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Hepato Effect of Argel Herb (*Solenostemma argel*) against Carbon Tetra Chloride Induced Liver damage in Albino Rats.

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Abstract:-

Argel (*Solenostemma argel*) is a traditional edible herb that is commonly used in folkloric medicine for the treatment of rheumatic pain, inflammation, hyperlipidaemia, hyperglycaemia and antimicrobial agent due to its rich in bioactive component which act as antioxidants.

Forty-eight male albino rats were used in biological experiments. Rats were fed the basal diet for two weeks prior to conducting the experiment. Initially, rats divided into six main groups and fed on rations for 42 days as follows: the negative control group (the first group) was fed on the basal diet. Forty male albino rats were fed on a basic diet and stimulated with carbon tetra chloride (CCl_4) in paraffin oil (50% v/v, 2 mL/Kg) twice a week by subcutaneous injection to induce chronic liver damage, then divided into 5 groups numbered from group 2 to group 6. Rats of the positive control group were fed basal diet until the final experiment. Group 3 and 4 rats were treated with dry leaves (0.5g and1.0g, respectively) of Argel. Also, group 5 and 6 were fed on 50 ppm and 100 ppm of hydroethanolic extracts orally, respectively.

Biochemical analyses as serum ALT, AST, ALP and MDA were significant decreased in rats fed on dried and extracts in Argel compared to positive group. In Contrast, it could be that SOD activity in rat groups was significant increased compared to positive group. Also, Argel extracts had highest effect for liver parameters than dried Argel.

These results are concluded that Argel could be suggested to the hepatoprotective effect of Argel may be due to its antioxidants content and free radical scavenging effect.

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Keywords: Argel *S. argel*; Hydroethanolic extracts; Hepatoprotective; Oxidative stress.

Introduction:-

Argel (*Solenostemma argel*) is a medicinal plant with several therapeutic properties, and its leaves, bark, and stems are used in traditional medicine for the treatment of coughs, gastrointestinal cramps, urinary tract infections, rheumatic pains, diabetes, cardiovascular disorders, kidney, inflammation, bronchitis, cold, gastrointestinal cramps and liver diseases .The Argel dried leaves or its extracts contains substantial quantities of a diverse range of phenolic compounds and possess antimicrobial and antioxidant activities. Argel leaves powder and extracts have been used to improve the oxidative stability of camel patties and chicken meatballs [1, 2, 3].

El-Kheir and Murwa [4] showed that the Argel leaves characterized by high carbohydrates (64.8%) and low crude fiber (6.5%). In addition, the leaves contained 15% protein, 1.6% crude oil, 7.7% ash and 4.4% moisture content. Also,Murwan and Murwa [5] added that, the leaves of this plant are characterized by having a high percentage of carbohydrates (64.8%), slightly low percentage of protein (15%), low percentage of crude fiber (6.5%), crude oil (1.6%), ash (7.7%) and moisture (4.4%).

Several Argel flavonoid glycosides have been tentatively identified and belonging mainly to the nuclei of flavonol, a class of flavonoids that have the 3-hydroxyflavone backbone (kaempferol *O*-glucoside, quercetin 3-*O*-neohesperoside and isorhamnetin *O*-rutinoside). Further, phenolic acids such as caffeoylquinic acid, feruloylquinic acid and vanillate glucoside have been also identified. Other miscellaneous metabolites such as organic acids and amino acids were also detected (citric acid, isocitric acid, phenylalanine and tryptophan [6].

The application of such extracts in dairy products is limited. Therefore, this study investigated the impact of argel leaf extract on the physicochemical properties (protein, ash, fat, pH, viscosity, color, acidity, texture, rheology, and water-holding capacity), antioxidant activity, microbial loads, and sensory quality of set-type yogurt during cold storage (4◦C) for 21 days. It was concluded that, Argel extracts (0.1 and 0.2 mg/100 mL) can be used as a natural ingredient to develop a functional yogurt with improved physical, nutritional, antioxidant, and sensorial qualities.

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Ahmed [7] showed that, Argel ethanolic extract (100mg/Kg) caused a significant decrease in serum protein and albumin, whereas ALT and AST and lipid peroxidation were increased following ethanol extract treatment. Argel treatment significantly ameliorated the previous parameters.

The aim of the present work was to high light the reverse effect of dried leaves Argel and its hydroethanolic extracts at various ratios against the toxicity of CCl_4, also to evaluate the various ratios on hepato functions changes that may occur on rats suffering from chronic liver disease.

