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Anti-tumor and antioxidant activities of *Rumex vesicarius l* extract against Ehrlich Ascites Carcinoma

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ABSTRACT

Background: *Rumex vesicarius l.* (polygonaceae) is an eatable herb developing in egypt. The plant has a significant value in folk medicine and it has been used to relieve many diseases. **Aim:** The aim of the study is to investigate the antitumor activity and evaluate the antioxidant potential of *Rumex vesicarius l.* extract in experimental animals. **Material & Methods:** the ethanolic extract of whole plant was prepared and then conducted by high performance liquid chromatography (HPLC) and 2,2-diphenyl-1-picryl hydrazil to evaluate its content and antioxidant potential. The exprimental animals were divided into the following groups: negative control, solvent group, ethanolic extract group, Ehrlich Ascites Carcinoma (EAC) positive group, preventive and therapeutic groups. The antitumor and antioxidant activities were carried out in female albino mice against EAC by measuring viability of EAC cells, nitric oxide (NO), Malondialdehyde (MDA) levels, catalase (CAT) and superoxide dismutase (SOD) activities and Arginase. **Results**: the ethanolic extract has a significant antioxidant activity and the HPLC fingerprints showed high contents of phenolic compoundes. Also The extract showed a significant reduction in the volume and count of EAC cells, increasing CAT and SOD activities and reduction in NO, MDA in studied groups compared to positive control. Also, the extract was found to be safe and effective in the treatment. **Conclusion*:*** The extract has potent antitumor, antioxidant, and decrease the survival of cancer cells.

Introduction:

Cancer remains a serious illness, responsible for almost a quarter of deaths, so it is one of the most advanced problems in the developed world and and remains a serious illness in both developed and developing countries. It is a high priority for research due to those vast number for deaths, extensive human enduring and related health awareness [(1)]. One of the hardest issues facing medicine just currently is the discovery for anti-tumor therapies. Therefore, the search for high-efficiency anti-tumor medicine can remain a fertile space for

scientific research. Due to its non-toxicity and absence of/minimal side effects, plants and plant-based products have been the main focus of attention in the fight against illnesses. Thus, it is vital and sensible to identify healthy plants. [2]. *Rumex vesicarius* Linn is a wild ealable plant used as a sorrel and collected in spring time and it may be consumed fresh [3], or cooked [4]. It belongs to perennial herbs to the family *(Polygonaceae*). For the length of the tap root, the plant will be generally upright. The plant is traditionally used to cure liver disorders, bronchitis, asthma, constipation, dyspepsia, and issues with the lymphatic and glandular systems. It is also used as a stomachic and diuretic. The plant abandons need aid rich for ascorbic acid, citric acid, and tartaric acid, What's more they also hold glycoside, alkaloid, flavonoids, tannins, and phenolic mixes[5]. It may be rich wellspring of β carotenes so it used as dietary supplement[6]. There are several important medical applications for *R vesicarius l.*, including the treatment of tumours, hepatic illnesses, calculi, heart problems, aches, spleen diseases, hiccoughs, flatulence, piles, scabies, leucoderma, toothache, and nausea. The plant is also used as a diuretic, astringent, purgative, antispasmodic, stomachic, cooling, laxative, stomachic, tonic, analgesic, appetiser, and antibacterial agent. The roasted seeds were consumed as a dysentery remedy. Finally, the plant can be used to regulate cholesterol levels and lessen biliary diseases. [7]. The medicinal role of this plant is a reflection to its compound arrangement since the plant holds Numerous bioactive substances for example, flavonoids (vitexin, isovitexin, orientin and isorientin). The plant also rich in anthraquinones particularly in roots (emodin and chrysophanol). The plant also contains carotenoids, vitamins (especially vitamin C), proteins, lipids and organic acids. This plant is a good source of minerals "K, Na, Ca, Mg, Fe, Mn, Cu" [8].The previously mentioned bioactive phytochemicals (like polyphenols, flavonoids, carotenoids, tocopherols furtheremore ascorbic acid) have antioxidant and detoxifying effects. The intake of dietary inhibitor phytochemicals similar to carotenodis, phenolic compounds and flavonoids can cause the protection against noncommunicable sicknesses done individauls "cancer, cardiovascular diseases and cataract" [9]. The lipoid constituents of *R. vesicarius l* were examined by both liquid chromatography/mass spectrometry (LC/MS) and by gas chromatography/mass spectrometry (GC/MS). Their essential oil compositions consisted mainly of thujene, limonene, fenchon, estragole, and anethole. The crude lipid extract and the methanol extract showed strong antioxidant activity and radical quenching potential against 2, 2-dipheny l-1-picrylhydrazyl (DPPH) systems [10],[11]. The purpose of this study was to look into the antioxidant and anticancer effects of R vesicarius l extract against EAC in female swiss albino mice.

