

Effect of Tarragon (*Artemisia dracunculus L.*) and Its Ethanolic Extracts on Chronic Liver Disease in Male Albino Rats

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Abstract

Tarragon (*Artemisia dracunculus L.*) is a herb belonging to the Asteraceae family with a long history of protective effect and other medicinal benefits. This study aiming for studying Effect of Tarragon dried leaves and its ethanolic extracts (80%) at different concentrations on chronic liver disease. Result of chemical composition of dried tarragon leaves (protein, carbohydrates, oil, fiber and ash (23.42, 48.53, 6.99 and 7.03 g/100g, respectively) .It is rich in polyphenol, flavonoids and carotenoids contents (28.5, 15.45 and 9.98 mg/100g, respectively). Antioxidants activity in dried of tarragon leaves was high(86.43%) the major phenolic compounds were Benzoic acid, Catechein, Salycillic acid and Pyrogallol (1405.42, 8080.80, 252.71 and 213.25ppm, respectively). Also, flavonoids fractions indicated the highest content in Acacetin neo.rutinoside, Apig.6-arbinose8-glactose, Apegnin, Luteolin 7-glucose and Hespirtin (907.70; 609.15; 576.54; 361.49 and 307.31 ppm, respectively). Sixty male albino rats weighted (180±20g) were fed on basal diet for two weeks before the experiment were divided to ten main groups for 60 days as follows: G1 (normal) and G2 induced CCl₄, in paraffin oil (50% v/v 2ml/Kg) twice weeks for chronic damage rats of liver rats and fed on basal diet. (G3 normal and G4 induced CCl₄) fed on diet containing tarragon powder 1% ,(G5 normal, and G6 induced CCl₄) fed on diet containing tarragon powder 2% ,(G7 normal and G8 induced CCl₄) treated orally with(100mg/Kg wt./day) tarragon extract. (G9 normal, G10 induced CCl₄) treated with 200mg/kg wt./day tarragon extract, orally . At the end of the experimental, serums were collected for determine liver and kidney functions. Results showed that decreased in AST , ALT and ALP in groups (9 and 10) which had oral tarragon extract (100 and 200mg /kg), while uric acid increased in G10 (47.67mg/dL), but was decreased creatinine in G6 (0.55mg/dL) which had taken 2% tarragon powder. Conclusively, tarragon extracts were significantly improved hepato-renal serum parameters in chronic liver disease rats groups.

Key words: Tarragon (*Artemisia dracunculus L.*), Dried Leaves, Polyphenols, Flavonoids, Chronic Liver Disease.

Introduction:

Chronic Liver Disease is a condition in which the liver slowly deteriorates and malfunctions due to chronic injury. Scar tissue replaces healthy liver tissue, partially blocking the flow of blood through the liver. Fibrosis begins with a long-lasting rather asymptomatic period (**Pinter et al., 2016**). It can lead to cirrhosis, hepatocellular carcinoma if left untreated and liver failure (**Rajathi and Jiji, 2019**).

People are interested in using herbal medicine because of side effects of chemical drugs. Plants are complementary and alternative medicine due to their ability for producing secondary metabolites such as; proteins, flavonoids, alkaloids, steroids, and phenolic compounds which are used to recover health (**Moradi and Esfahani, 2016**).

Tarragon (*Artemisia dracunculus L.*) is a perennial herb belonging to the Asteraceae family with a long history in culinary tradition and medicinal, it also possesses wide range of health benefits (**Ribnický, et al., 2014**). Its taste is herbaceous with anise like (**Obolskiy et al., 2011**). Leaves smooth green and grown in warm, dry areas, grows in Egypt on Middle Sinai region (**El-Sayed et al., 2009**). Tarragon has a long history in food industry (flavoring of meat, sauces and vinegar) and cosmetics industry as well as medicinal use (**Ekiert et al., 2021**). It contains antioxidants including monoterpenoids, sesquiterpenoids and isocoumarins, flavonoids, coumarin and alkaloids (**Eisenman et al., 2011**). The extract of tarragon has anti-parasitic, anti-fungal, sedative, anti-cough activity, immunomodulating and anti-tumour activities (**Wang et al., 2011**). Flavonoids have gained great interest as potential therapeutic agents against a wide variety of diseases. The most common flavonoids present in medicinal plants include quercetin, kaempferol, luteolin and apigenin (**Duric et al., 2015**).