Materials

Dried Argel (*Solenostemma argel*) herb was obtained from Horticultural Institute, Agricultural Research Center, Giza, Egypt.

2.2. Methods

2.2.1. Chemical analyses of raw materials

The chemical analyses such as, moisture, ash,crude oil, crude protein, crude fiber were determined according the procedures described in AOAC[8].While ,total carbohydrates were calculated by difference according to Mathew et al.[9]

Total polyphenolic compounds were determined by the Folin-Ciocalteau method [10], the absorbance was measure at 760 nm. Results were express as gallic acid equivalents (GAE) per 100 g sample. Also, the content of flavonoids was determined according to [11] at a wavelength of 510 nm. Total flavonoids content of herb extracts were calculated using a standard curve prepared as rutin per 100 g sample.

Total chlorophyll and carotenoids were extracted from dried Argel according to methods of Schopfer [12]. Then, samples were measured at 664nm and 662 nm for chlorophyll and at 480 nm and 510 nm for carotenoids.

Also, tannins were evaluated using the Folin Denis method as described by [13]. Absorbance was measured with a spectrophotometer at 725 nm.

While, fractionated Polyphenolic and flavonoids were determined by HPLC respectively according to method [14] and [15] as follow: 5g of dried Argel leaves were mixed with methanol and centrifuged at 1000 rpm for 10 min and the

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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.

DPPH scavenging activity tests were carried out according to the method of [16]. 0.01 g of sample was dissolved in 10 ml (dimethyl sulphoxide) DMSO and seven different concentrations (1 mg/ml to 0.015 mg/ml) were prepared with ½ dilutions. 2.9 ml DPPH solution (10-4 M in ethanol) was added into 0.1 ml of sample solutions. The mixture was shaken vigorously and incubated 30 minute at 30°C in water bath. Absorbance of the resulting solution was measured at 517 nm UV/visible spectrophotometer (Shimadzu). Percentage of inhibition (DPPH scavenging activity) determined as follows:
% DPPH radical-scavenging activity = [(Absorbance of DPPH -Absorbance of sample) / Absorbance of DPPH] x 100

2.2.2. Preparation of extracts

Argel leaves were washed with tab water several times to remove any adhering flesh, dried in oven under vacuum, then ground well. Ground Argel powder was dipping in ethanol 80% (1:100 w/v) or in distilled water (1:100 w/v) in dark bottle for 48h in refrigerator at 4ºC temperature. To obtain extracts, then mixtures were filtered by filter paper (Whatman1). Hydroethanolic extracts were evaporated in rotary evaporator at 40ºC [17].

2.2.3. Diet composition and animal groups

2.2.3.1. Diet composition*:* Basal diet was prepared according to [18]. The vitamin and mineral mixture had the prepared according to [19].

2.2.3.2. Experimental design: This experiment in Animal house Unit, Food, Technology Research Institute, Agriculture Research Center. Male albino rats weighed 180 ±10g were adapted for one week prior to commencement of the experiment, housed in well aerated cages under hygienic condition and water was *ad-libitum*. After this week, rats were divided into 6 main groups (eight rats for each) and fed on diets for four weeks as follows: Group 1: Negative control group fed on basal diet. Forty rats fed on basal diet and injected with CCl_4, in paraffin oil (50 % v/v 2 mL/Kg) twice a week subcutaneous injection to induce chronic damage in the liver [20], 5 groups numbered from 2 to group 6. Group2: Positive control group had fed on basal diet till final experiment. Group 3: Treated with 0.5% dried Argel leaves. Group 4: treated as group 3 with 1.0% dried Argel leaves daily. Group 5: treated with 50 ppm Argel leaves hydro ethanolic extract

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daily, orally. Group 6: treated with 100 ppm Argel leaves hydro ethanolic extract [21].

2.2.3.3. Blood Sampling: At the end of the experiment period, the rats were fasted overnight then anaesthetized, 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.

2.2.4. Biochemical analyses of serum

2.2.4.1. Liver functions: Aspartate amine transaminase (AST), Alanine amine transaminase (ALT), and Alkaline phosphatase (ALP) were measured according to the method described by [22].

2.2.4.2. Antioxidant biomarker: Malondialehyde (MDA) was determined in the serum according to the colorimetric method described by [23]. Super oxide dismutase (SOD) was determined in serum according to on awrah [24]

2.3. Statistical analysis

Results were expressed as the mean with standard deviation ± SD. Data were statistically analyzed for variance "ANOVA" test at $P \leq (0.05)$ using SPSS statistical software, "version 20" will be used for these calculations [25].