Material and Methods:

Materials:

Solvents: dimethyl sulfoxide (DMSO), ethyl alchol, ethyl acetate and n.Hexane were provided from Algomhoria chemical compony

Kits: Biodiagnostic kits for (MDA), (NO), (CAT), (SOD) and Arginase

Biodiagnostic kits for liver functions (total proteins, Bilirubin, albumin, ALT and AST) and Kidney functions (urea and creatinine) were provided from Bio diagnostic company, Dokki, Giza, Egypt.

Tumor: Ehrlich ascites carcinoma (EAC): EAC cells were initially supplied from the National Cancer Institute, Cairo, Egypt (only for the first transplantation), and maintained in female Swiss albino mice through serial intraperitoneal (I.P.) inoculation at 7 -10 days intervals in our laboratory in an ascites form.

Plant material

Plant of *R vesicarius l.* was collected from Egyptian fields (Al-sharkia government) from November to December (2014).

Animals

A total number of 60 swiss albino mice weighting (25-30g) were used. Mice were purchased from (Aborwash, Giza). The animals were housed in plastic cages at room temperature in experimental animal house of faculty of science Zagazig university under normal condition for adaption. Animals were allowed for free access of tap water *ad libitum* and fed on commercial pellet diet.

Methods:

Extaction

The plant material of *R vesicarius l* was air dried, powdered coarsely. A weighed amount (98g) of the dried powder was subjected to extraction with diverse solvents (ethyl alchol, ethyl acetat, dist.water, n.Hexan) respectively in a closed flask for 24hrs shaking each 6hrs. All extracts were separated through Whatmann number 1 filter paper and then were subjected to continuous hot extraction in Soxhelt apparatus. The extract was evaporated under reduced pressure using rotary evaporator until all the solvent has been evaporated to give an extract sample according to (**Raghavendra & Reddy** method.[12]

high performance thin layer chromatography (HPLC) profile:

HPLC was used to separate and identificate the phenolic compounds present in the extract with HPTLC (Hewlett Packard series 1050,USA), the column (Hypersil BDS 5 um C18). Asampling injector by using quaternary HP pump (Series 1100), solvent degasser, iso gradient separation was carried out with methanol and acetonitrile as amobile phase at flow rate of 1ml/min, temperature was maintained at 35c. The ultraviolet UV detector set at wavelength 280 and 330 nm for phenolic and flavonoid compounds. Standards were obtained from sigma co.were dissolved in a mobile phase and injected into HPLC. Retention time and peak area were used to calculation of phenolic and flavonoid compounds concentration by the data analysis of HEWLLET packared software, according to **(Goupy *et al*** method). [13]

Determination of antioxidants activity of extracts:

The antioxidant activity of extract was determined through free radical scavenging activity (DPPH assay): The free radical scavenging of different extracts was measured by the 2, 2-diphenyl-1-picryl hydrazil (DPPH) method in which the hydrogen atoms or electrons donation ability of the corresponding extracts were measured from the bleaching of purple colored ethanol solution of DPPH. This spectrophotometric assay uses the stable radical DPPH as a reagent according to (**Tepe and Daferera**) [14], 3ml of 0.1ml Methanolic solution of DPPH was added to 1ml of ethanolic extracts at concentration 100Mg/ml. The absorbance was measured against a blank at 517nm at 0, 30, 60 and 120min. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity.

***Determination of median lethal dose (LD_{50}) of* extract**

Approximate LD_{50} of extract in mice was determined according to method described by Meier and Theakston [15].

Dose response curve

The most effective dose was determinedaccording to (**crump *et al*** method. [16]

Experimental design

60 female swiss albino mice were divided into six groups (10 mice/ each group):
Group (1) (Negative control): Animals were injected intraperitoneal (i.p.) with sterile saline solution along experiment. **Group (2) (DMSO):** Animals were injected intraperitoneal (i.p.) with 0.2 ml of DMSO for the entire experimental period. **Group (3) (Extact):** Animals were injected intraperitoneal (i.p.) with plant *R vesicarius l* extract at dose (5mg/kg). **Group (4) (Positive control):** Intraperitoneal (i.p.) injections of EAC ($2.5\ 10^6$ cells/0.3 ml/mouse) were administered to the animals. **Group (5) (Preventive)**: Animals were injected with of plant *R vesicarius l* extract (5 mg/kg) before EAC transplantation then day after day along experiment. **Group (6) (Therapeutic)**: Animals were injected intapeitoneal (i.p.) with EAC, then were injected with extract of plant *R vesicarius l* at dose (5mg/kg) day after day along experiment.