Ibrahem, (2017) found that tarragon has a potential chemo-preventive effect against carcinogen-induced skin cancer in mice. The methanolic extract of tarragon dried aerial parts contains glycosides, volatile oils, alkaloids, terpenoids, phenolic compounds and flavonoids. **Msaada et al., (2015)** added that gallic acid, widely used as an additive to prevent food spoilage, is renowned for its anti-carcinogenic, anti-inflammatory, and antimutagenic activities. Study showed the presence of other flavonoid glycosides (isoquercitrin and quercetin-3-O- β -D-glucoside) in the samples. **Kafshboran et al., (2011)** showed that flavonoids are a class of secondary plant phenolic, which act as pharmacological active compounds in many medicinal plants with their powerful antioxidant properties. Flavonoids have the basic skeleton of diphenyl propanes (C6–C3–C6) with various oxidation levels of the central pyran ring, they could provide strong antioxidant activities associated with their capacity to scavenge free radical and terminate radical chain. Therefore, this study aimed to evaluate Tarragon herb and its extracts as a hepato-protective plant.

2.1. Materials:

Tarragon (*Artemisia dracunculus L.*) leaves was obtained from Herbs and Spices Company in Obour City El Kalubia, Egypt. Carbon tetrachloride (CCl₄) and ethanol (80%) were obtained from Middle East Company Ind. Cairo, Egypt. Animals: sixty male albino rats weighed 180 ±20 g obtained from Experimental Animals unit in Food Technology Research Institute, Agricultural Research Center, Giza- Egypt.

2.2. Methods:

2.2.1. Chemical Analyses of Dried Leaves Tarragon:

- Chemical composition of Tarragon dried leaves (moisture, crude fiber, oil, protein, and ash contents) was determined according to methods of **(A.O.A.C., 2000)**. Total carbohydrates were calculated by difference.
- Tarragon leaves was washed in aqueous several times to remove any adhering flesh, dried in oven under vacuum, then ground well. Ground tarragon was dipping in ethanol 80% (1:100 w/v) in dark bottle for 48h in refrigerator at 4°C temperature. To obtain extracts, then mixtures were filtered by filter paper (Whatman1). Ethanolic solutions were evaporated in rotary evaporator at 50°C **(El-Hadidy et al., 2018)**.

2.2.2. Determination of Antioxidants Content:

The content of total polyphenols in the Tarragon dried leaves was determined by using the Folin-Ciocalteu phenol reagent **Amarowicz et al., (2004)**. Flavonoids were determined according to the methods of **Chang et al., (2002)**. The free radical scavenging capacity of extracts was determined using DPPH by **Burits and Bucar (2000)**. Also, Carotenoids as β-Carotene was determined according to the method of **Nagata and Yamashita (1992)**. While, Volatile oil was determined by hydordistillation method **(ISO 6571: 2009)**.

The capacity to scavenge activity the “stable” free radical DPPH (2,2-Diphenyl-1-picrylhydrazyl) was monitored according to the method of **Takao et al. (1994)**.

2.2.3. HPLC Determination of Phenolic and Flavonoid Compounds:

Polyphenolic and Flavonoid compounds determined by HPLC according to method **Mattila et al., (2000) and Goupy et al., (1999)**.

2.2.4. Diet Composition and Fed Animal Groups:

1) Diet composition: Basal diet was prepared according to *Reeves et al., (1993)*. The vitamin and mineral mixture had the prepared according to *NCR, (1995)*.