Results and Discussion:-

Table (1):- Chemical composition of Argel dried leaves (g/100g on dry weight basis)

Results in table (1) shows that Argel dried leaves had a high content of carbohydrate and protein (76.19 and 14.10 g/100g on dry weight) respectively. While, ash and fiber contents were 7.20 and 6.92 g/100g on dry weight), also total calories in 100g Argel dried leaves was 374.75 Kcal.

Content	(g/100g)dry weight
Moisture	1.00 ± 0.12
Protein	14.10 ± 0.45
Crude oil	1.51 ± 0.14
Ash	7.20 ± 0.09
Crude fiber	6.92 ± 1.02
Carbohydrate	76.19 ± 3.25
Total calories (Kcal)	374.75

All results are expressed as mean ± SD.

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These results are comply to[4]they found that Argel is a rich source of phytochemical constituents, the leaves are rich in carbohydrates, while low in fibers, protein, oil and minerals contents.

Table (2):- Total phenolic, total flavonoids contents and (DPPH) activity on dried leaves Argel (mg/100g dry weight basis):-

Contest	mg/g
Total polyphenolic	62.58±0.70
Total Flavonoids	23.37±0.23
Carotenoids	13.50±0.58
Tannins	42.37±0.32
Chlorophyll	28.32±1.23
1,2diphenyl-1-picryl hydrazyl DPPH	63.48±0.58

All results are expressed as mean ± SD.

The results in table (2) indicated the dried Argel was rich with total polyphenolic, flavonoids and tannins (62.58, 23.37 and 42.37 mg/g, respectively on dry weight) .Furthermore, chlorophyll and carotenoids contents were 28.32 and 13.50 mg/g, respectively on dry weight. While, antioxidant activity in Argel by DPPH methods was 63.48. The activity of antioxidant may be due to the increment of polyphenolic, flavonoids, carotenoids and tannins.

The high total phenolic content of Argel leaves indicates that these leaves can be used as a good and cheap source of bioactive ingredients. Moreover, S.Argel leaves extract has *in vitro* antioxidant activity by inhibiting microsomal lipid peroxidation, and removing DPPH radicals [7]

These results adapted by [4] they found that dried Argel is high levels of bioactive properties TPC, 73.02 g gallic acid equivalents (GAE)/kg and DPPH inhibition was 85.56% in their extracts.Antioxidant activity of Argel in this study showed that Argel had antioxidant activity on DPPH radicals, microsomal lipid peroxidation and the iron-chelating ability and this activity was in a dose-dependent manner.

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Table (3):-Polyphenolic compounds fractionation of Argel (mg/100g on dry weight basis):-

Phenolic Compounds	mg/100g*
Gallic acid	53.70
Pyrogallol	466.63
4-amino benzoic acid	20.62
Protocatechuic acid	67.71
Chlorogenic acid	251.53
Catechol	129.22
Epicatechein	74.98
Caffeine	37.25
***P*-hydroxy benzoic acid**	194.07
Caffeic acid	62.38
Vanillic acid	93.88
Ferulic acid	261.13
Iso-ferulic acid	36.40
E-vanillic acid	2628.94
Reversetrol	11.52
Ellagic acid	345.18
α-coumaric acid	63.84
Benzoic acid	215.37
3,4,5-methoxy cinnamic	145.80
Coumarin	38.64
***P*-cumaric acid**	18.94
Cinnamic acid	47.77

**mg/100g dry weight*

Argel extracts was high content of vanilic acid. While, pyrogallol, Ellagic acid and Chlorogenic acid (466.63, 345.18 and 251.53mg/100g). Also the contents of p-coumaric acid and reverstrol were18.94 and 11.52mg/100g, respectively (Table 3)

The fact that Argel leaves have high quantities of phenolics with antioxidant potentials, limited information is available on their use in meat and meat products for extending shelf-life and preventing lipid oxidation .

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Table (4):-Flavonoids compounds fractionation of Argel dried leaves (mg/100g on dry weight basis):-

Flavonoids compounds	mg/100g
Narengin	146.21
Rutin	94.71
Hisperdin	386.33
Quercetin	34.61
Narengenin	69.97
Rosmarinic	121.33
Quercetrin	21.68
Kampferol	37.25
Hespertin	99.88
Apegenin	78.30

But there another angel to Argel herbs, it was high content in Hisperdin, Narengin and Quercetin 386.33, 146.21, 121.33 mg/100g, repsectively. While, Hespertin and Rutin was 99.88 and 94.71 mg/100g, respectively. Also, Narengenin and Kampferol contents were 21.68 and 37.25 mg/100g, respectively.