Collection and sampling of blood

At the end of the experiment, the blood samples were withdrawn from aorta under light ether anaesthesia to obtain serum according to (**Joslin**, [17]. Serum was prepared by centrifuging collected blood at 3000 r.p.m for 10 minutes. Serum samples were aliquoted and stored at -20°C until biochemical analysis. Also **EAC cells** were harvested from each mouse in centrifuge tube contains heparinized saline.

Tissues (**Liver and kidney**) were excised from each mouse preserved in 10 % Formalin solution until histological examination.

Viability of EAC cells: Trypan Blue Exclusion Method (McLiman et al., [18] was used to assess the vitality of EAC cells**.**

Life span prolongation: Life span prolongate was carried out according to the method described by (**Mazumdar *et al*** [19].

Antioxidant assays: Malondialdehyde (MDA), Nitric Oxide (NO) levels, Superoxide dismutase (SOD), Catalase (CAT), and Arginase activities were measured using the techniques described by Satoh [20], Montgomery and Dymock [21], Nishikimi et al. [22], Aebi [23], and Marsch et al. [24]; Respectively.

Estimation of liver and kidney functions:

Estimation of (serum total potiens, serum bilirubin, serum albumin, the serum activities of ALT and AST) and estimation of serum (urea and creatinine) were determined according to "**(Doumas *et al.*, [25], Doumas *et* al., [26], (Schumann *et al.*, [27]; Karmen *et al.*, [28], Chaney *et al.*, [29], (Murray, [30]**. Respectively.

Histophathological analysis

After blood collection, liver and kidney tissues were quickly excised from the mice and were fixed in 10% buffered formalin solution, then embedded in paraffin by placing tissue into 50% paraffin at 47˚C. For 2 hours. The embedded sections were immersed into the melted paraffin with the lesion towards the bottom of the mold; the hard blocks were saved for sections. The blocks were mounted on the object carrier or the microtone to section thickness of 5 microns, the sections was stretched on the surface of Worm water bath. The flattened sections were placed on the surface of

clean microscope slide according to **(Lillie**[31] .

Statistical analysis:

All statistical analyses were done by a statistical for social science package "SPSS" 15.0 for Microsoft Windows, SPSS Inc **(levsque** .[32] and considered statistically significant at a two-sided P < 0.05.Numerical data were expressed as mean ± SD.

Results

Solubility of extract

Yield of ethanolic extract of was found to be 27.4g. Extract was souble in dimethyl sufoxide (DMSO).

HPTLC finger print profiles of extract:

The HPTLC finger print profiles of extract of *R vesicarius l* showed the presence of (24) major compounds as shown in Table (1) and illustrated in fig. (1). (Benzoic) and (Pyrogallol) were found in maximum concentration (2660.201ppm,1704.771ppm) respectively, (Gallic 26.050ppm), (4-Amino-benzoic 22.119ppm), (3-oh-Tyrosol 354.279ppm), (Protocatchuic148.619pp), (Catechein 47.509ppm), (Chlorogenic 732.899ppm), (Catechol 206.074ppm), (Epicatechein 555.520ppm), (Caffeine 70.063ppm), (P-OH-benzoic 315.511ppm), (Caffeic 121.432ppm), (Vanillic 488.093ppm), (Ferulic110.645ppm), (Iso-ferulic 61.976ppm), (Reversetrol35.000ppm), (Ellagic 161.016ppm), (3,4,5-methoxy-cinnamic 20.912ppm), (Coumarin 25.244ppm), (Salycilic 418.189ppm), (P-coumaric 23.569ppm), (Cinnamic 207.655ppm) and (Alpha coumaric10.822ppm) was in minimum concentrations.

DPPH radical scavenging activity:

Table (**2**) and **fig** (**2**) illustrated the DPPH radical scavenging of extract. The results showed that ethanolic extract possess high scavenging capacity compared to TBHQ (tertiary butylhydroquinone).

The median lethal dose (LD_{50})

Our results revealed that, doses up to 2000 mg /kg were considered to be safe, where no mortality was observed for extract.

Dose response curve

The most effective dose of *R vesicarius l* extract was found to be "5 mg/kg" dose response curve illustrated in the fig. (3).

