml/min for air. Detector and injector temperatures were 300°C and 250°C, respectively. The obtained chromatogram and report of GC analysis for each sample were analyzed to calculate the percentage of main components of volatile oil (*British Pharmacopoeia, 1963*).

### **Preparation of dried hydroethanolic extract of Juniper leaves and berries:**

Juniper leaves and berries were washed in water several times to remove any adhering parts, dried in oven under vacuum (50°C), then ground well. Ground leaves or berries Juniper were mixed with 80% ethanol (1:100 w/v) and stored in dark bottle for 48h/4°C. The mixtures were filtered through filter paper (Whatman No. 1). Hydroethanolic solution was evaporated in rotary evaporator at 50°C and dried leaves or berries Juniper extracts were collected. Different Juniper extracts were prepared from the dried leaves using different concentrations (50 and 100 ppm) for biological experiments (*El-Hadidy et al., 2018*).

### **Animal Experiment:**

Basal diet, vitamins and minerals mixture were prepared according to *Reeves et al., (1993)*. Sixty male albino rats were adapted for one week prior to commencement of the experiment, housed in well aerated cages under hygienic condition and water was introduced *ad-libitum*. After this week, rats were divided into 10 groups (6 rats of each) and fed on diets for six weeks as

follows: Negative control group fed on basal diet. Fifty-four rats fed on basal diet and injected with 2ml/kg CCl<sub>4</sub>, in paraffin oil (50%v/v) twice/week subcutaneous to induce chronic damage in the liver (*Jayasekhar et al., 1997*) after the confirmation of the damage through liver enzymes test the 9 groups numbered from 2 to 10 were classified as follows: Group 2 positive control group was fed on basal diet till final of experiment (4 weeks).

Group 3 and 4 fed on dried juniper leaves (0.5 and 1%, respectively), while group 5 and 6 fed on dried Juniper berries (0.5 and 1%, respectively). Also, group 7 and 8 treated with 50 and 100 ppm ethanolic extract orally every day from juniper leaves. In addition, groups 9 and 10 treated with 50, and 100 ppm ethanolic extract orally from juniper berries.

Serum aspartate amino transaminase (AST), alanine amino transaminases (ALT), and Alkaline phosphates (ALP) were measured every 2 weeks according to the method described by *Tietz et al (1999)*. Serum samples were used for the determination of total cholesterol, triglycerides, total lipids, and HDL-C according to the methods described by *Young and Friedman (2001)*. Serum LDL-C and VLDL-C were calculated according to *Lee and Nieman (1996)* by the following equation:

$$\text{VLDL-C Concentration (mg/dL)} = \text{TG (mg/dl)}/5$$

$$\text{LDL-C} = \text{Total Cholesterol} - \{(\text{VLDL-C}) + (\text{HDL-C})\}$$

Determination of serum Superoxide dismutase (SOD) (*Kakkar et al., 1984*). Malondialdehyde (MDA) was determined in the serum according to the colorimetric method described by *Yagi, (1998)* Glutathione reductase (GSH-RX) was determined in the serum according to *Kamencic (2000)*.

#### **Cytotoxicity activity of Juniper leaves and berries:-**

Measurement of potential cytotoxicity activity Juniper against the liver carcinoma cell line (HEPG-2), was test by SRB assay using the method of (*Skahane et al., 1990*). This

experiment was conducted in Egyptian cancer institute, Cairo, Egypt.

### Statistical analysis:

Descriptive values of data were expressed as the mean $\pm$ SD and they were applied to the (ANOVA); followed by Duncan's test. In all cases  $p < 0.05$  was used as the criterion of statistical significance by SAS program (SAS, 2003)

### Results and Discussion:

Juniper leaves and berries rich in some of bioactive components as polyphenols, flavonoids, carotenoids, especially volatile oils content. These antioxidants synergists may effective to prevent some of global diseases as hepato-cancer. The followed results will illustrate that.