The results were parallel to Shafek and Michael [26] the presence of chemical ingredients such as pyrgene glycosides, flavonoids, kaempferol, quercetin, rutin, flavonols, flavanones, alkaloids.

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Table (5):- Effect of dried leaves Argel and its extracts on AST (IU/L) during experiment period (6 weeks):-

Group	Zero Time	2 weeks	4 weeks	6 weeks	Decreament%
Negative control	$56.00^{a}\pm3.41$	$50.00^{b}\pm2.37$	$48.83^{b}\pm2.32$	$49.50^{b}\pm3.39$	11.61
Positive control	$79.67^{a}\pm2.16$	$76.50^{b}\pm1.78$	$71.17^{c}\pm1.47$	$68.50^{d}\pm1.78$	14.02
Dried leaves of Argel (0.5%)	$82.50^{a}\pm2.43$	$74.50^{b}\pm1.87$	$64.17^{c}\pm1.94$	$58.50^{d}\pm2.43$	29.09
Dried leaves of Argel (1.0%)	$84.67^{a}\pm3.08$	$75.50^{b}\pm1.78$	$71.17^{c}\pm1.87$	$55.83^{d}\pm2.99$	33.98
Argel extract 50 ppm	$79.67^{a}\pm2.16$	$72.50^{b}\pm1.87$	$60.50^{c}\pm1.87$	$47.50^{b}\pm2.88$	40.37
Argel extract 100 ppm	$82.50^{a}\pm2.45$	$73.67^{b}\pm1.63$	$62.17^{c}\pm1.94$	$48.17^{d}\pm2.32$	41.25

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 (5) showed the significant decreament of AST between (29.09 to 41.25) compared to positive control group (14.02%).

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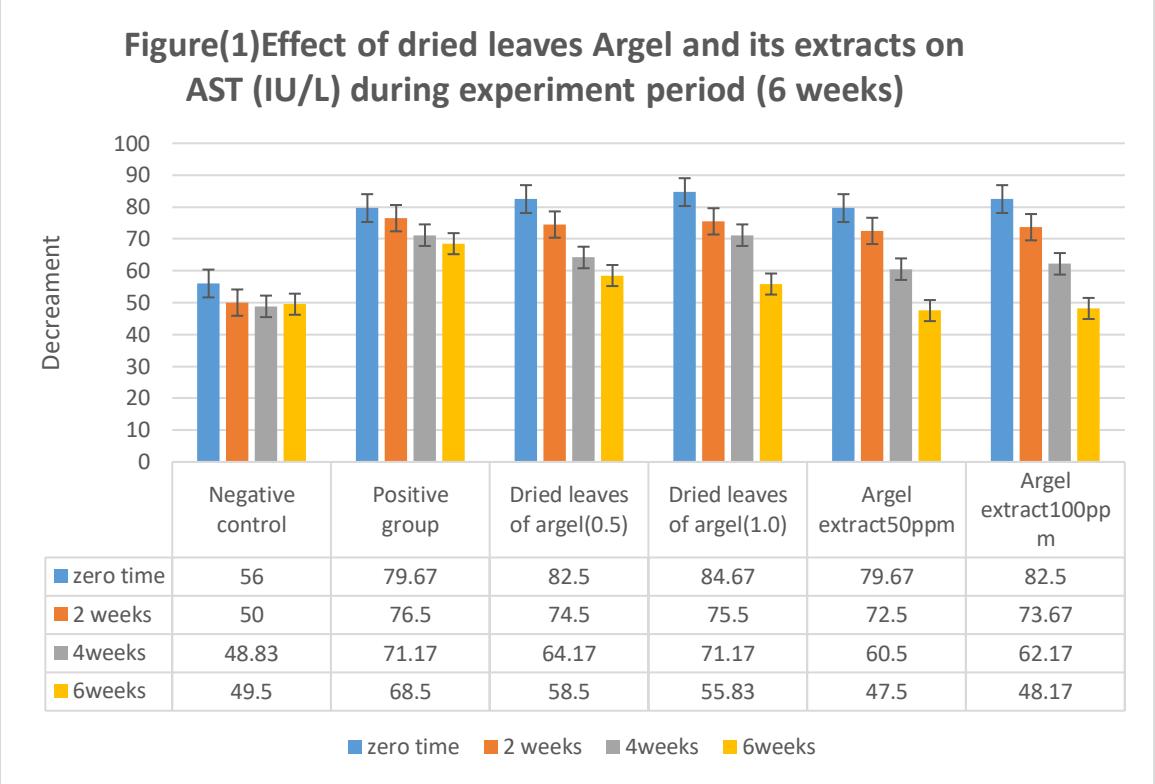

