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جامعة عين شمس، كلية التربية النوعية، القاهرة، مصر

تحية طيبة وبعد،،،

يسر **معامل التأثير والاستشهادات المرجعية للمجلات العلمية العربية (ارسيف – ARCIF)**، أحد مبادرات قاعدة بيانات **"معرفة"** للإنتاج والمحتوى العلمي، إعلامكم بأنه قد **أطلق التقرير السنوي التاسع للمجلات للعام 2024**.

ويسرنا تهنئتكم وإعلامكم بأن **المجلة المصرية للدراسات المتخصصة** الصادرة عن **جامعة عين شمس، كلية التربية النوعية، القاهرة، مصر، قد نجحت في تحقيق معايير اعتماد معامل "ارسيف 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/

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

ختاماً، نرجو في حال رغبتكم الحصول على شهادة رسمية إلكترونية خاصة بنجاحكم في معامل " **ارسيف** "، التواصل معنا مشكورين.

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Using of Ajwain (Tracyspermum ammi L.) and Its Extracts in Prevention of Hepato-Renal Toxicity in Experimental Rats

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Using of Ajwain (Tracyspermum ammi L.) and Its Extracts in Prevention of Hepato-Renal Toxicity in Experimental Rats

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Prof. Eshak Mourad El-Hadidy
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Abstract

This study was aimed at examining the role of Ajwain and its extracts on toxic kidneys and livers. Rats (64) were divided into two main groups. The first is the control (8 rats), and the second (56 rats) were injected with CCL4 and Gentamicin to induce liver and kidney toxicity. The second group was divided into 7 groups: control (+) fed on basal diet, (3, 4) had (0.5%,1.0%) Ajwain powdered, (5, 6) had (50, 100 ppm) ethanolic extract and (7, 8) had (50, 100 ppm) aqueous extract. Results showed improvement of liver and kidney functions. IC50 in hepatic cells cytotoxcity was (189 µg/ml).

Keywords: Ajwain, Polyphenols, Flavonoids

ملخص:

العنوان : إستخدام الكمون الملكي (Trachyspermum ammi L.) ومستخلصاته فى الوقاية من التسمم الكبدي الكلوي في فئران التجارب

المؤلفون : ايفيلين سعيد عبدالله ، أيمن فتحي خليل ، اسحق مراد الحديدي ، منال ميلاد عبد الشهيد بخيت

هدفت هذه الدراسة إلى: دراسة دور الكمون الملكي و مستخلصاته في سموم الكبد و الكلى. (64) فأر قسمت إلى مجموعتين رئيسيتين: الرئيسية الأولى: ضابطة (-) (8 فئران)، الرئيسية الثانية : (56 فأراً) تم حقنها بـ: CCL4 والجنتاميسين لتسبب تسمم الكبد والكلى. قسمت المجموعة الثانية إلى 7 مجموعات: الضابطة (+) ، و (3 و4) دعمت ب(0.5% و1.0%) من مسحوق الكمون الملكي ، و (5 و6) دعمت ب(50 و100جزء/ المليون) من المستخلص الإيثانولي ، و (7 و8) دعمت ب(50 و100 جزء/ المليون) من المستخلص المائي. وأظهرت النتائج تحسين وظائف الكبد والكلى. بلغت نسبة التسمم الخلوي IC50 في خلايا الكبد (189 ميكروغرام/مل).

الكلمات الدالة : الكمون الملكى ، البوليفينول ، الفلافونيدات

Introduction

Liver and kidney are well known and strictly connected in a reciprocal manner in both the physiological and pathological condition. Liver is a vital organ that plays a key role in the detoxification of endogenous and exogenous substances. Liver is responsible for excreting cholesterol, bilirubin, drugs and hormones, as well as producing and excreting bile ***(Ajjawi, 2022).***

A variety of pathological factors including viral hepatitis (especially hepatitis B and C), alcohol and drug abuse, metabolic diseases, autoimmune diseases and congenital abnormalities can cause hepatic injury. Chronic hepatic diseases are quite common in daily clinical practice. Liver cirrhosis is the final stage of all chronic hepatic diseases. Liver transplantation is the treatment of choice for chronic liver failure, although it faces several difficulties ***(Haytham et al., 2019).***

The kidneys control many biological mechanisms such as fluid, electrolyte, pH balance, blood pressure, excretion of toxins and waste, vitamin D metabolism, and hormone synthesis. Chronic kidney disease often goes undiagnosed due to a lack of apparent symptoms in its early stages. An estimated 94% with mild to moderate decline in renal function and about 48% of individuals with severe renal dysfunction go undiagnosed ***(Adair and Bowden, 2020).***

Medicinal plants are a rich source of valuable biochemical and bioactive compounds and, therefore, have been widely utilized for the management a variety of disorders. Natural antioxidants present in several medicinal plants are responsible for inhibiting the harmful effects of oxidative stress. These plants contain polyphenols and flavonoids that act as free radical scavengers and reduce oxidative stress and may be an alternative remedy to cure various harmful human diseases ***(Phuyal et al., 2020).***

Ajwain is a traditional medicinal plant belonging to the family Apiaceae ***(Nabi et al., 2023).*** Ajwain seeds have enormous health benefits. The therapeutic, medicinal and pharmaceutical potential of Ajwain seeds is attributed to their phytochemical composition and their bioavailability ***(Singh and Meghwal, 2019),*** this plant contains a variety of bioactive compounds of pharmacological importance, involving carbohydrates, fat, fiber, volatile oil, glycosides, protein, phenolic compounds, saponins, and mineral content ***(Goyal et al., 2022).***

The aim of this study is to use Ajwain and its extracts to prevent hepato-renal toxicity in experimental rats.