Viability and life span prolongation

The mean values of EAC volume and count were found to be 4.1±0.49 (ml) and 244.4 ± 31.7 ($\times 10^6$ cells/ml) in positive group as **Freitas *et al*** [33] . While, preventive and therapeutic groups were demonstrated a significant decrease in EAC volume to (No EAC and 1.4±0.45) by (100% and 66.1%) respectively, There was significant reduction in EAC cells count in both preventive and therapeutic groups to (No growth, and 104.4 ±10.8) by (100% and 57.2%); respectively compared to positive control group (EAC bearing tumor) as showen in table (3).

The mean life span prolongation in the positive control group was found to be 16 days. Therapeutic and preventive groups showed a significant increase in the life span prolongation to 17 days by 6.25 % (T/ C ratio = **106.25** %), and 24 days by 50% (T/ C ratio = **150** %); respectively; compared to the positive control group **table(4).**

Effect of extract on antioxidants across all groups under investigation:

Table (5) compiled the average values and levels of MDA, NO, SOD, and CAT activities across all groups.The mean value of MDA and NO levels were found to be 40.93±1.84273 (nmol/ml), and 50.15±3.24457 (µmol/l) in positive control group; respectively, ($p<0.001$) compared to negative control group. DMSO & extract groups showed increasing to 14.02+1.05704 and 12.25+1.46225 (nmol/ml) and 40.87+3.63631 and 30.64+1.29889 (µmol/ml); respectively in contrast to the negative control. While in the extract,

preventive , and therapeutic groups there are a significantly decreased in MDA levels to 12.25 ±1.14, 21.69±1.37, and 36.67±2.16 (nmol/ml), ($p<0.001$) respectively; comparing with the positive control group. Additionally, NO levels considerably dropped in the extract, preventative, and treatment groups to 30.64 ±1.29, 17.11±1.88, and 23.28±2.38 (µmol/l), respectively, ($p<0.001$) compared to positive control group.

However, CAT and SOD activity levels fell from 285.77±10.82, 217.56±6.31 (U/ml) in negative control group to 100.11±6.42, 104.67±6.76 in positive control group; respectively, ($p<0.001$). While, their activities were significantly increased to 223.11±18.43, and 325.46±29.20 in extract group, to 726.23±43.17 and 891.03±66.80 in preventive group, and to 499.81±12.82, and 635.73±29.47 in therapeutic group; respectively, ($p<0.001$) compared to positive control group.

Additionally, there was significantly increased in Arginase activity in positive control group from 95.32±4.38 to 253.01±21.68 compared to negative control group as illustrated in table (6). These values were significantly decreased to 157.06±22.37 in extract group, to 88.44±3.36 in preventive group, and to 113.78±4.87 in therapeutic group; respectively, ($p<0.001$) compared to positive control group.

Effect of extract on liver and kidney functions in all studied groups:

Table (7) showed the effect of extract on serum liver and kidney functions.

There are significant increase in ALT, and AST activities in positive control group to 104.23 ±6.53504, and 138.04 ±11.40742 U/L; respectively compared to negative control group, ($p<0.001$).

These high activities of liver enzymes were significantly reduced to 25.73±2.76 and, 85.09±2.01 in preventive group, and to 32.54±2.20, and 95.53±2.22 in therapeutic group; respectively, ($p<0.001$) compared to positive control group.

Total proteins and albumin concentrations were significantly decreased in positive control group to 5.30 ±0.24 (g/dl), and to 2.5±0.33 (g/dl); respectively, ($p<0.01$) compared to negative control group. to 6.99 ±0.36 and, 3.74±0.11 in preventive group, and to 6.84±0.31, and 3.2±0.16 in therapeutic group; respectively, ($p<0.001$) compared to positive control group. There were alterations in ALT and AST activities and Total proteins and albumin concentrations in extract group and DMSO groups, table (7).

While the level of total bilirubin showed significantly increased in positive control group from 0.51 ±0.0.07 to 0.977±0.05 compared to negative control group. Also, to 0.151 ±0.03 in preventive group, and to 0.362±0.05 in therapeutic group; respectively, ($p<0.001$) compared to positive control group.

These results were confirmed by the histopathological study of liver and kidney tissues, illustrated in fig.(4). As Preventive group of liver and kidney tissues showing dilated sinusoids with atrophied, disorganized hepatocytes and interstitial blood vessel congestion in renal tubules. Therapeutic group showing hyperplasia of bile duct with portal tract fibrosis, and vacuolated glomerular tuft and degenerated renal tubules.