	Negative control	Positive group	Dried leaves of argel(0.5)	Dried leaves of argel(1.0)	Argel extract50ppm	Argel extract100ppm
zero time	56	79.67	82.5	84.67	79.67	82.5
2 weeks	50	76.5	74.5	75.5	72.5	73.67
4weeks	48.83	71.17	64.17	71.17	60.5	62.17
6weeks	49.5	68.5	58.5	55.83	47.5	48.17

Also, Results showed that dried leaves of Argel was decreased significant percentage (33.98%) in AST followed by 0.5% dried Argel (29.09%) compared to positive control group (14.02%). While, rats fed on Argel extract100ppm had the highest decrease significant (41.25%) than rats fed on 50ppm (40.37%) orally compared to positive control group (14.02%).

Results indicated that, the gradually significant decreament of AST compared to positive control group. Also, Argel extracts (100 and 50 ppm) were better than dried Argel to decrease the AST levels. (Figure-1)

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Table (6):- Effect of dried leaves Argel and its extracts on ALT (IU/L) during experiment period (6 weeks)

Group	Zero time	2 weeks	4weeks	6weeks	Decreament%
Negative	$22.17^{a}\pm3.76$	$20.67^{a}\pm3.39$	$19.67^{a}\pm2.16$	$19.50^{a}\pm1.87$	12.04
Positive	$39.00^{a}\pm5.73$	$36.00^{a}\pm2.61$	$37.50^{a}\pm1.87$	$26.50^{b}\pm1.87$	32.05
Dried leaves of Argel (0.5%)	$39.00^{a}\pm5.73$	$33.17^{b}\pm2.48$	$25.67^{c}\pm1.97$	$19.50^{d}\pm1.87$	50.00
Dried leaves of Argel (1.0%)	$39.83^{a}\pm4.7$	$35.00^{b}\pm2.61$	$30.17^{c}\pm2.32$	$19.17^{d}\pm2.48$	51.87
Argel extract 50 ppm	$45.17^{a}\pm6.11$	$33.00^{b}\pm1.79$	$24.33^{c}\pm3.14$	$21.50^{c}\pm1.87$	52.40
Argel extract 100 ppm	$44.50^{a}\pm6.57$	$34.50^{b}\pm1.87$	$29.17^{c}\pm2.32$	$20.50^{d}\pm1.87$	53.93

All results are expressed as mean ± SD.

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

Dry Argel and its extracts were significant decreament effect on ALT of rats groups ranged from 50.00 to 53.93% compared to decrement effect of positive group (32.05%). (Table6)

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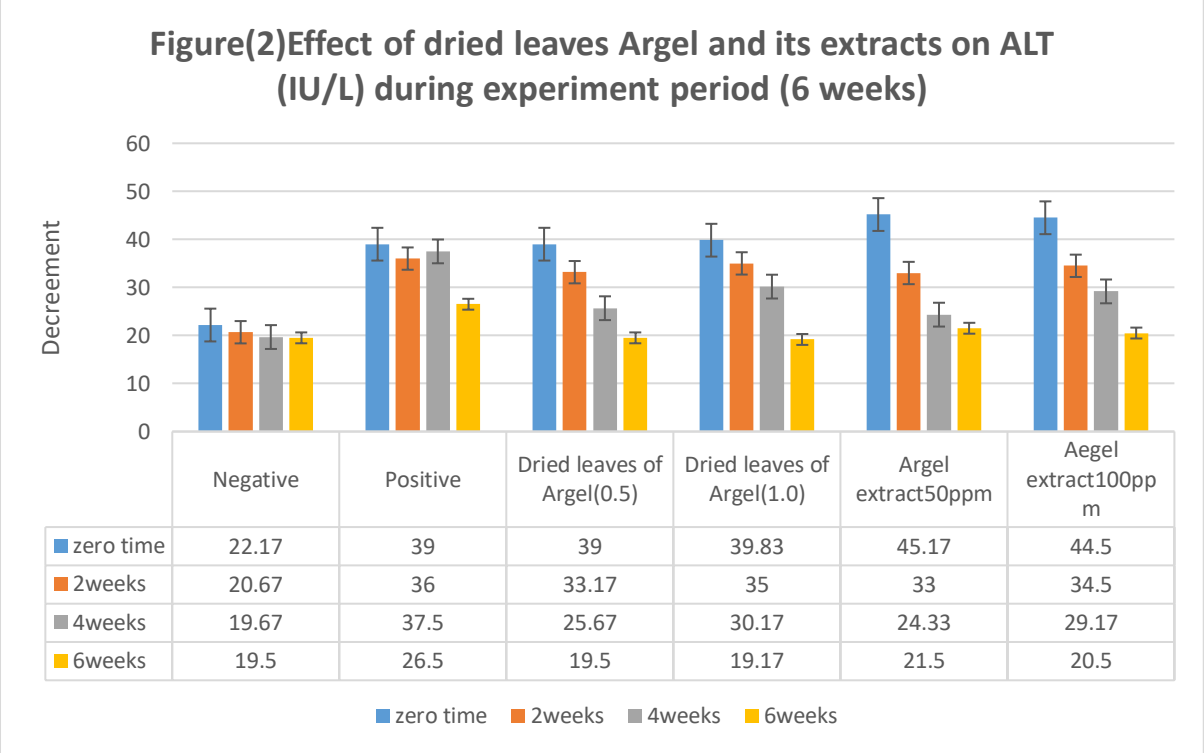