2. Materials and Methods

2.1. Materials

Ajwain seeds were obtained from the Horticultural Research Institute, Agricultural Research Center, Giza, Egypt. Two kg of transparent plastic bags were kept refrigerated at 4˚C. Carbon tetrachloride (CCl_4), Gentamicin, Ethanol 90%, Casein, Vitamins, Minerals, Cellulose and Choline Chloride were purchased from El- Gomhoreya Company, Cairo, Egypt. Sixty-four male albino rats were obtained from Animal House, Food Technology Research Institute, Agricultural Research Center, Giza, Egypt. The average rat weight was 190±10 g. The basal diet was prepared according to ***Reeves et al., (1993).*** The kites of serum cholesterol, triglycerides, high density lipoprotein (HDL), low density lipoprotein (LDL), uric acid, urea, creatinine, glutamate pyruvate transaminase (ALT), glutamate oxaloacetate transaminase (AST), alkaline phosphatase (ALP), superoxidase dismutase activity (SOD) and malonaldehyde (MDA) were purchased from Bio-Diagnostic Company in Egypt.

2.2. Methods

Volatile oil was determined by the International Standard Organization method ***(ISO, 2009).*** Total polyphenol content was

measured using Folin–Ciocalteu method described by ***(Singleton and Rossi, 1965).***

Gallic acid was used as standard and samples were read in triplicate at 730 nm by a spectrophotometer. Total flavonoids were determined according to the method of ***(Chu et al., 2000).*** Carotenoids (as β-Carotene) was determined according to the method of ***(Nagata and Yamashita, 1992).*** Total tannins were determined according to the method of ***(Polshettiwar et al., 2007).*** The antioxidant activity of Ajwain was determined by 2,2 diphenyl-picrylhydrazyl (DPPH) method described by ***(Chu et al., 2000).***

Ajwain seeds volatile oil Analysis

The gas chromatography-mass spectrometry (GC-MS) was used for analysis the essential oil samples, 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.) polysillphenylene-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 200ºC. 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 300ºC and 250ºC, respectively. The obtained chromatogram and report of GC-MS analysis for each sample were analyzed to calculate the percentage of main components of volatile oil ***(British Pharmacopoeia, 1963).***

Preparation of ethanolic extract of Ajwain seeds

Ajwain seeds were properly washed with water to remove dust particles then dried in under vacuum oven (40 ºC). The dried seeds were ground, stored at 4 ºC and protected from light prior to further use. The ajwain (seeds) were extracted. Briefly, the powder from the seeds was dipped in 100 ml of distilled water and 100 ml ethanol (90%), then refrigerated (4 ºC) for 48 h. The extracts were filtered and the extraction was repeated twice. The

extract was stored in a glass container in refrigerator ***(El-Hadidy et al., 2018).***

Fractionation of Polyphenolic and flavonoid contents

Determination of Polyphenolic Components by HPLC

Total polyphenolic compounds were determined by the Folin-Ciocalteau method ***(Slinkard and Singleton, 1997),*** the absorbance was measured at 760 nm. Results were expressed as gallic acid equivalents (GAE) per 100 g sample. Also, the content of flavonoids was determined according to ***(Chen and Li, 2007)*** at a wavelength of 510 nm. Total flavonoids content of herb extracts was calculated using a standard curve prepared as rutin per 100 g sample.

Determination of Flavonoid compounds by HPLC

While fractionated polyphenolic and flavonoids were determined by HPLC, respectively, according to the methods ***(Goupy et al., 1999)*** and ***(Mattila et al., 2000***) as follows: 5g of dried Ajwain seeds powder was mixed with methanol and centrifuged at 1000 rpm for 10 min and the supernatant was filtrated through 0.2µm Millipore membrane filter, then 1-3ml was collected in avail for injection in HPLC Hellwet Puckered (series 1050) equipped with auto-samplier injector, solvent degasser, ultraviolet (UV) detector set as 289nm and 330nm and quarter HP pump (series 1050). The Colum temperature was maintained at 35ºC. Gradient separation was carried out with methanol and acetonitrile as a mobile phase at flow rate 1ml/min.

Experimental Design

Sixty-four male albino rats weighing an average of 190±10 g, were purchased from Animal House, Agricultural Research Center, Giza. Rats were kept under normal healthy conditions and fed on the basal diet ***(Reeves et al., 1993)*** without any treatment for one week before the experiments for adaptation to laboratory conditions, all practical parts are applied in agriculture research center, Giza, Egypt.

Rats were divided into two main groups. The first group is the negative control (-) consists of 8 rats. The second group, consists of 56 rats were injected with: Carbon tetrachloride (1ml/Kg body weight) and gentamicin (1ml/Kg body weight) to induce liver and kidney toxicity. The same second group was divided into 7groups: the control (+) group, which was fed on basal diet till the end of the experiment. Hepato-renal toxic groups (3 and 4) had 0.5%, 1.0% of the diet of Ajwain powdered seeds, respectively. Also, groups (5 and 6) had 50 ppm, 100 ppm of Ajwain ethanolic extract, respectively, and groups (7 and 8) had 50 ppm, 100 ppm of Ajwain aqueous extract, respectively. During the experimental period (6 weeks), rats were individually weighed every two weeks. The dose of ethanolic and aqueous extracts in rats fed on orally was 2 ml extract/day for each rat.

Collection of Blood Samples

At the end of the experimental period, the rats were fasted overnight then, anaesthetized and sacrificed and blood samples were collected from the aorta. The blood samples were centrifuged for 15 minutes at 3000 rpm to separate the serum. The serum was carefully separated into dry clean Wassermann tubes by using a Pasteur pipette and kept frozen till analysis at -20°C.

Serum Analysis

Liver functions: aspartate amine transaminase (AST), Alanine amine transaminase (ALT) were measured according to the method described by ***Tietz et al., (1999).*** Alkaline phosphates (ALP) were measured according to ***Belfield and Goldberg (1971).***

Determination of Total Protein: Total protein was determined according to the method described by ***(Gornall et al., 1949)***, albumin was determined according to the method described by ***(Doumas, 1971),*** and globulin calculation:

Total Protein - Albumin = Globulin

Renal functions: Uric acid was determined in the serum according to the method described by ***(Fossati et al., 1980)***, urea nitrogen was determined according to ***(Batton and Crouch, 1977).*** Serum creatinine was determined according to ***(Bowers and Wong, 1980).***

Estimation of Malondialdehyde (MDA): Lipid peroxide (Malondialdehyde) was determined in the serum according to the colorimetric method described by ***Ohkawa et al., (1979).*** The determination of superoxide dismutase activity (SOD) was examined according to the method of ***Misra and Fridovich (1972).***

Cytotoxicity activity of Ajwain extract

Measurement of potential cytotoxicity activity of Ajwain against the liver carcinoma cell line (HepG-2), was done by SRB assay using the method of ***Skehane et al., (1990).*** This experiment was conducted at the Egyptian Cancer Institute in Cairo, Egypt.