**Table (1): Bioactive contents and its antioxidant activity in dried juniper leaves and Berries**

Items	Juniper Leaves (mg/g)	Juniper Berries (mg/g)
Total polyphenols	63.73 $\pm$ 3.25	43.22 $\pm$ 2.55
Total flavonoids	14.55 $\pm$ 0.82	20.32 $\pm$ 1.02
Total carotenoids	0.19 $\pm$ 0.002	0.22 $\pm$ 0.002
Vitamin C	32.54 $\pm$ 1.23	65.90 $\pm$ 2.43
Saponins	15.22 $\pm$ 0.96	23.12 $\pm$ 1.04
Tannins	41.32 $\pm$ 3.42	24.55 $\pm$ 1.87
Volatile oil**	1.12 $\pm$ 0.002	1.92 $\pm$ 0.005
Antioxidant activity DPPH %	58.20 $\pm$ 3.25	66.80 $\pm$ 4.52

\*Means of 3 samples  $\pm$  SD

\*\*ml/g v/w

Results in table (1) showed the juniper leaves were the highest content of total polyphenols and tannins 63.73 and 41.32 mg/g, respectively. While, the content of total flavonoids (mg/g), saponins (mg/g) and volatile oil (ml/g) were higher in Juniper berries than juniper leaves (20.32, 23.12, 1.92 and 14.55, 15.22, 1.12), respectively. Therefore, the antioxidant activity in juniper berries was nearly result in juniper leaves (66.80 and 58.20,

respectively). These results were comply with *Miceli et al, (2009)*.

**Table (2): Volatile oil components of Juniper dried leaves and berries (Area%)**

Items	Juniper Leaves	Juniper Berries
$\alpha$ -Thujene	-	0.81
$\alpha$ -Pinene	48.00	34.90
Camphene	0.12	0.52
Sabinene	8.9	7.63
$\beta$ -Pinene	2.10	3.29
p-Mentha-2,8-diene	0.40	0.32
Myrcene	18.94	15.42
p-Mentha-1(7),8-diene	1.88	0.68
$\alpha$ -Terpinene	0.70	0.47
p-Cymene	3.84	2.22
Limonene	9.25	7.38
(E)-Ocimene	1.03	0.18
$\gamma$ -Terpinene	1.77	1.85
Terpinolene	1.42	1.17
Linalool	0.73	0.20
(E)-Pinocarveol	-	0.10
Terpinen-4-ol	0.90	2.26
$\alpha$ -Terpineol	0.12	0.80
$\beta$ -Elemene	0.81	0.57
$\beta$ -Caryophyllene	6.25	0.25

Juniper leaves and berries were rich in  $\alpha$ -Pinene (48% and 34.90%, respectively), myrcence and limonine. While, leaves were higher contents in  $\alpha$ -Pinene, Sabinene, p-Cymene, Limonene, (E)-Ocimene,  $\beta$ -Elemene and  $\beta$ -Caryophyllene than Juniper berries. Also, Juniper berries were highest in  $\gamma$ -Terpinene (1.85%), Terpinen-4-ol (2.26%) and  $\alpha$ -Terpineol (0.80%) compared to Juniper leaves. Some of components were present in berries only as  $\alpha$ -Thujene and E-Pinocarveol. These results were adapted to who found that *Hoferl, (2014)* and *Mansouri et al., (2010)*.

**Table (3): Change in sAST of serum rats fed on Juniper dried leaves or berries and their ethanolic extracts (IU/L).**

Items	After CCl <sub>4</sub> induction	2 weeks	4 weeks	% Decrement
G1 (Negative Control)	43.91h±3.42	45.26h±2.92	44.83h±3.97	-
G2 (Positive Control)	141.83a±14.07	134.33ab±11.75	129.52b±9.87	8.68
G3 Dried Leaves 0.5%	135.83ab±11.54	98.50c±9.50	58.16f±8.07	61.60
G4 Dried leaves 1%	130.78ab±10.64	99.83c±5.45	51.83g±7.83	78.95
G5 Dried Berries 0.5%	133.20ab±11.38	104.16c±9.06	45.33g±5.50	59.21
G6 Dried Berries 1%	128.33b±9.16	96.51c±6.27	55.16f±7.99	73.17
G7 Leaves Extract 50 ppm	123.00b±8.60	95.16c±7.30	46.84g±5.31	61.91
G8 Leaves Extract 100 ppm	132.61ab±10.86	90.51b±7.17	40.00h±5.74	69.83
G9 Berries Extract 50 ppm	129.83ab±10.78	106.16b±6.43	75.20e±13.8	42.08
G10 Berries Extract 100 ppm	128.70b±9.64	105.66c±8.13	61.40f ±4.32	52.29

All results are expressed as Mean ± SD.