2) Experimental design: Animal house in Food Technology Research Institute, Agriculture Research Center albino rats were adapted for one week prior to commencement of the experiment, housed in well aerated cages under hygienic condition and water introduced *ad-libitum*. After this week, thirty rats fed on basal diet and treated with CCl₄ in paraffin oil (50% v/v 2ml/Kg) twice weeks subcutaneous injection to induce chronic damage in the liver rats (*Jayasekher et al., 1997*) were divided into ten groups, six rats each as follow: G1, rats basal diet (Negative control), while G 2, rats induced CCl₄ and basal diet till the end of experiment (Positive control). G3, rats basal diet+1% tarragon powder. G4: rats induced CCl₄ and basal diet + 1% tarragon powder. G5, rats basal diet+2% tarragon powder. G 6, rats induced CCl₄ and basal diet+2% tarragon powder. G7, rats fed on basal diet+ Tarragon extract 100mg/Kg b.w./day, orally .G8, rats induced CCl₄ and basal diet+ Tarragon extract 100mg/ Kg b. w. /day, orally. *Obolskiy et al., (2011) and Zarasvand et al., (2016)* G9 rats basal diet+ Tarragon extract 200mg/ Kg b. w. /day, orally. G10, rats induced CCl₄ and basal diet + Tarragon extract 200mg/ Kg b.w./day, orally (*Modaresi et al., 2011*).

3-Assessment of Liver and Kidney Functions:

At the end of experiment (after 2months), rats were fasted overnight and anesthetized and blood samples were taken for determination of Glutamate pyruvate Transaminase (ALT/GPT) and Glutamate Oxaloacetate Transaminase (AST/GOT) were determined according to *Sherwin,(1984)* .Serum Alkaline Phosphatase (ALP) was determined (IU/L) according to *Tietz et al., (1999)*. Uric acid was determined according to *Sherwin, (1984)*. Serum Creatinine was determined according to *Bartles et al., (1973)*.

Statistical Analysis: The results were expressed as mean± SD. Data were analyzed by one-way analysis of variance (ANOVA).The differences between means were tested for significance using Bon ferroni-Dunn test at (P<0.05) according to *pc-stat., (1985)*.

Results and Discussion:

Chemical Compositions and Antioxidants of Dried Leaves Tarragon: The proximal composition and nutritive value of tarragon was presented in table (1). Leaves tarragon as shown in table (1) protein (23.42±2.53 g/100g), carbohydrate (48.53±5.32 g/100g). Moisture, fat, fiber and ash were 7.74, 6.99, 8.48 and 7.03g/100g, respectively.

Table :(1): Chemical Composition, Antioxidants Content and Its Activity of Dried Leaves Tarragon (g/100g) on Dry Weight Basis.

Composition	g/100g
Moisture	7.74 ± 0.93*
Crude protein	23.42±2.53
Crude Oil	6.99±1.28
Carbohydrate	48.53±5.32
Crude fiber	8.48±0.93
Ash	7.03±1.25
Total Polyphenols(TPC)	28.5 ± 1.85
Total Flavonoids (TFC)	15.54 ± 2.83
Total Carotenoids	9.98 ± 0.87
Volatile oil (v/w, %)	0.30 ± 0.03
Radical scavenging activities(DPPH)%	86.43 ± 2.85

*Means (triplicate sample) ± SD

Iqbal et al., (2012) found that chemical compositions of (*Artemisia Annu*a leaves)other type of tarragon summarized in percentage of protein (24.37 g/100g) dry weight, carbohydrate 48.3, fat 6.07, fiber 14.2, moisture 11.4 g/100 g and protein). However, the ash content suggests a high amount of inorganic minerals in *A. annua* leaves. On the other hand, **Brisibe et al., (2009)**, in the same type *A. Annu*a leaves were the richest protein content; (27.1%) and equally showed the compositions of fat.