Statistical Analysis

The data obtained was subjected to an analysis of variance (ANOVA) followed by Duncan Multiple Range test to compare treatment means; differences were considered significant at 95% ($P \leq 0.05$) (SPSS V21 software) ***(Owoicho et al., 2020).***

3. Results and Discussion

3.1. Antioxidant Activity

Ajwain has the potential to be used as a source of natural antioxidants and this property is directly related to the amount of total phenols and flavonoids ***(Saei et al., 2021).*** Phenolic and flavonoid compounds have been known as natural products with high antioxidant activity. Nowadays, such components are frequently used and emphasized in food and some industrial products because of their health properties. These compounds also have a crucial role in scavenging of the free radicals that are considered as serious risk factors for human health. Therefore,

polyphenolic components extracted from natural sources of plant species are of great importance and thence, there is a growing interest to use the natural sources of antioxidants instead of synthetic ones ***(Gharibi et al., 2019).***

DPPH radical scavenging activity

Table (1): Antioxidant Contents and Its Activity (DPPH) in Ajwain Seeds

Component	(mg/100g)
Total phenolic	64.58 ± 2.70
Total Flavonoids	23.27 ± 2.23
Carotenoids	13.60 ± 0.58
Tannins	11.26 ± 0.052
1,2diphenyl-1-picryl hydrazyl (DPPH)	43.48 ± 2.58

All results are expressed as mean (3 samples) ± SD.

The results in Table (1) showed the highest content of total phenolic compounds. While, the content of total flavonoids (23.27mg/100g), carotenoids (13.60 mg/100g) and tannins (11.26 mg/100g).

3.2. Essential Oil Constituents of Ajwain Seeds

The essential oils extracted from different plants have different antibacterial activities, which can be attributed to different chemical compounds in the essential oils. The antibacterial properties of essential oils are greatly influenced by alkaloids, phenolic compounds, flavonoids, cyanidins, and saponins ***(Kiarsi et al., 2020).***

Table (2) Essential Oil Composition of Ajwain Seeds

Component of Essential oil	(mg/100g)
Thymol	39.46
Carvacrol	3.45
α-thujene	0.39
α-pinene	0.72
β-pinene	3.18
Myrcene	0.96
ρ-cymene	29.14
Limonene	0.65

γ-terpinene	20.42
Terpinen-4-ol	0.35
Carvone	0.46
Unknown	0.82

Eleven compounds were identified in the oil by GC analyses. The identified compounds and their percentages have been given in Table (2). The major compounds were thymol(39.46mg/100g), p-cymene (29.14 mg/100g) and γ-terpinene (20.42 mg/100g).

Fractionation of Polyphenolic and flavonoid contents

3.3. Quantification of Polyphenolic Components in Ajwain Seeds Extract

Polyphenols play an important role in maintaining your health and wellness. Polyphenols are a hot topic among functional food proponents due to the increasing evidence that they can impact your health in positive ways. Phenolic compound exerted antioxidant, anticancer, antidiabetic, cardiovascular effect, anti-inflammatory, protective effects in neurodegenerative disorders and many others therapeutic effects ***(Al-Snafi, 2018).***

Table (3): Polyphenolic Compounds of Ajwain Seeds Extract

Phenolic Compounds	(mg/100g)
Gallic acid	55.35
Pyrogallol	45.25
4-amino benzoic acid	22.16
Protocatechuic acid	68.53
Chlorogenic acid	25.53
Catechol	27.89
Epicatechein	72.75
Caffeine	38.14
Caffeic acid	63.54
Vanillic acid	92.37
Ferulic acid	62.38
Iso-Ferulic acid	34.45
E-vanillic acid	60.75
Reversetrol	12.32
Ellagic acid	46.27
α-coumaric acid	61.92
Benzoic acid	26.54
3,4,5-methoxy cinnamic acid	44.87

Coumaric acid	1.19
P-cumaric acid	19.35
Cinnamic acid	48.29
Quercetin	11.12
Catechin	4.42

Results in Table (3) indicated the polyphenols content in Ajwain, which showed that vanillic acid (92.37mg/100 g), pyrogallol (45.25mg/100 g), epicatechin (72.75mg/100 g), caffeic acid (63.54mg/100 g). On the other hand, the results are recorded that e-vanillic acid (60.75mg/100 g), protocatechuic acid (68.53mg/100 g), ellagic acid (46.27mg/100 g) and ferulic acid (62.38mg/100 g).

3.4. Quantification of Flavonoid Components in Ajwain Seeds Extract

In recent years, more attention has been paid to natural sources of antioxidants. Flavonoids are natural substances synthesized in several parts of plants that exhibit a high antioxidant capacity. They are a large family, presenting several classes based on their basic structure. Flavonoids have the ability to control the accumulation of reactive oxygen species (ROS) via scavenger ROS when they are formed. Therefore, these antioxidant compounds have an important role in plant stress tolerance and a high relevance in human health, mainly due to their anti-inflammatory and antimicrobial properties ***(Dias et al., 2021).***

Table (4): Flavonoids Compounds of Ajwain Seeds Extract

Flavonoid compounds	(mg/100g)
Rutin	2.93
Naringenin	1.12
Hesperidin	7.84
Quercetrin	2.47
Apigenin	1.12
Kaempferol	0.65
Luteoline-6-arabinose-8-glucose	10.76
Luteoline -6-glucose-8-arabinose	2.95
Narengin	2.11
Apigenin-6-arabinose-8-galactose	7.04

**mg/100g dry weight basis*

Data in table (4) showed that Luteoline-6-arabinose-8-glucose (10.76 mg/100g), hesperidin (7.84 mg/100g) and Rutin (2.93 mg/100g).