Values in columns and rows which have different letters are significantly different (p<0.05)

Results in table (3) showed that sAST activity significant decrease percentage in rats fed on diets 0.5% and 1.0% juniper dried leaves (61.60 and 78.95%), respectively and 0.5% and 1% juniper dried berries (59.21 and 73.17%) respectively, while in rats fed orally on 50 and 100 ppm juniper leaves extracts (61.91 and 69.83%), respectively and 50 and 100 ppm juniper berries extracts (42.08 and 52.29%) respectively compared to the significant decrement percentage of sAST in rats induced to CCl<sub>4</sub> which fed on basal diet during experimental period (8.68%). These decrement calculated at the end of experiment (After 4 weeks) compared to zero time of experiment (After rats induced CCl<sub>4</sub>).

These results indicated that, rats fed on diet containing dried leaves or berries 1% were better than it's fed on diet containing 0.5%. While, rats fed on diets containing dried leaves were better than dried berries. Also, dried leaves and berries are better effect than its extracts and 100 ppm extracts were more effective than 50 ppm.

Generally, dried Juniper leaves were better than berries to significant decrease sAST activity compared to rats group, which fed on basal diet after induced CCl<sub>4</sub> till final of experiment. These

results may due to leaves rich in polyphenols also structure differentiation of volatile oil components.

**Table (4): Change in sALT of serum rats fed on Juniper dried leaves or berries and their ethanolic extracts (IU/L).**

Items	After CCl <sub>4</sub> induction	2 weeks	4 weeks	% Decrement
G1 (Negative Control)	37.50f ± 1.05	38.16f ± 1.63	39.07f ± 1.47	-
G2 (Positive Control)	126.50a ± 9.62	121.02a ± 8.71	118.17a ± 8.93	6.54
G3 Dried Leaves 0.5%	119.30a ± 6.24	74.83c ± 5.47	60.50d ± 8.27	48.29
G4 Dried leaves 1%	125.65a ± 9.66	74.50c ± 7.50	58.83d ± 6.17	53.18
G5 Dried Berries 0.5%	128.67a ± 9.60	84.00b ± 7.73	76.67bc ± 5.16	40.41
G6 Dried Berries 1%	112.50a ± 8.88	80.33b ± 6.58	61.33d ± 7.40	45.48
G7 Leaves Extract 50 ppm	124.18a ± 9.84	76.50bc ± 7.87	58.67d ± 8.81	52.75
G8 Leaves Extract 100 ppm	130.50a ± 10.23	78.67bc ± 5.01	66.52cd ± 5.87	49.04
G9 Berries Extract 50 ppm	123.67a ± 8.16	78.00bc ± 5.10	49.33e ± 6.80	60.11
G10 Berries Extract 100 ppm	118.50a ± 8.87	77.34bc ± 7.88	47.45e ± 6.87	59.96

All results are expressed as Mean ± SD.

Values in columns and rows which have different letters are significantly different (p<0.05).

Table (4) showed a higher significant decrease in sAST in all groups treated with juniper leaves and berries and their extracts (from 40.41 to 60.11%) than rats group induced in CCl<sub>4</sub> then it's fed on basal diet until the end of experiment (G2 – 6.54%). These decrement calculated at the end of experiment (After 4 weeks) compared to zero time of experiment (After rats induced CCl<sub>4</sub>).

Results indicated that, rats fed on Juniper berries extracts were better than rats fed on juniper leaves extracts (100ppm and 50 ppm. Respectively) orally followed by groups of rats fed on diets containing 1% then that fed on diets containing 0.5% dried leaves and berries.

**Table (5): Change in Alkaline Phosphatase (sALP) of serum rats fed on Juniper dried leaves or berries and their ethanolic extracts (IU/L).**

Items	After CCl <sub>4</sub> induction	2 weeks	4 weeks	% Decrement
G1 (Negative Control)	154.87g ± 18.07	151.24g ± 8.20	149.12g ± 6.30	-
G2 (Positive Control)	304.48a ± 7.86	296.33a ± 14.63	291.25a ± 10.93	4.35
G3 Dried Leaves 0.5%	296.33a ± 14.71	265.62c ± 14.52	218.83d ± 15.19	26.15
G4 Dried leaves 1%	292.16a ± 15.31	270.52b ± 18.20	210.66f ± 17.80	27.90



G5 Dried Berries 0.5%	305.52a ± 15.24	276.16b ± 13.24	228.22d ± 17.87	25.30
G6 Dried Berries 1%	295.40a ± 7.89	280.46b ± 8.96	216.83d ± 8.79	26.60
G7 Leaves Extract 50 ppm	289.83a ± 11.86	270.00b ± 10.63	210.33f ± 11.63	27.43
G8 Leaves Extract 100 ppm	296.31a ± 12.12	274.26b ± 13.31	209.54f ± 10.89	29.28
G9 Berries Extract 50 ppm	295.16a ± 9.30	284.16b ± 13.76	219.00d ± 14.09	25.72
G10 Berries Extract 100 ppm	299.66a ± 11.87	282.83b ± 8.08	213.20df ± 9.81	28.85

All results are expressed as Mean ± SD.