Antioxidant Contents and Its Activity of Tarragon Extract: Tarragon herbs is rich in antioxidant contents as polyphenols, flavonoids, carotenoids, volatile oil and other items as shown is table (1) Results showed that total phenol (28.5 ± 1.85mg/g), flavonoids (1.54±2.83 mg/g), total carotenoids (9.98 ± 0.87mg/100g), volatile oil (0.30±0.03% v/w) and DPPH (86.43 ± 2.85 %). Several studies proved that tarragon is strong antioxidants (**Behbahani et al, 2017**). **Sahreen et al., (2010)** mentioned that The antioxidant properties of phenolic and flavonoid are potent chelators of redox-active metal ions and they can inactivate free radical chain reactions by hindering the conversion of hydroperoxides to reactive oxyradicals. While, **Gawlik-Dziki, (2012)** found that tarragon total phenolic content in aqueous extract of *A. dracuncul*us (26.2 mg/g) and the phenolic content of ethanolic extracts of tarragon leaves about 27 mg/g. Another study, **Bandi and Hidari, (2014)** mentioned that phenolic and flavonoid contents of methanol extracts of tarragon leaves showed higher values than the other two extracts . According to **Sengul et al., (2011)** studied the total phenolic content in *Artemisia absinthum* was (9.79 mg/g) followed by *Artemisia santonicum* (15.38 mg/g) and *Saponaria officinalis* (6.57 mg/g).while hydroxycinnamates were the main phenolic

components of tarragon leaves (*Lin and Harnly, 2012*). Also, *Zarezade et al., (2018)* added that total phenolic content was 197.22 ± 3.73 mg/g from gallic acid equivalent/g the hydro-alcoholic extract of aerial parts of *Artemisia dracunculus* dry weight. It's clear that, total phenolic of *Artemisia* 6.57 to 27 mg/g in the previous study types of tarragon.

Polyphenols Fractions:

A- Analysis of Polyphenolic and Flavonoids Compounds by HPLC:

Quantitative analysis of the identified compounds (Table 2) showed that the phenolic Compounds of the studied tarragon extract were predominated by Benzoic acid, Catechein, Pyrogallol, and Caffeine (1405.42, 808.80, 213.25 and 190.54 ppm, respectively). Concerning, flavonoids compounds of tarragon extract by Acacetin neo.rutinoside, Apig.6-arbinose8-galactose, Apegnin and Luteolin 7-glucose (907.70, 609.15, 576.54 and 361.49 ppm, respectively).

These data were adapted by *Ibrahim, (2017), Msaada et al., (2015) and Kafshboran et al., (2011)*.

Table (2): Analysis of Polyphenolic and Flavonoids Compounds by HPLC in Tarragon Dried Leaves.

Polyphenolic Compounds	ppm	Flavonoids	ppm
Pyrogallol	213.25	Apig.6-arbinose8-galactose	609.15
Gallic acid	13.01	Luteolin 7-glucose	361.49
Protocatchuic acid	70.36	Naringin	298.92
4-Aminobenzoic acid	9.85	Rutin	175.86
Catechein	808.80	Quercetrin-3-O-glucose	21.98
Chlorogenic acid	20.33	Apigenin-7-glucose	56.48
Catechol	40.12	Apigenin-7-O-neohespiroside	119.94
P-OH-benzoic acid	95.85	Kampferol 3-7-diramoside	255.92
Caffeic acid	28.26	Quercetrin	124.17
Vanillic acid	47.64	Quercetin	147.00
Caffeine	190.54	Naringenin	15.39
P-coumaric acid	9.59	Acacetin neo.rutinoside	907.70

Ferulic acid	42.96	Hespirtin	307.31
Iso-Ferulic acid	12.88	Kampferol	169.63
Salycillic acid	252.71	Apegnin	576.54
Benzoicacid	1405.42	-	-
Coumarin	45.22	-	-
3,4,5-methoxy- cinnamic acid	143.62	-	-
Cinnamic acid	33.14	-	-

Effect of Dried Tarragon and Its Extract on Serum Liver Parameters:

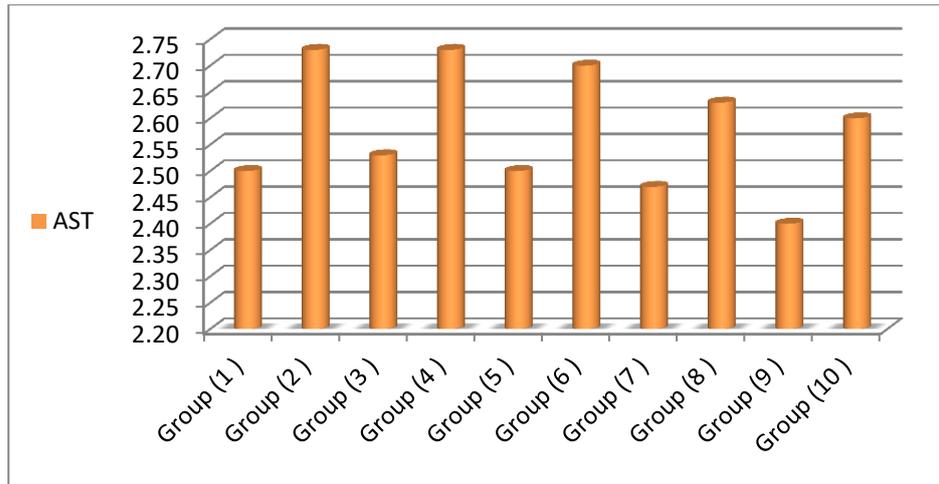
Result of table (3) revealed the effects of tarragon powder and tarragon extract on the liver enzymes of normal and chronic liver diseases groups. It showed that tarragon powder and tarragon extract in normal groups G3, G5, G7 and G9 did not alter any values of the measured parameters ALT,AST and ALP significantly when compared to normal group (G1). However, tarragon powder and tarragon extract in G8, and G10 induced a markedly significant reduction ($p < 0.05$) of the serum activities of the enzymes ALT (36.00 and 34.33 IU/L), AST (2.63 and 2.60 IU/L); when they are compared to the chronic liver disease G2 ALT (41.33 IU/L) and AST (2.73 IU/L), respectively. On the other hand, ALP results showed a significant reduced in chronic liver disease groups G8 and G10 (1.88, and 1.86 IU/L) respectively, regarding G2 (2.14 IU/L).

Table (3): Effect of Tarragon Dried Leaves and Its Extracts on Serum Liver Parameters

Groups	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
G1	2.50 a ± 0.18	29.67 de ± 2.07	1.85 ab ± 0.19
G2	2.73 a ± 0.46	41.33 a ± 9.40	2.14 a ± 0.04
G3	2.53 a ± 0.31	28.33 e ± 4.13	1.83 ab ± 0.04
G4	2.73 a ± 0.46	41.00 ab ± 0.00	2.05 a ± 0.10
G5	2.50 a ± 0.15	28.33 e ± 4.13	1.76 ab ± 0.20
G6	2.70 a ± 0.50	37.67 abc ± 2.58	2.02 a ± 0.20
G7	2.47 a ± 0.31	27.00 e ± 3.58	1.76 ab ± 0.43
G8	2.63 a ± 0.29	36.00 bc ± 4.47	1.88 ab ± 0.26
G9	2.40 a ± 0.24	25.67 e ± 2.07	1.53 b ± 0.71
G10	2.60 a ± 0.24	34.33 cd ± 2.58	1.86 ab ± 0.23

Data are presented as mean (n=6 rats) .All results are expressed as mean ± SD .Values in each column which have different letters are significantly different ($p < 0.05$) .

G1: normal no treatment , G2: induced CCL₄ + no treatment , G3: normal 1% tarragon dry
G4: induced CCL₄ + 1% tarragon dry ,G5: normal 2% tarragon dry , G6: induced CCL₄ + 2%tarragon
dry, G7: normal 100mg tarragon extract, G8: induced CCL₄ +100mg tarragon extract, G9: normal
200mg tarragon extract G10: induced CCL₄ +200mg tarragon extract.