3.5. Effect of Ajwain Seeds Powder and Its Extracts on Serum Liver Parameters

Ajwain was considered a liver tonic, effective in treating liver diseases and colds. The methanolic extract of Ajwain also contains hepatoprotective properties. An in vivo study reported that the methanolic extract of Ajwain could protect the liver by avoiding an increase in the serum levels of liver enzymes of AST, ALT, and ALP ***(Shafiezadeh et al., 2020).***

Table (5): Effect of Ajwain Seeds Powder and Its Extracts on Serum Liver Parameters

Groups	AST				ALT				ALP			
	0 time	After 2 weeks	After 4 weeks	After 6 weeks	0 time	After 2 weeks	After 4 weeks	After 6 weeks	0 time	After 2 weeks	After 4 weeks	After 6 weeks
Negative Control	37.62 b ± 1.23	37.01 g ± 1.13	37.26 g ± 1.11	36.72 g ± 1.30	23.27 b ± 1.35	23.24 g ± 0.83	23.36 g ± 0.86	23.54 g ± 0.91	126.72 b ± 1.62	126.14 f ± 1.43	125.75 e ± 1.34	125.49 e ± 1.67
Positive Control	68.77 a ± 0.99	68.07 a ± 1.08	67.38 a ± 0.71	66.56 a ± 0.90	57.83 a ± 2.28	56.91 a ± 2.27	56.05 a ± 2.46	55.01 a ± 2.51	262.95 a ± 3.85	262.06 a ± 3.68	268.75 a ± 10.79	268.03 a ± 10.96
Ajwain Seeds Powder (0.5%)	68.43 a ± 0.95	64.38 c ± 0.37	56.11 c ± 0.40	46.11 c ± 0.41	57.00 a ± 4.33	50.22 cd ± 0.10	44.14 cd ± 0.18	38.67 cd ± 0.26	262.20 a ± 8.62	236.20 c ± 0.66	216.02 bc ± 0.32	196.29 bc ± 0.51
Ajwain Seeds Powder (1.0%)	68.23 a ± 1.40	63.26 d ± 0.34	55.01 d ± 0.37	45.12 d ± 0.41	57.05 a ± 3.64	49.51 de ± 0.34	43.08 de ± 0.28	37.59 de ± 0.40	262.07 a ± 8.20	234.03 d ± 0.31	214.87 bcd ± 0.28	194.04 bcd ± 0.25
Ajwain Ethanolic Extract (50 ppm)	68.52 a ± 1.43	61.81 e ± 0.29	53.03 e ± 0.34	43.02 e ± 0.40	57.07 a ± 2.70	48.76 ef ± 0.17	42.01 e ± 0.15	36.58 ef ± 0.43	262.22 a ± 10.62	233.00 d ± 0.41	212.71 cd ± 0.28	192.69 cd ± 0.30
Ajwain Ethanolic Extract (100 ppm)	68.41 a ± 1.23	60.97 f ± 0.65	51.17 f ± 0.23	41.00 f ± 0.29	57.01 a ± 3.75	48.13 f ± 0.21	40.77 f ± 0.22	35.43 f ± 0.31	262.34 a ± 10.17	230.93 e ± 0.28	211.06 d ± 0.22	190.95 d ± 0.23
Ajwain Aqueous Extract (50 ppm)	68.46 a ± 1.24	65.81 b ± 0.23	60.02 a ± 0.36	48.05 a ± 0.20	57.35 a ± 2.76	52.12 b ± 0.16	46.09 b ± 0.35	40.93 b ± 0.20	262.64 a ± 12.00	239.48 b ± 0.39	219.59 b ± 0.35	198.70 b ± 0.75
Ajwain Aqueous Extract (100 ppm)	68.38 a ± 1.36	64.91 c ± 0.23	58.08 b ± 0.36	47.08 b ± 0.23	57.90 a ± 2.18	51.06 c ± 0.14	45.02 bc ± 0.19	39.55 c ± 0.37	262.14 a ± 11.02	237.93 b ± 0.40	218.07 b ± 0.29	197.92 b ± 0.33

All results are expressed as mean ± SD.

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

The effect of Ajwain seeds powder and its extracts on liver function tests: AST, ALT and ALP are shown in Table (5). Administration of CCl4 significantly ($p < 0.05$) increased the liver enzymes AST (68.77 IU/L), ALT (57.83 IU/L) and ALP (262.06 IU/L) in all the treated rats when compared to control rats (37.62,

56.91, and 126.72 IU/L, respectively). Ajwain seeds powder and its extracts treated groups tended to significantly reverse the effects of CCl_4 on the AST, ALT and ALP in a dose- dependent manner.

From the data illustrated in table (5) and fig. (1,2&3) it could be showed that the highest effect on Liver function parameters in rats group fed on ethanolic extract 100ppm compared to the decrement of positive control group.