Values in columns and rows which have different letters are significantly different ( $p < 0.05$ ).

The results in table (5) showed a significant increase in ALP in rats group 2 (Positive control) compared to rats in Group 1 (Negative control). While, it's noted that the significant decrease in different groups which fed on dried leaves and berries and its ethanolic extracts which ranged from 25.30 to 29.28% compared to rats induced to  $CCl_4$  and fed on basal diet during experimental period (4.35%). Leaves and berries 100 ppm extracts were the best significant decrement effect compared to all groups. These decrement calculated at the end of experiment (After 4 weeks) compared to zero time of experiment (After rats induced  $CCl_4$ ).

Conclusively, it's noticed that, Juniper leaves, berries and its extracts more effect in sGOT and sGPT than sALP activities.

**Table (6): Change of Serum total lipids, triglycerides and total cholesterol in rats fed on Juniper dried leaves and berries and their ethanolic extracts at the end of experiment.**

Items	Total Lipids		Triglycerides		Total Cholesterol	
	mg/dL	%	mg/dL	%	mg/dL	%
G1 (Negative Control)	310.50e ± 14.05		215.33e ± 13.27		77.66d ± 18.14	
G2 (Positive Control)	622.84a ± 20.16		310.70a ± 17.42		220.40a ± 16.68	
G3 Dried Leaves 0.5%	465.44c ± 18.42	- 25.27	160.42c ± 11.33	- 48.37	124.08b ± 13.49	-57.31
G4 Dried leaves 1%	488.95b ± 16.00	- 21.50	149.52 ± 16.09	- 51.88	103.12c ± 12.79	-53.21
G5 Dried Berries 0.5%	498.88b ± 15.82	- 19.96	181.60b ± 18.54	- 41.55	100.60c ± 11.17	-56.64
G6 Dried Berries 1%	494.27b ± 17.50	- 20.64	176.72b ± 18.32	- 43.12	93.56c ± 16.24	-57.55
G7 Leaves Extract 50 ppm	452.59c ± 17.14	- 27.33	124.92e ± 19.06	- 59.79	98.33c ± 14.26	-55.38
G8 Leaves Extract 100 ppm	440.50cd ± 16.08	- 29.28	109.72e ± 14.40	-64.69	82.75cd ± 11.47	-62.45

G9 Berries Extract 50 ppm	445.62cd ± 18.40	- 28.45	145.84d ± 19.70	- 53.06	103.30c ± 14.03	-53.23
G10 Berries Extract 100 ppm	425.56d ± 12.92	- 31.67	139.88d ± 12.82	-54.98	98.58c ± 11.12	55.27

All results are expressed as Mean ± SD.

Values in each column which have different letters are significantly different ( $p < 0.05$ ).

Table (6) results showed that the highest effect of serum total lipids in rats fed on Berries extract 100ppm (425.56 mg/dL) followed by leaves extracts 100ppm, berries extract 50ppm orally (440.50 and 445.62 mg/dL, respectively) then rats fed on diet containing 1% dried berries (452.59 mg/dL) compared to G2 (622.84 mg/dL).

On the other view, triglycerides level in rats fed on leaves extracts 100ppm and 50 ppm (109.72 and 124.92 mg/dL, respectively) were significant decrease than berries extracts (139.88 and 145.84 mg/dL, respectively) orally compared to G2 (310.70 mg/dL). Also, the highest decreased significant in total cholesterol was observed in rats groups fed on juniper leaves extracts 100 ppm (82.75 mg/dL) while the decrease significant were ranged from 82.75 to 124.08 mg/dL in other groups compared to G2 (220.40 mg/dL). The percentage of these results calculated at in all rats groups (From G3 to G10) compared to positive control group (G2) at the end of experiment.