Blood urea nitrogen and serum creatinine were significantly increased in positive control from 19.40 ±2.88 to 51.40±3.97 (mg/dl), and from 0.438±0.046 to 1.063±0.098 (mg/dl); respectively, ($p<0.01$) compared to negative control group. These levels were significantly decreased to 18.6±1.505 and 0.691±0.041 in preventive group, and to 19.2±1.549, and 0.801±0.026 in therapeutic group; respectively, ($p<0.001$) compared to positive control. Also, there were some alterations in extract and DMSO group compared to negative control group.

Discussion

At present, tumor is that the most typical in both patient and death rates across the nation what's more during the state level [34]. Cancer occurrence may be 6 million for every year, therefore cancer could be a developing issue within the field of public health. An expansive amount of plant, marine, and microbial wellsprings have been tested as threads and number of compounds survived possible threads [5]. Hence, the present study was designed to explore the possible anticancer activity of extract of *R. vesicarius l* and also antioxidant activity. Our ethanolic extract of *R. vesicarius l* showed significant reduction in volume and count of cancer cells and arginase activity in the studied groups. It showed decreasing in volume and count of EAC cells in both therapeutic and preventive groups compared to positive group. Medicine with extracte diminished those the tumor volume, viable tumor cell count and redouble the life span of the tumor bearing mice and it might be expected should decline those dietary liquid volume what's more delay those cellular division. [35] Also, effective in antioxidant enzymes. This effect may be due to the presence of high content of phenolic compounds (pyrogallol, catechein, benzoic, Gallic, 4- Amino-benzoic, 3-oh-Tyroso l, Protocatchuic, Chlorogenic, Catechol, Epicatechein, Caffeine, P-OH-benzoic, Caffeic, Vanillic, Ferulic, Iso-ferulic, Reversetrol, Ellagic, 3,4,5-methoxy-cinnamic, Coumarin, Salycilic, P-coumaric, Cinnamic and Alpha-coumaric). This results also supplement the folkloric usage of the studied plant, which possesses several known bioactive compounds. This result was in a harmony with **(Khan *et al.*,** [36] who reported that The HPTLC finger print profiles of methanolic extract of *R vesicarius l* showed the existence of eight major components. These compounds especially those with the highest concentration may be the reason for its biological activities (inhibitory activities). Our results revealed that *R vesicarius l* extract exhibited noticeable antioxidant activity compared to Tertiary butylhydroquinone (TBHQ). (TBHQ) is an essential antioxidant for highly effective oxidation in the room or moderate temperatures and it used in the food industry on a large scale [37]. Compared with TBHQ, extract of *R vesicarius l* demonstrated higher radical scavenging abilities against DPPH assays, whereas for the lipid peroxidation assay, the extract had lower activity than TBHQ. This result demonstrated that the extract of *R vesicarius l* had good radical scavenging activity. Antioxidant activity and total phenolic content results agreed with **(El-Hawary *et al.*,** [38], where *R vesicarius l* had antioxidant and hepato-protective activities because of the existence of phenolics and flavonoids also with results of **(Tavares *et al.*,** [39], since they found that, flavonoids and poly phenolics in *R maderensis* were related to antioxidant capacity, total flavonoids and phenolics content reflecting the antioxidant capacity of the plant. Antioxidant activity of *Rumex* corroborates the findings of (**El-Bakry *et al.*,)** [10]. Our results said that, extract was considered to be safe up to 2000mg/kg b.w., where no mortality was observed for extract, these results were consistent with **(Raghavendra & Reddy)** [12] who reported that there was no toxicity were observed at the dose of 2000mg/kg b.w., and the plant is safe for utilization and for medicinal utilization. Our studies cleared that, 5mg/kg was the most effective dose where it induced a significant increase in life span prolongation of both therapeutic and preventive groups compared to positive control group. Prolongation of life span of animals could be a constant criterion for deciding the strength of any malignant neoplasm drug [40]**.** It can be concluded that the ethanolic extract of

the demonstrated remarkable antitumor activity against EAC in mice. And this result was in harmony with **(Alam *et al.*, (41)** who established that the methanolic fractions of the aerial elements of *Polygonum viscosum* (MAPV) (family of Polygonaceae) considerably minimized tumor growth and viability of tumor cells and normalized serum biochemical profiles, increasing life span as compared with those of EAC management mice. Phytochemical examine explained the presence of steroids, tannins, phenoles What's more flavonoid parts Also glycosides previously, rough extract of *Polygonum viscosum*. Variation in scientific reports recommend certain steroids and phenolic compounds like tannins, caumarins and flavonoids even have a chemopreventive role in cancer **(Kumar *et al* (42).** This effect could be related to its anti-tumor activity, potent antiangiogenic activity and effective antiproliferative potential as well. Our result was in harmony with **(Shahat *et al.*, (1)** who reported that plants of genus *Rumex vesicarius l* had antitumor activity against alternative cancer cell lines holding colon, ovary, melanoma, breast, focal sensory system and gastric tumor and this effect might due to its the antiproliferative activity**.**