Fig(1): Effect of tarragon powder and tarragon extract on AST (IU/L) for normal and chronic liver disease rats.

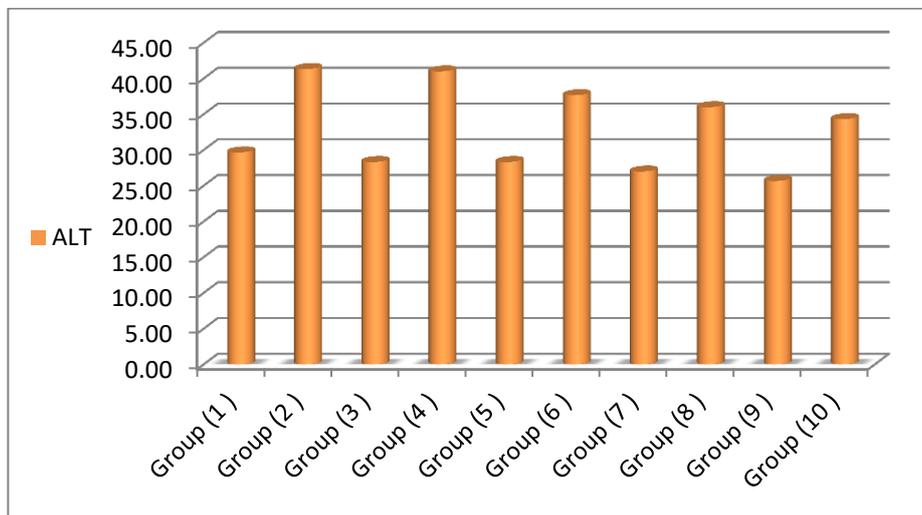


Fig. (2): Effect of tarragon powder and tarragon extract on ALT (IU/L) for normal and chronic liver disease rats

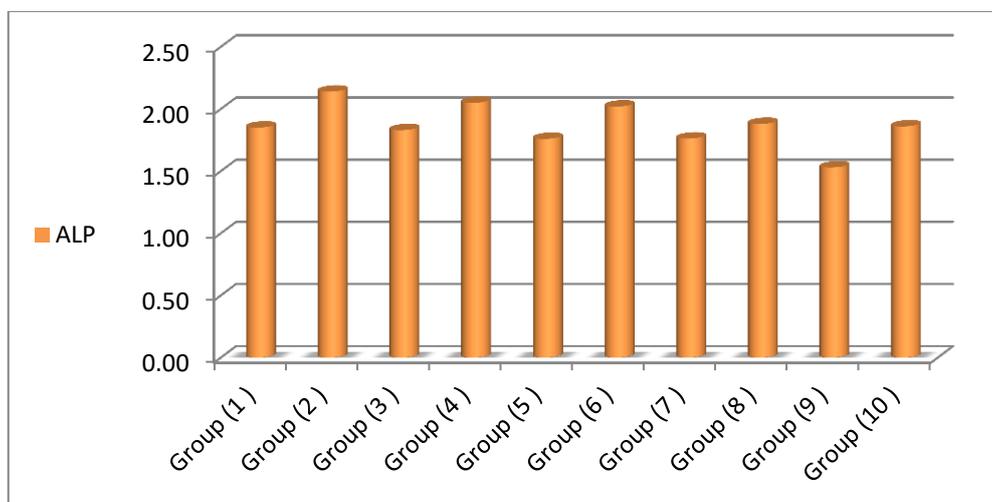


Fig (3): Effect of tarragon powder and tarragon extract on ALP (IU/L) for normal and chronic liver disease rats

Effect of Dried Leaves Tarragon and Tarragon Extract on Kidneys Functions for Normal and Chronic Liver Disease Rats:

tarragon powder and tarragon extract in normal groups G3 and G5 increased a significant alteration in serum uric acid and creatinine levels regarding the normal G1 the chronic liver disease groups G4, and G8 showed significant reduction of serum Uric acid levels (30.67 and 25.33) mg/dL respectively, in comparison with the mean value recorded in chronic liver disease G2 (35.33 mg/ dL). A statistically significant decrease ($p < 0.05$) in the level of creatinine for chronic liver disease groups G6 (0.55mg/dL), when compared with group (G2).