These results complied to *Bais (2014)* he found that, the hepatoprotective activity of *J. communis* in rats was determined by given  $\text{CCl}_4$ . In  $\text{CCl}_4$  treatment group was showed significant increase in serum glutamic oxaloacetic transaminase (sGOT), serum glutamic pyruvic transaminase (sGPT), total bilirubin (TB), and alkaline phosphatase (ALP) values when compared to control group. There was significant decrease in the level of sGPT, sGOT, TB, and ALP in silymarin treated group. The abnormal high level of sGOT, sGPT, ALP, and bilirubin observed was due to  $\text{CCl}_4$  induced hepatotoxicity. *J. communis* reduced the increased levels of sGPT, sGOT, ALP, and bilirubin, which showed protection against hepatic cells (ethanol and aqueous extract show better protection). Generally, Juniper berries and

leaves extracts were better effect on serum total lipids, triglycerides and total cholesterol than dried juniper berries and leaves.

**Table (7): Change in serum VLDL, LDL and HDL in rats fed on Juniper dried leaves and berries and their ethanolic extracts (mg/dL).**

Items	VLDL-C	LDL-C	HDL-C
G1 (Negative Control)	21.25c ± 4.55	70.04c ± 3.59	65.10c ± 1.50
G2 (Positive Control)	42.56a ± 6.22	90.52a ± 2.42	55.21a ± 1.69
G3 Dried Leaves 0.5%	38.92ab ± 3.87	71.04c ± 2.82	56.00a ± 3.10
G4 Dried leaves 1%	34.00ab ± 6.10	72.15c ± 2.13	59.96b ± 1.77
G5 Dried Berries 0.5%	32.90ab ± 6.80	75.00b ± 3.64	57.40b ± 2.75
G6 Dried Berries 1%	30.82c ± 3.90	75.18b ± 1.86	59.52b ± 1.60
G7 Leaves Extract 50 ppm	35.22ab ± 2.80	67.08c ± 1.32	60.14b ± 3.24
G8 Leaves Extract 100 ppm	32.24ab ± 2.96	67.55c ± 2.78	61.52 ± 2.24
G9 Berries Extract 50 ppm	29.80c ± 3.08	77.42b ± 2.78	62.45bc ± 2.21
G10 Berries Extract 100 ppm	27.00c ± 1.58	75.50b ± 4.10	63.00bc ± 1.24

All results are expressed as Mean ± SD.

Values in each column which have different letters are significantly different ( $p < 0.05$ ).

Results in table (7) showed that there are gradually statistically decrease significant differences between the negative control group and other groups in the level of V-LDL, its ranged from 38.92 to 27.00 mg/dL compared to rats group induced  $\text{CCl}_4$  then fed on basal diet (42.65 mg/dL). While, groups G9 and G10, which were fed on the ethanolic extract of juniper leaves and berries orally at 50 and 100 ppm, achieved the highest decrease in the level of V-LDL, followed by groups G7 and G8. At the same trend, rats fed on dried juniper leaves and berries, concentration of 0.5% and 1.0%. It is clear that the rats fed on berries ethanolic extract orally at 100 ppm and 50ppm were the best results to lowering the level of V-LDL

There are statistically significant differences in total harmful cholesterol (LDL) between the negative control group and the positive control group, also all groups are reduced the level of harmful cholesterol compared to the negative control (G2). while groups 7 and 8, which were fed on the ethanolic extract of juniper leaves and berries at 50 and 100 ppm, achieved

the highest reduction in the level of sLDL followed by the rats fed on 50 and 100 ppm berries extracts. Its also observed that juniper leaves or berries extracts were better than dried plants.

In contrast, there are statistically increase significant differences in the level of good cholesterol between positive control group (G2) in the level of good cholesterol, compared to other groups. Furthermore that, groups 9 and 10, which were fed on the ethanolic extract of juniper leaves and fruits at a concentration of 50 and 100 parts per million, achieved the highest percentage of increase in the level of good cholesterol, followed rats fed on diets containing dried leaves and berries.

These results adapted by *Barzegarnejad et al., (2014)*, they found that the methanolic extract of *Juniperus communis* (100 and 200mg/kg bw) showed significant ( $P<0.01$ ) reduction in blood glucose levels total cholesterol, triglyceride, LDL, VLDL, with elevation. *Bais (2014)* added that when the cholesterol was given along with 200mg/kg *J. communis* then there was no significant increase in the level of Ox-LDL. Therefore, the study showed anti-hyper cholesterolemic effect of Juniper leaves and berries and its extracts.