Conclusion:

The extract of *R vesicarius l* showed anticancer and anti-oxidant activities.

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Table (1) HPLC analysis the phenolic contents of ethanolic Rumex vesicarius l extract:

	Phenolic compounds	Test results of phenolic compounds (ppm)
1	Gallic	26.050
2	Pyrogallol	1704.771
3	4- Amino-benzoic	22.119
4	3-oh-Tyrosol	354.279
5	Protocatchuic	148.619
6	Catechein	47.509
7	Chlorogenic	732.899
8	Catechol	206.074
9	Epicatechein	555.520
10	Caffeine	70.063
11	P-OH-benzoic	315.511
12	Caffeic	121.432
13	Vanillic	488.093
14	Ferulic	110.645
15	Iso-ferulic	61.976
16	Reversetrol	35.000
17	Ellagic	161.016
18	Alpha-coumaric	10.822
19	Benzoic	2660.201
20	3,4,5-methoxy-cinnamic	20.912
21	Coumarin	25.244
22	Salycilic	418.189
23	P-coumaric	23.569
24	Cinnamic	207.655

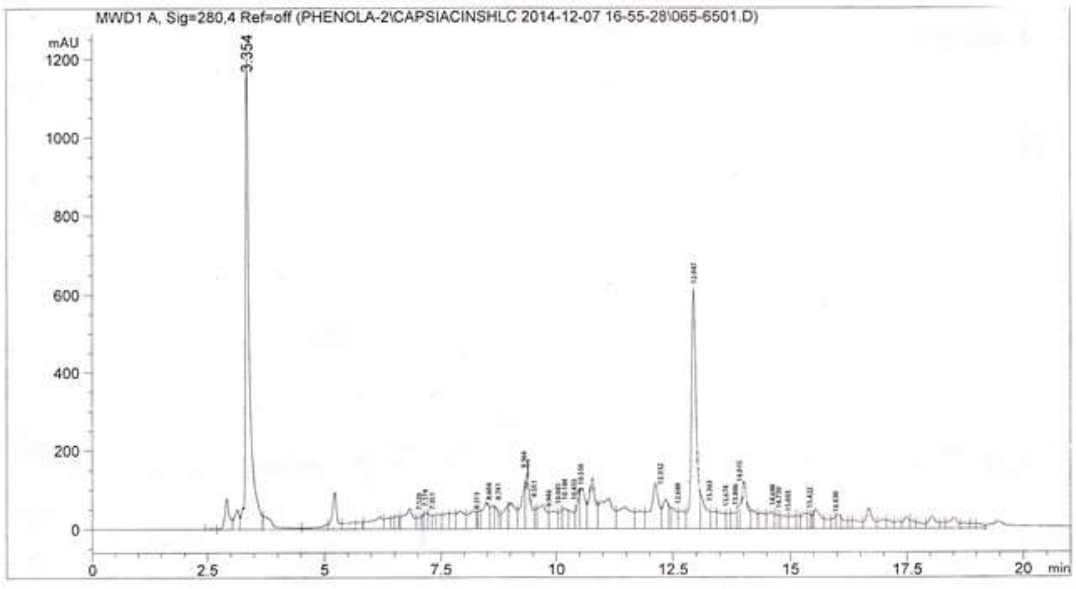


Fig (1) A chromatogram of Rumex vesicarius l extract by HPLC

Table (2): Antioxidant activity of Rumex vesicarius l extract by DPPH:

Sample	Time(0)	(30)min	(60)min	(120)min
TBHQ	84.7	94.1	85.1	92.9
Extract	66.5	76.7	70.8	76.5