Table(4): Effect of Tarragon Dried Leaves and Its Extracts on Kidneys Functions

Groups	Uric acid (mg/dL)	Creatinine (mg/dL)
G1	32.33 c ± 1.03	0.74 ab ± 0.08
G2	35.33 bc ± 4.41	0.60 cd ± 0.04
G3	40.00 b ± 5.59	0.61 cd ± 0.10
G4	30.67 c ± 1.86	0.61 bcd ± 0.08
G5	32.33 c ± 4.13	0.67 abcd ± 0.09
G6	31.67 c ± 5.75	0.55 d ± 0.09
G7	25.00 d ± 6.75	0.75 a ± 0.15
G8	25.33 d ± 6.47	0.79 a ± 0.05
G9	39.33 b ± 1.03	0.72 abc ± 0.15
G10	47.67 a ± 4.03	0.70 abc ± 0.06

Data are presented as mean (n=6 rats) .All results are expressed as mean ± SD .Values in .each column which have different letters are significantly different ($p < 0.05$).

G1: normal no treated , G2: induced CCL4 + no treated , G3: normal 1% tarragon dry G4: induced CCL4 + 1% tarragon dry ,G5: normal 2% tarragon dry , G6: induced CCL4 + 2%tarragon dry, G7: normal 100mg tarragon extract, G8: induced CCL4 +100mg tarragon extract, G9: normal 200mg tarragon extract G10: induced CCL4 +200mg tarragon extract.

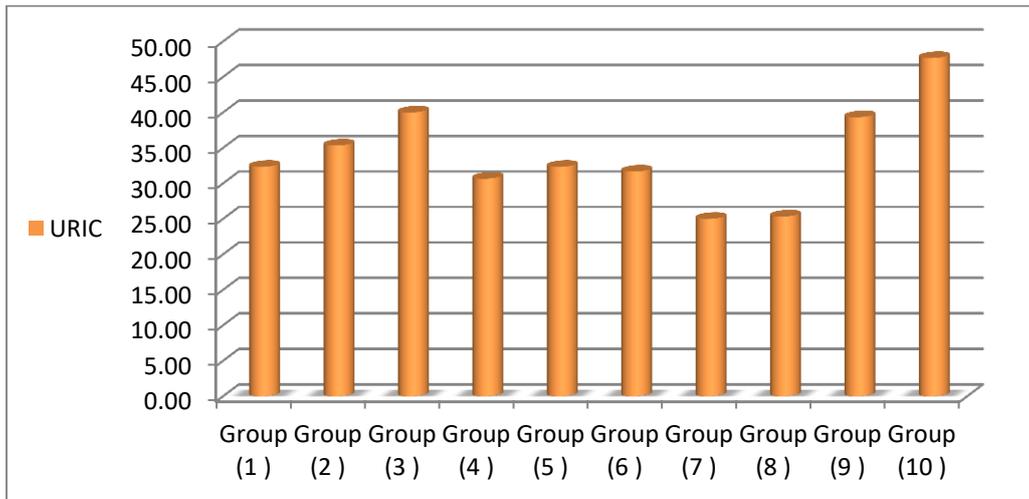


Fig. (4): Effect of tarragon powder and tarragon extract on Uric acid (mg/dL) for normal and chronic liver disease rats.