**Table (8): Changes in serum SOD, MDA, GSH activities in rats fed on Juniper dried leaves or berries and their ethanolic extracts (IU/L).**

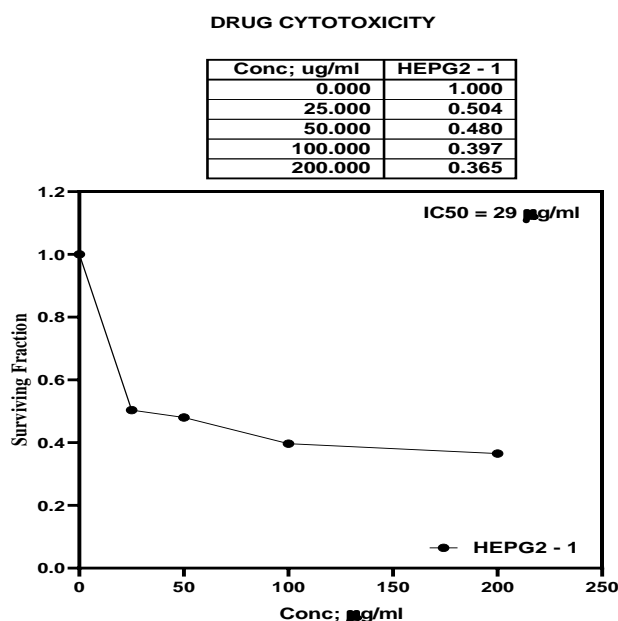
Items	SOD	MDA	GSH
G1 (Negative Control)	29.0c ± 0.89	1.60d ± 0.21	1.75c ± 0.12
G2 (Positive Control)	45.5a ± 1.90	2.50a ± 0.31	2.35a ± 0.08
G3 Dried Leaves 0.5%	40.2ab ± 2.59	1.61d ± 0.25	1.74c ± 0.02
G4 Dried leaves 1%	38.6b ± 0.70	1.35cd ± 0.13	1.52d ± 0.02
G5 Dried Berries 0.5%	36.5bc ± 1.14	1.85bc ± 0.23	1.86b ± 0.19
G6 Dried Berries 1%	34.0bc ± 1.76	1.73c ± 0.04	1.80b ± 0.18
G7 Leaves Extract 50 ppm	32.7c ± 1.24	1.15d ± 0.01	1.29d ± 0.01
G8 Leaves Extract 100 ppm	32.0c ± 1.50	0.65f ± 0.07	1.26c ± 0.03
G9 Berries Extract 50 ppm	40.0ab ± 1.50	1.91b ± 0.47	2.30a ± 0.24
G10 Berries Extract 100 ppm	40.5ab ± 2.14	1.95b ± 0.30	2.15ab ± 0.23

All results are expressed as Mean ± SD.

Values in each column which have different letters are significantly different ( $p<0.05$ ).

It is clear from that there are statistically significant differences between the negative control group and the positive control group in the SOD level (table 8), in favor of the positive control group. There are also statistically significant differences between the negative control group and all experimental groups, in favor of the experimental groups. Groups 7 and 8, which were fed on juniper leaves and berries, achieved concentrations of 50 and 100. ppm, the highest percentage of decrease in levels, followed by group 5 and 6, which were fed on juniper seeds, concentration of 50% and 100%, followed by group 3 and 4, which were fed on dried juniper leaves, concentration of 50% and 100%, and finally group 9 and 10, which were fed on extract, concentration of 50 and 100 ppm. Juniper leaves and berries.

**Table (9) Effect of Juniper leaves extracts on heptocytotoxicity (HEPG-2):-**



Results in tables (9 and 10) illustrated the effect of different concentration of Juniper leaves or berries extract (25, 50 100 and 200 µg/ml, respectively) on hepatic cytotoxicity cells (HPG-2). IC<sub>50</sub> was 29 and 170 µg/ml in juniper leaves and berries extracts, respectively. Results showed leaves extracts more effect than

berries may due to the bioactive components as antioxidants and volatile oil contents.

**Table (10) Effect of Juniper berries extracts on hepatocytotoxicity (HPG2):-**

Generally, the unique structure of antioxidant contents and its synergists in leaves and berries as polyphenols, flavonoids and especially volatile oils reflect to its role of hepatoprotective and anti-hepatocancer effects. Therefore, it can be added to food or juices.

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Instructor at the Ontario institute for studies in  
education (OISE) at the university of Toronto  
and consultant to UNESCO

**Prof. Nicos Souleles** (Greece)

Multimedia and graphic arts, faculty member,  
Cyprus, university technology



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البريد الإلكتروني:

[egvjournal@sedu.asu.edu.eg](mailto:egvjournal@sedu.asu.edu.eg)

الترقيم الدولي الموحد للطباعة : 1687 - 6164

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