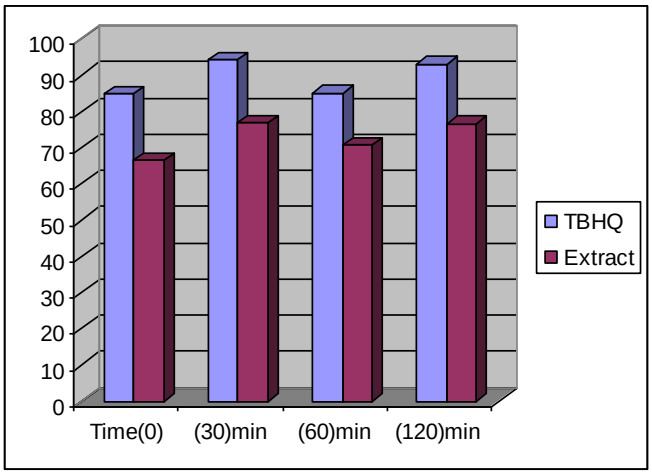


Fig. (2): Antioxidant activity of Rumex vesicarius l extract by DPPH:

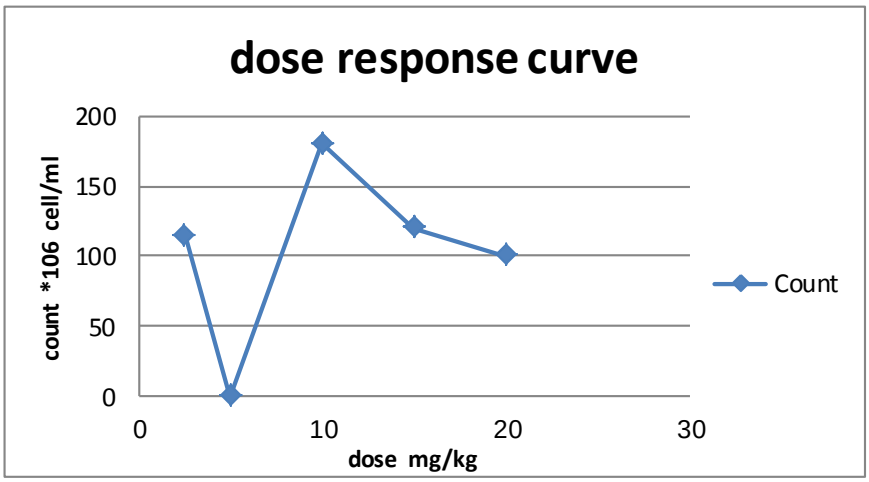


Fig (3) Dose response curve of Rumex vesicarius l extract.

Table (3) Effect of extract on volume and viability of EAC cell in studied groups:

Group	Positive Control Group		Therapeutic Group		Preventive Group	
Parameter	Tumor Volume (ml)	EAC cells Count ($\times 10^6$)	Tumor Volume (ml)	EAC cells Count ($\times 10^6$)	Tumor Volume (ml)	EAC cells Count ($\times 10^6$)
Mean ± SD.	4.1±0.49	244.4 ± 31.7	1.4±0.45	104.4 ±10.8	0	0
% Change	-----	-----	66.1%	57.2%	100	100

Table (4): Effect of Rumex vesicarius l on life span prolongation

	Positive control	Therapeutic group	Preventive group
Days	16	17	24
% change	--------	6.25%	50%
T/C ratio (%)	--------	106.25%	150%

Table (5): Change in oxidative stress and antioxidant in all studied groups

variable	Groups	Negative control	Extract group	DMSO group	Positive control	Therapeutic group	Preventive group
MDA (nmol/ml)	Mean ± SD	10.99+1.14547	12.25+1.46225	14.02+1.05704	40.93+1.84273	36.67+2.169	21.69+.1.37796
	% of change	-	27.57	11.46	272.42	-10.41	-47.01
	P value	-	0.068	0.000	0.000	0.000	0.000
NO (μmol/ml)	Mean ± SD	10.99+0.92502	30.64+1.29889	40.87+3.63631	50.15+3.24457	23.28+2.38737	17.11+1.88353
	% of change	-	59.00	112.09	160.25	-53.58	-65.88
	P value	-	0.000	0.000	0.000	0.000	0.000
SOD (U/ml)	Mean ± SD	285.77±10.82888	223.11+18.43128	199.14+4.43877	100.11+6.42849	499.81+12.82346	726.23+43.17423
	% of change	-	-21.92	-30.31	-64.96	399.26	625.59
	P value	-	0.000	0.000	0.000	0.000	0.000
CAT (U/L)	Mean ± SD	217.56±6.31052	325.46±29.20883	214.12±13.0675	104.67±6.7759	635.73+29.47575	891.03+66.80135
	% of change	-	49.59	-1.58	-51.88	507.36	751.27
	P value	-	0.000	0.815	0.000	0.000	0.000