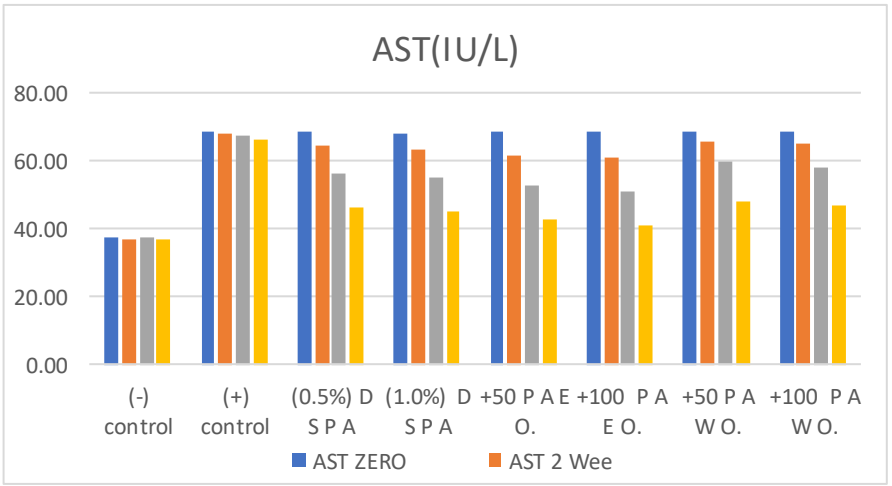

Fig.1: Effect of Ajwain Seeds Powder and Its Extracts on AST

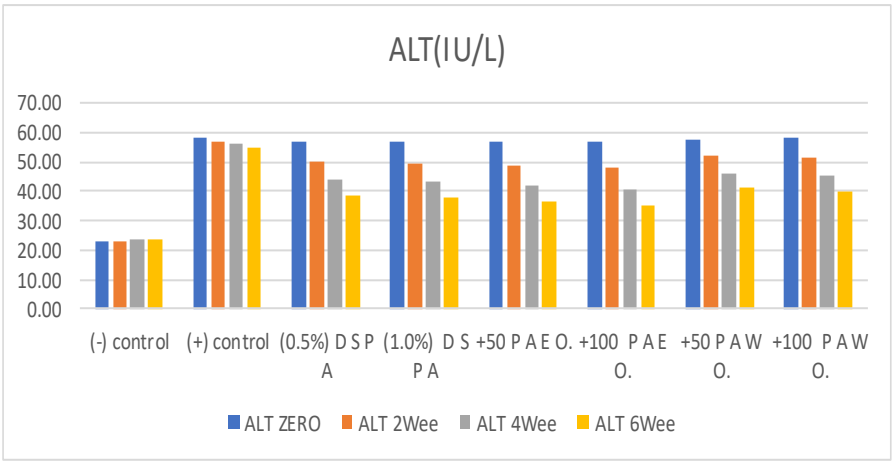

Fig.2: Effect of Ajwain Seeds Powder and Its Extracts on ALT

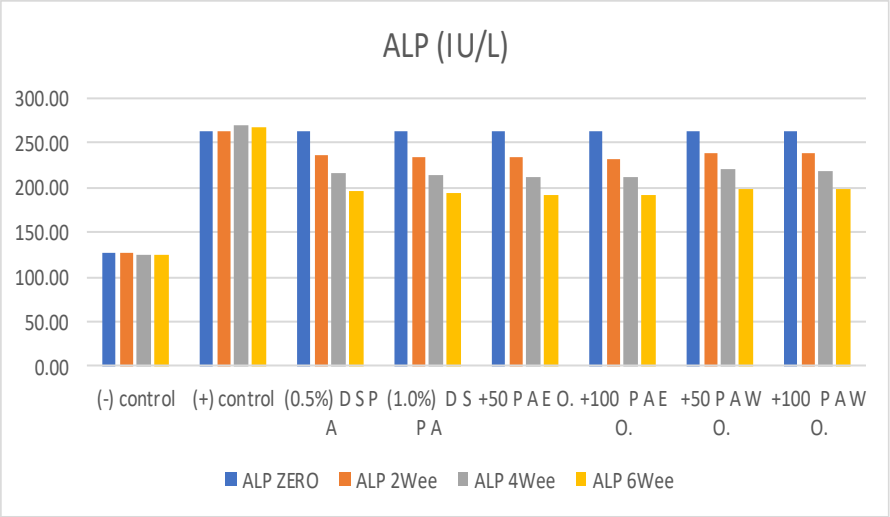

Fig.3: Effect of Ajwain seeds powder and its extracts on ALP

Along with the potent antioxidant activity, the Ajwain methanolic extract was revealed to exhibit *in vivo* hepatoprotective activity with 80% defense against an in general deadly dose of paracetamol in pests. The extract also had preventive effects against CCl4-induced prolongation of pentobarbital sleeping time as well as the levels of alkaline phosphatase (ALP), aminotransferases (AST and ALT) hepatic enzymes, and liver damage ***(Saraswat et al.,2020).***

3.6. Effect of Ajwain Seeds Powder and Its Extracts on Serum Kidney Parameters

Urea, creatinine and uric acid are the most important indicators of kidney function ***(Muhammad et al., 2014).***

Concentration of urea and creatinine was significantly increased ($p \leq 0.05$) in serum with induced nephrotoxicity as compared to the control group. Coadministration of and different doses of Ajwain significantly improved the changed levels of urea and creatinine causing a subsequent recovery towards normalization. However, there was no significant difference between different doses of T. ammi ***(Farzaei et al., 2018).***

Table (6): Effect of Ajwain Seeds Powder and Its Extracts on Serum Kidney Parameters

Groups	Urea (mg/dl)				Creatinine (mg/dl)				Uric Acid (mg/dl)			
	0 time	After 2 weeks	After 4 weeks	After 6 weeks	0 time	After 2 weeks	After 4 weeks	After 6 weeks	0 time	After 2weeks	After 4 weeks	After 6 weeks
Negative Control	44.77 b ± 2.19	44.05 g ± 2.13	44.60 g ± 2.15	44.55 f ± 2.42	0.75 b ± 0.02	0.74 e ± 0.02	0.75 d ± 0.02	0.74 d ± 0.02	1.90 c ± 0.04	1.83 e ± 0.04	1.85 d ± 0.06	1.81 d ± 0.07
Positive Control	64.53 a ± 1.77	64.13 a ± 1.93	66.68 a ± 0.74	66.16 a ± 0.81	1.27 a ± 0.03	0.99 a ± 0.06	0.99 a ± 0.05	1.01 a ± 0.06	3.75 a ± 0.10	3.66 a ± 0.15	3.76 a ± 0.15	3.51 a ± 0.20
Ajwain Seeds Powder (0.5%)	64.26 a ± 2.67	58.05 cd ± 0.33	53.02 d ± 0.14	50.00 cd ± 0.20	1.26 a ± 0.04	0.93 bc ± 0.03	0.91 b ± 0.03	0.86 b ± 0.03	3.06 b ± 0.27	2.86 b ± 0.09	2.80 b ± 0.39	2.76 b ± 0.61
Ajwain Seeds Powder (1.0%)	62.52 a ± 2.87	57.01 de ± 0.25	52.01 e ± 0.37	49.90 cd ± 0.14	1.28 a ± 0.05	0.89 cd ± 0.06	0.89 b ± 0.04	0.85 bc ± 0.03	3.24 b ± 0.56	2.75 bc ± 0.37	2.89 b ± 0.41	2.73 b ± 0.37
Ajwain Ethanolic Extract (50 ppm)	63.05 a ± 3.47	56.04 ef ± 0.27	51.03 ef ± 0.22	49.00 de ± 0.21	1.29 a ± 0.03	0.90 bc ± 0.03	0.89 b ± 0.03	0.84 bc ± 0.06	3.21 b ± 0.37	2.47 cd ± 0.28	2.40 c ± 0.46	2.34 c ± 0.50
Ajwain Ethanolic Extract (100 ppm)	63.04 a ± 2.74	55.40 f ± 0.27	50.23 f ± 0.28	48.25 e ± 0.29	1.26 a ± 0.06	0.84 d ± 0.05	0.81 c ± 0.06	0.80 cd ± 0.05	3.06 b ± 0.50	2.40 d ± 0.43	2.14 cd ± 0.25	2.01 cd ± 0.08
Ajwain Aqueous Extract (50 ppm)	64.12 a ± 1.74	60.25 b ± 0.81	55.61 b ± 0.32	52.02 b ± 0.26	1.25 a ± 0.04	0.95 ab ± 0.03	0.93 b ± 0.04	0.90 b ± 0.05	3.02 b ± 0.36	2.88 b ± 0.25	2.90 b ± 0.08	2.82 b ± 0.11
Ajwain Aqueous Extract (100 ppm)	64.47 a ± 2.49	59.04 bc ± 0.30	54.60 c ± 0.37	51.02 bc ± 0.26	1.27 a ± 0.03	0.95 ab ± 0.03	0.91 b ± 0.02	0.89 b ± 0.07	3.30 b ± 0.48	2.87 b ± 0.16	2.90 b ± 0.12	2.84 b ± 0.08