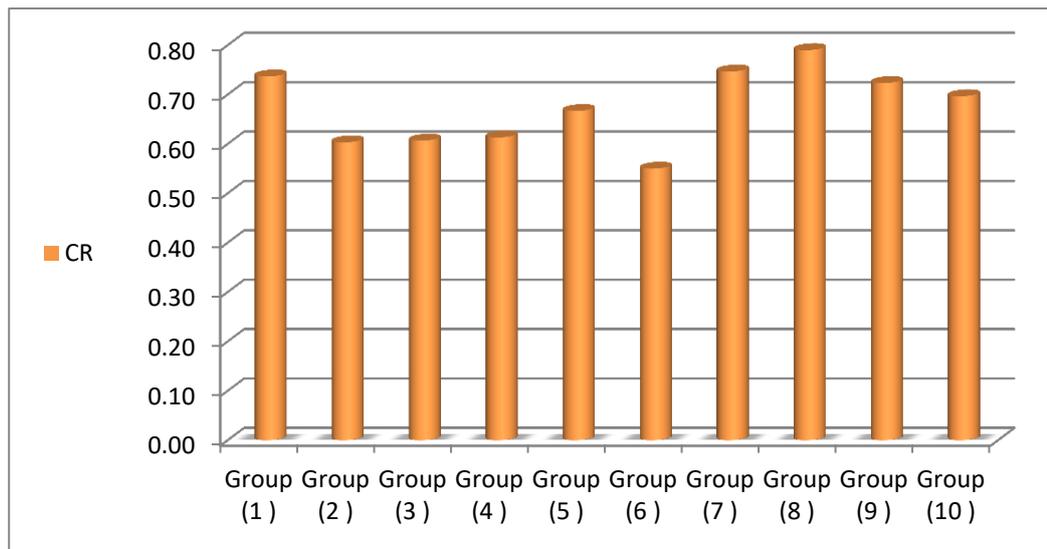


Fig. (5): Effect of tarragon powder and tarragon extract on Creatinine (mg/dL) for normal and chronic liver disease rats.

These data were adapted by **Zarezade et al., (2018)** the hepatoprotective activity of a hydro-alcoholic extract of the herb of tarragon ,rats were given 50, 100, or 200 mg/kg of the extract for 15 days, followed by a single dose of carbon tetrachloride. Evidence was documented of a reduction in the levels of alanine transaminase, aspartate transaminase, alkaline phosphatase and total bilirubin, as well as a total protein increase. At different doses offered hepatoprotection, but 200 mg/kg of HEAD was more effective than the other doses. These data were adapted by **Yazdani et al., (2013)** the study showed that No significant changes in liver factors were observed with treatment of extract of *Artemisia deserti*. Whereas **Iriadam et al., (2006)** indicated that *A. herba alba* aerial parts aqueous extract caused reductions in ALT and AST. In the other study no significant changes were observed in the serum ALT activities, however, the value of AST increased after 3 month oral administration of single dose (100 and 1000 mg/kg) of *Artemisia afra* aqueous extract. Also, suggesting that the AST activity levels increased with time, but ongoing treatment with high dose of aqueous extract removed this elevation, also, the extract may have a liver protecting effect **Mukinda and Syce, (2007)**. Also, **Soqeer (2011)** conclude that the activity of AST was significantly reduced next management of *A. monosperma* extract in rat. This extract was increased antioxidant enzymes so for this reason, the rate of AST was reduced **Kim et al., (2012)**.

These data were adapted by **Choi et al., (2013)**, they reported that the clinical uses of *Artemisia capillaries* is beneficial for hepatic disorders which is associated with alcohol and its mechanisms may involve both augmentation of antioxidant actions and modulation of proinflammatory cytokines. reported that administration of *Artemisia capillaris* extract in high-fat diet (HFD) serum AST and ALT significantly decreased compared to HFD-control group (**Habib et al., 2013**). Oral treatment with the hydro-alcoholic extract of aerial parts of tarragon exhibited a significant decrease in the levels of AST, ALT, ALP, the extract showed a good concentration-dependent reducing power, which was consistent with the findings of **Rajabian et al., (2016)**.

Conclusively, tarragon extracts 200ppm was better than other treatment compared to normal treatment.

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