Rats injected with CCl_4 then fed basal diet had decrease significant in ALT (26.50IU/L) compared to (19.50IU/L) at the end of experiments. Normal rats fed on basal diet during experimental period (Table 6). While, rats fed on dried Argel and its extracts were decrease significant in ALT level (from50.00to 53.93IU/L) compared to (32.05IU/L). (Figure2)

The protective effect of Argel against ethanol-induced hepatotoxicity demonstrated by the significant reduction of serum AST and ALT as well as significant decrease of PC, TNF-α, NO and CYP2E1 in liver tissues. Also, Argel may protect the liver from oxidative damages induced by ethanol through ant oxidative effects [27].

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Table (7):- Effect of dried leaves Argel and its extracts on ALP level during experiment period (6 weeks)

Group	Zero time	2weeks	4 weeks	6weeks	decreament%
Negative	$145.12^{a}\pm6.30$	$154.17^{a}\pm18.07$	$151.63^{a}\pm8.20$	$127.67^{b}\pm16.56$	12.02
Positive	$471.83^{a}\pm14.08$	$465.33^{a}\pm21.72$	$447.33^{b}\pm12.32$	$323.13^{c}\pm10.12$	31.51
Dried leaves of Argel (0.5%)	$472.00^{a}\pm14.01$	$468.33^{a}\pm24.72$	$408.14^{b}\pm41.23$	$244.78^{c}\pm13.97$	48.13
Dried leaves of Argel (1.0%)	$482.17^{a}\pm33.77$	$465.83^{a}\pm24.29$	$392.67^{b}\pm25.77$	$227.50^{c}\pm7.11$	52.81
Argel extract 50 ppm	$474.00^{a}\pm7.48$	$462.33^{a}\pm25.16$	$389.50^{b}\pm26.13$	$214.75^{c}\pm2.46$	54.69
Argel extract 100ppm	$478.00^{a}\pm18.36$	$455.33^{b}\pm25.16$	$345.50^{c}\pm12.53$	$213.00^{d}\pm9.40$	55.43

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

While, the decreament effect in ALP of rats group ranged from 48.13 to 55.43%comperd to the decrement effect of positive group (31.51%). (Table7)

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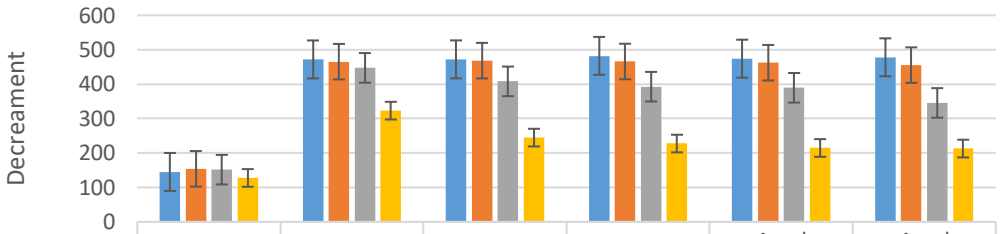
Figure(3)Effect of dried leaves Argel and its extracts on ALP level during experiment period (6 weeks)