All results are expressed as mean ± SD.

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

In the present study, the serum levels of urea, creatinine and uric acid in gentamicin-treated group were significantly high ($P \leq 0.01$), indicating nephrotoxic effects of gentamicin. This increased serum urea, creatinine and uric acid levels by gentamicin were antagonized by Ajwain seeds powder and its extracts in a dose-dependent manner as shown in Table (6).

Administration of Ajwain seeds and their extracts to rats significantly ($p < 0.05$) decreased the serum levels of uric acid, creatinine and urea when compared to positive control group (3.51, 1.01 and 66.16 mg/dl, respectively).

Data presented in table (6) and fig. (4-5&6) showed that groups 5 and 6 the groups of rats fed with the ethanolic extract orally (50 and 100 ppm) achieved the highest percentage of decrease in serum levels of Uric acid, Creatinine and Urea followed by group 3 and 4 which were fed on dried Ajwain powder (0.5 and 1%), then group 7 and 8 which were fed on the aqueous extract orally.

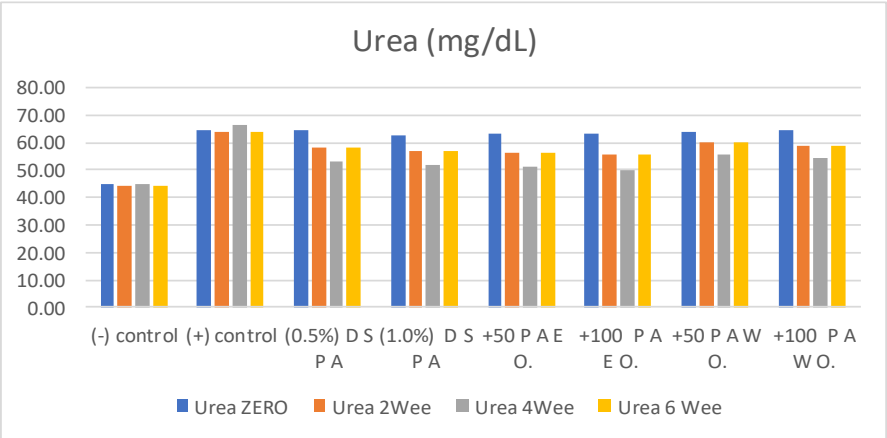

Fig.4: Effect of Ajwain Seeds Powder and Its Extracts on Urea

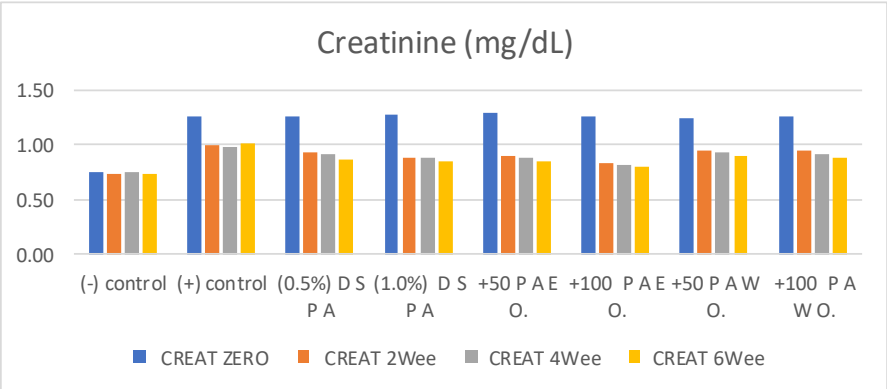

Fig.5: Effect of Ajwain Seeds Powder and Its Extracts on Creatinine

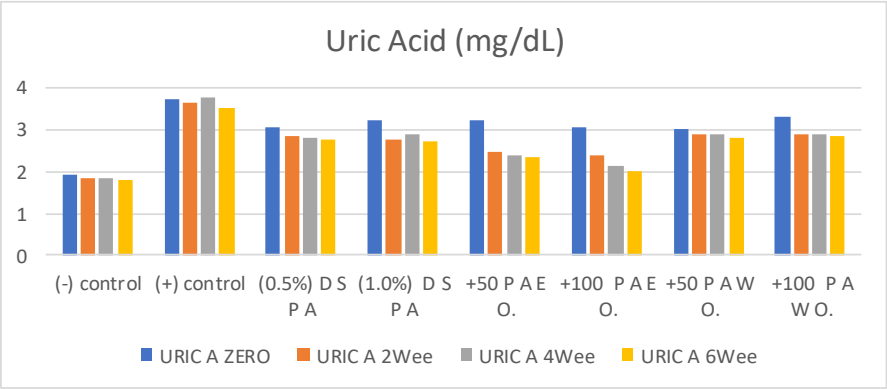

Fig.6: Effect of Ajwain Seeds Powder and Its Extracts on Uric Acid

Administration of gentamicin results in kidney damage, which is counteracted by co-administration of plant extract, which shows nephroprotective activity. It has been shown that polyphenolic compounds prevent nephrotoxicity induced by oxidative stress and restore renal marker levels. The phytochemical constituents in Ajwain might be responsible for its antioxidant activity and protective effects in gentamicin-induced nephrotoxicity ***(Ishaq et al., 2015).***

3.7. Effect of Ajwain Seeds and Its Extracts on Serum Total Protein, Albumin and Globulin

Table (7): Effect of Ajwain seeds powder and Its Extracts on Total Protein, Albumin and Globulin

Groups	Total Protein	Albumin	Globulin
Negative Control	4.88 g ± 0.16	2.48 g ± 0.03	2.40 e ± 0.07
Positive Control	7.44 a ± 0.10	3.58 a ± 0.04	3.85 a ± 0.44
Ajwain Seeds Powder (0.5%)	6.1 c ± 0.05	3.28 c ± 0.03	2.82 cd ± 0.04
Ajwain Seeds Powder (1.0%)	5.8 d ± 0.17	3.11 d ± 0.04	2.69 cd ± 0.05
Ajwain Ethanolic Extract Orally (50 ppm)	5.65 e ± 0.03	2.90 e ± 0.04	2.75 cd ± 0.09
Ajwain Ethanolic Extract Orally (100 ppm)	5.45 f ± 0.03	2.77 f ± 0.04	2.68 d ± 0.05
Ajwain Aqueous Extract Orally (50 ppm)	6. 5 b ± 0.01	3.37 b ± 0.01	3.13 b ± 0.34
Ajwain Aqueous Extract Orally (100 ppm)	6.26 c ± 0.22	3.31 c ± 0.03	2.95 bc ± 0.14

All results are expressed as mean ± SD.

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

The data in Table (7) showed a significant decrease in total protein in rats fed on Ajwain seeds powder ethanolic extract orally at 50 and 100 ppm (5.65 and 5.45 mg/dl) followed by dried Ajwain seeds powder at 0.5% and 1% (6.1 and 5.8 mg/dl), then Ajwain aqueous extract orally at 50 and 100 ppm (6. 5 and 6.26 mg/dl), respectively compared to the positive group (7.44mg/dl).

Also, the results at the same table showed the albumin had been decreased in rats fed with dried powder (0.5% and 1.0%) of Ajwain, the decrease were 3.28 and 3.11 mg/dL, respectively. While, the rats had been fed with Ajwain ethanolic extracts

(50ppm and 100ppm) orally were 2.90 and 2.77 mg/dL compared to positive group (3.58 mg/dL).

At the same trend, dried Ajwain powder and its ethanolic extracts were significant decrease effect on globulin of rats group (0.5 and 1.0% dried Ajwain powder and 50 and 100 ppm ethanolic extracts) were 2.82, 2.69, 2.75 and 2.68 mg/dL, respectively compared to positive group (3.85 mg/dL).

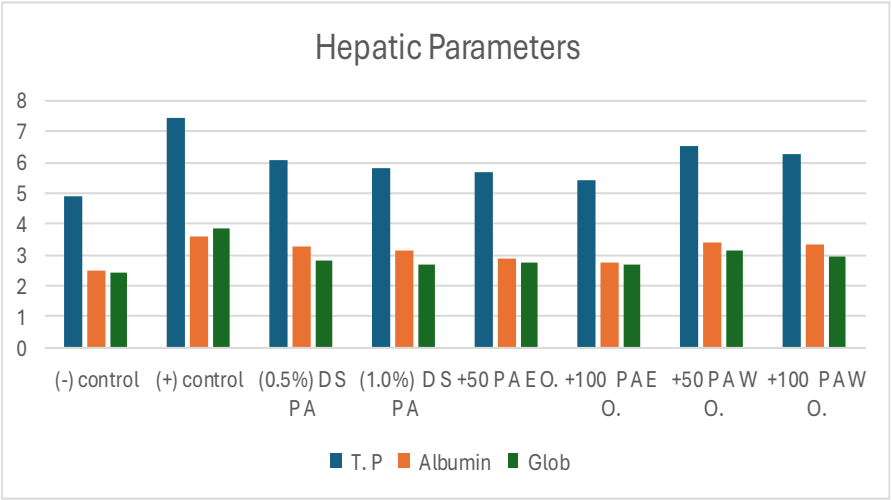

Fig.7: Effect of Ajwain Seeds Powder and Its Extracts on Total Protein, Albumin and Globulin

3.8. Enzymatic determination

Effect of Ajwain Seeds Powder and Its Extracts on Serum SOD And MDA

(SOD) superoxide dismutase and (MDA) malondialdehyde are parameters of oxidative stress that relate to organ capacity.

The effect of Ajwain seeds powder and its extracts on superoxide dismutase (SOD) and malondialdehyde (MDA) is shown on Table (8).

Table (8): Effect of Ajwain Seeds Powder and its extracts on SOD (IU/mL) and MDA (µmol/L) after 6 weeks

Groups	SOD (IU/mL)	MDA (µmol/L)
Negative Control	58.55a ± 0.08	3.46c ± 0.38
Positive Control	35.49d ± 4.14	5.18a ± 0.75
Ajwain Seeds Powder (0.5%)	46.30c ± 1.77	4.25b ± 0.13
Ajwain Seeds Powder (1.0%)	48.02c ± 1.61	4.19b ± 0.06
Ajwain Ethanolic Extract Orally (50 ppm)	50.15b ± 3.71	3.86c ± 0.07
Ajwain Ethanolic Extract Orally (100 ppm)	52.13b ±1.82	3.65c ± 0.07
Ajwain Aqueous Extract Orally (50 ppm)	44.29 b ± 0.01	4.37 b ± 0.01
Ajwain Aqueous Extract Orally (100 ppm)	45.56 c ± 0.22	4.31 c ± 0.03

All results are expressed as mean ± SD.

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

The effect of Ajwain on the superoxide dismutase (SOD) and malondialdehyde (MDA) in liver and kidney is shown in table (8).

Rats fed on different diet which contained Ajwain seeds Powder or its extracts were significant increase in SOD activity compared to positive group table (15).