	Negative	Positive	Dried leaves of Argel(0.5)	Dried leave of Argel(1.0)	Argel extract50ppm	Argel extract100ppm
zero time	145.12	471.83	472	482.17	474	478
2 weeks	154.17	465.33	468.33	465.83	462.33	455.33
4weeks	151.63	447.33	408.14	392.67	389.5	345.5
6 weeks	127.67	323.13	244.78	227.5	214.75	213

zero time 2 weeks 4weeks 6 weeks

Effects of Argel on activities of ALT and AST, as indication of hepatic injuries were significantly increased in the EtOH-treated group compared with those of the control group ($p < 0.05$). S. argel treatment decreased their levels significantly ($p < 0.05$) compared with EtOH-treated group (Figure3)

Argel leaves extract showed a hepatoprotective effect that could be attributed to the free radical scavenging activity of Argel extract due to the activity of these phytochemicals [28]

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Table (8):- Effect of dried leaves Argel and its extracts on SOD (IU/ml) and MDA (µmol/L) after 6 weeks:-

Super oxid dismutase and Melondialdehyde are parameter of oxidative stress and its relate to organs capacity.

Group	SOD (IU/mL)	MDA (µmol/L)
Negative	$68.55^{a}\pm0.08$	$2.46^{c}\pm0.38$
Positive	$25.49^{d}\pm4.14$	$4.18^{a}\pm0.75$
Dried leaves of Argel (0.5%)	$45.30^{c}\pm1.77$	$3.35^{b}\pm0.13$
Dried leaves of Argel (1.0%)	$46.02^{c}\pm1.61$	$3.29^{b}\pm0.06$
Argel extract 50 ppm	$49.15^{b}\pm3.71$	$2.86^{c}\pm0.07$
Argel extract 100ppm	$50.13^{b}\pm1.82$	$2.65^{c}\pm0.07$

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

Rats fed on different diet which contained dried Argel or its extracts were significant increase in SOD activity compared to positive group table (8).

Result stated that Argel extracts 50ppm and 100ppm had higher significant increments SOD than (92.82 and 96.66%, respectively) dried Argel 0.5and 1.0% (77.7% and 80.54%, repsctively).

In contrast, rats fed on dried Argel and its extracts were decreased in MDA compared to positive group .Also, rats group fed on extracts were orally higher decreament in MDA than rats fed on dried Argel.

Rats fed on different diets which contained dried Argel or its extracts were significant decrease in MDA activity compared to positive group table(8).
Result pointed to Argel extracts 50ppm and 100ppm had higher significant decrease than (31.57% and 36.60%, respectively) dried Argel 0.5and 1.0% (19.85% and 21.29%, repsctively).

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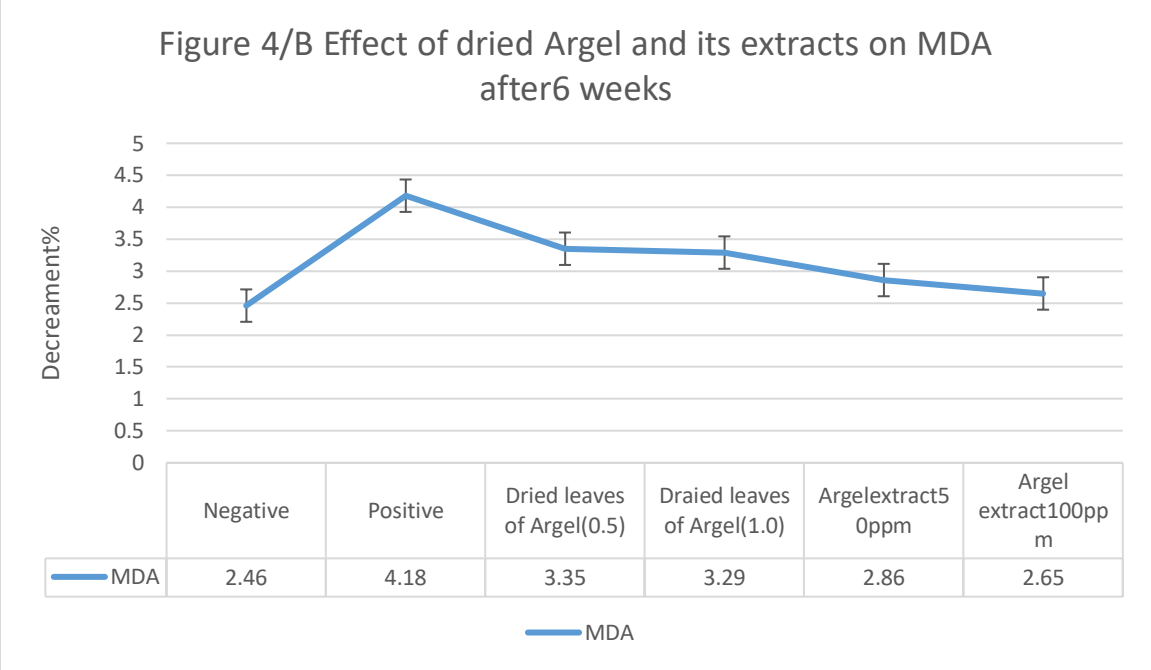