Result stated that Ethanolic Extract 50ppm and 100ppm had higher significant increase SOD (50.15 and 52.13%, respectively) than Ajwain seeds Powder 0.5 and 1.0% (46.30% and 48.02%, respectively).

In contrast, rats fed on Ajwain seeds powder or its extracts were decreased in MDA compared to positive group. Also, rats group fed on ethanolic extract orally were higher decrement in MDA than rats fed on Ajwain seeds powder.

Ajwain administration decreased the level of MDA and increased the level of SOD in the CCl4 treated groups significantly in dose dependent manner.

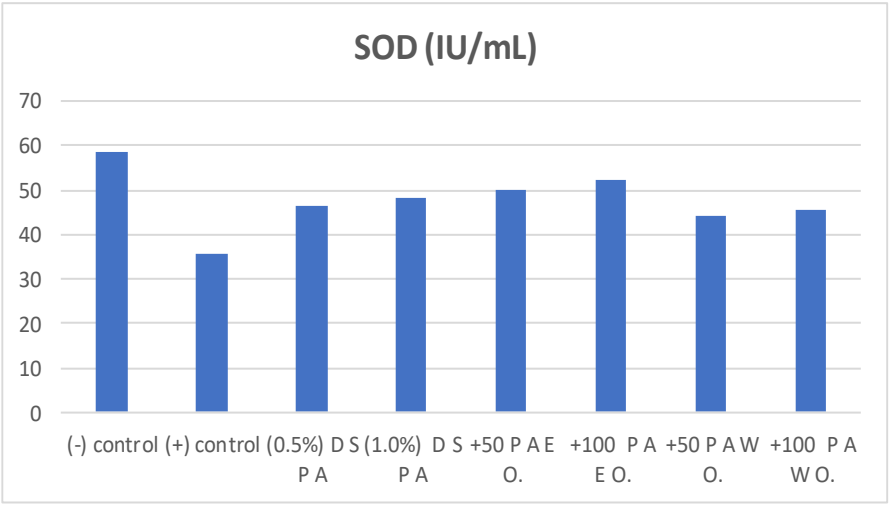

Fig.8: Effect of Ajwain Seeds Powder and its Extracts on SOD (IU/ml)

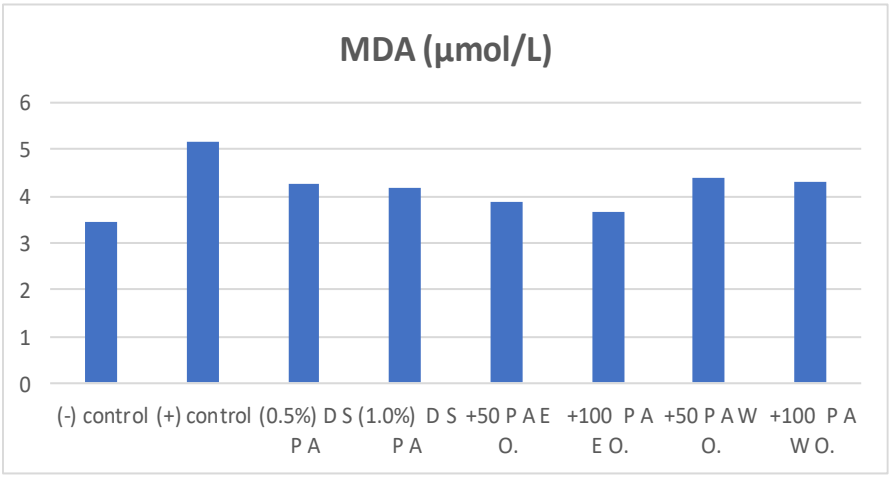

Fig.9: Effect of Ajwain Seeds Powder and Its Extracts on MDA (µmol/L)

3.9. Effect of Ajwain on Hepatic Carcinogenic

Table (9): Effect of Ajwain extracts on hepato-cytotoxicity (HepG-2)

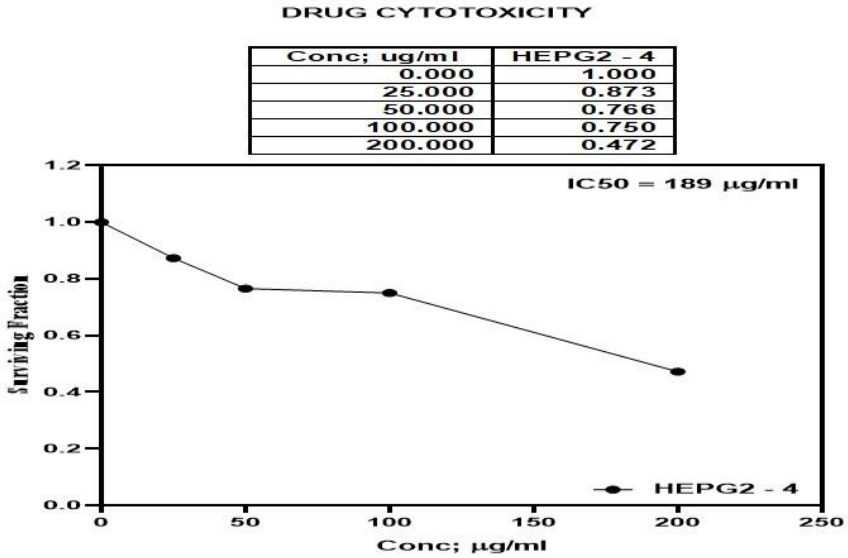

Fig. 10: Effect of Ajwain extracts on hepato-cytotoxicity (HEPG-2)

Results in table (10) illustrated the effect of different concentration of Ajwain extracts (25, 50 100 and 200 µg/ml, respectively) on hepatic cytotoxicity cells (Hep G -2). IC50 was 189 µg/ml in Ajwain extracts. This result was indicated Ajwain had an effect on cancer (HepG-2 cells).

Recommendations

- More research is needed for using Ajwain in other diseases in rats and human.
- Nutritional education programs must be carried out through media sources about the benefits of Ajwain and its antioxidants.

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ترسل المراسلات باسم الأستاذ الدكتور/ رئيس التحرير، على العنوان التالى

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يوليو ٢٠٢٥

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