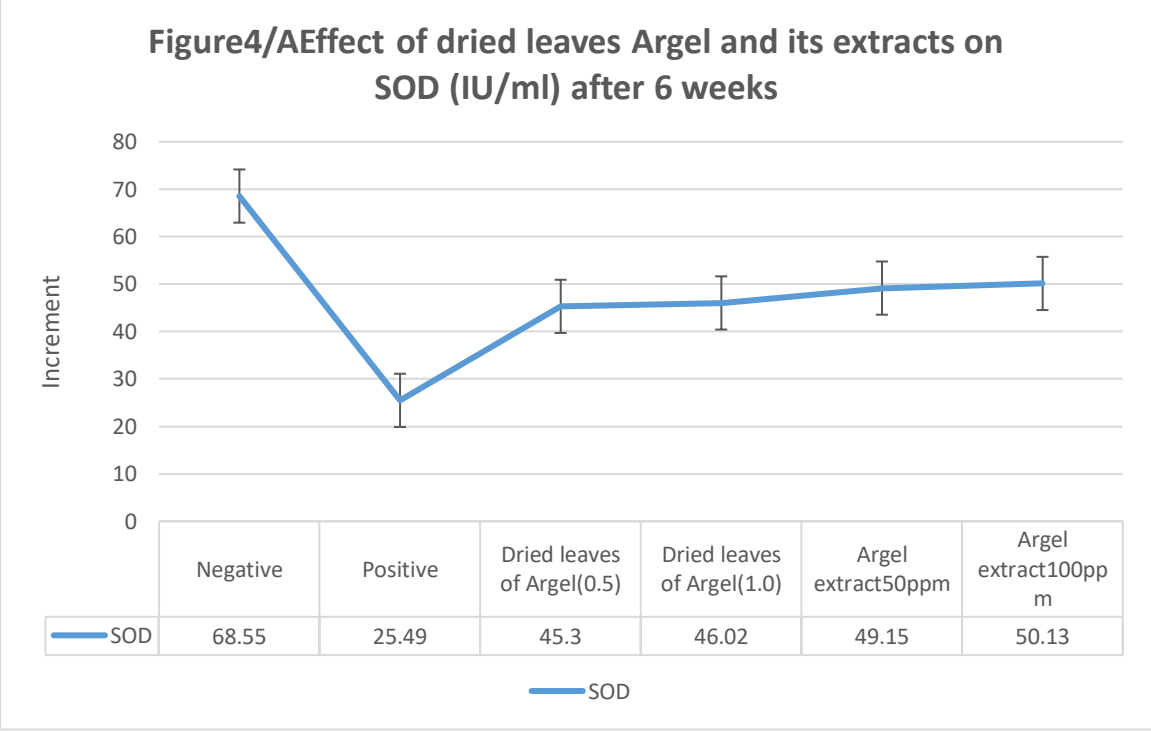

It could be noticed that the presented data in table (8) that positive control showed a significant decrease of the mean values, there was significant increase of all treated groups fed on Argel Also, data showed marked decrease in the serum Malondialdehyde (MDA) level of rats in the positive group with (induced CCl_4) compared with rats in other groups (treated with dried Argel and its extracts) which recorded a significant decrease in the serum MDA level. These data may

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be due to polyphenolic and flavonoids content, which effect working as a scavenging free radical to prevent liver cell damage [29].

In conclusion, our investigation shown that the Argel extract had potent hepatoprotective effects against ccl_4 induced hepatotoxicity in rats. This extract inhibited the hepatic damage accompanied by decreased activity of serum liver enzymes. Treatment with Argel extract resulted in restoration of SOD and MDA, the antioxidant defense system, which was impaired by ccl_4 exposure, and suppressed SOD activity. Furthermore, the protective effect of Argel against ethanol-induced hepatotoxicity demonstrated by the significant reduction of AST, ALT, ALP, SOD and MDA in serum.

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