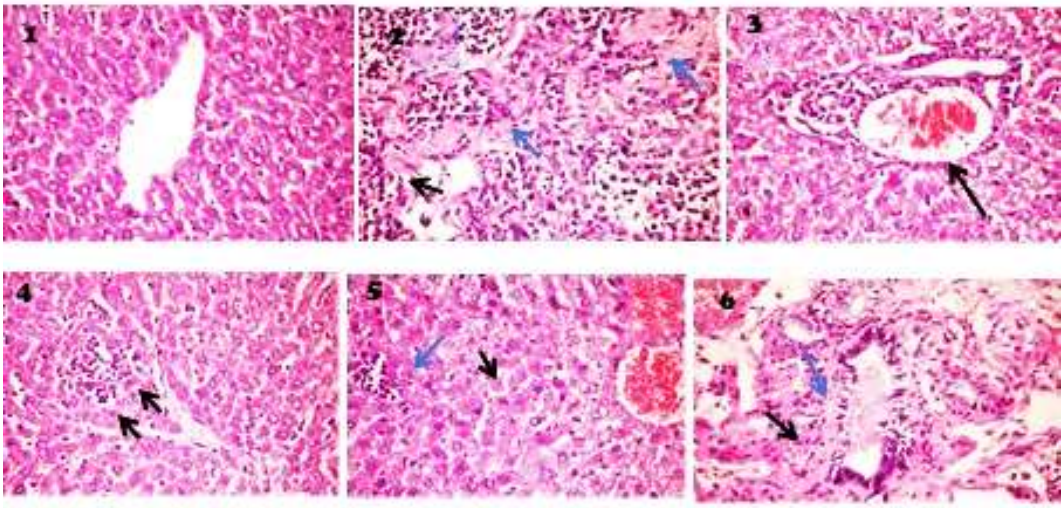
Table (6): Arginase levels in all studied groups

Group / Arginase (U/ml)	Negative control	Extract group	DMSO group	Positive control	Therapeutic group	Preventive group
Mean±S.D	95.32±4.38629	157.06±22.37723	138.57±10.01566*	253.01±21.68478*	113.78+4.87802*	88.44+3.36822
% change	-------	64.77	45.37	82.58	-17.88	-36.17
P value	-------	0.000	0.000	0.000	0.000	0.000

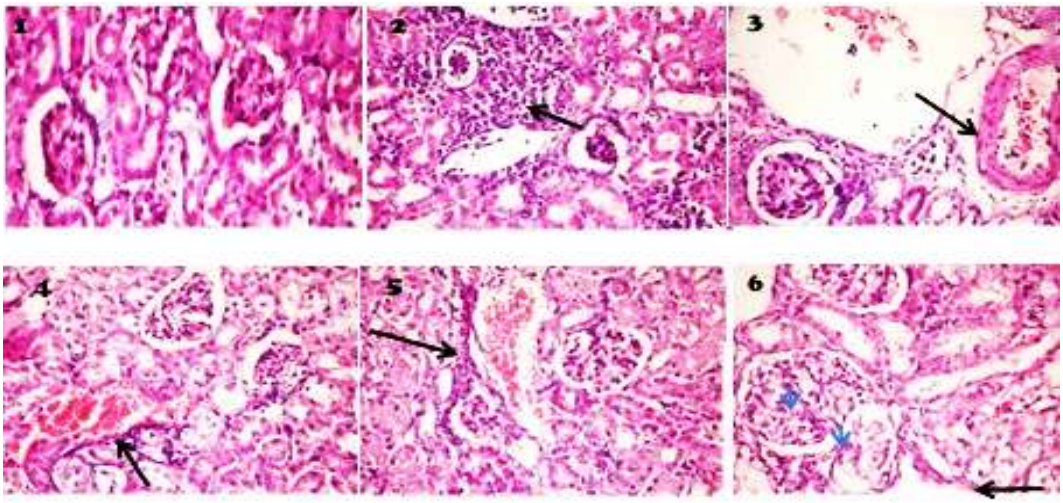
* Highly significant difference from control value at P < 0.001

Table (7): Effect of extract on liver and kidney functions in all studied groups

variable		Negative control	Extract group	DMSO group	Positive control	Therapeutic group	Preventive group
ALT ((U/L)	Mean ± SD	39.17±1.04886	38.94±2.5321	33.17+2.25342	104.23+6.5350	32.54+2.20867	25.73+2.76086
	% of change	-	-0.58	-15.31	166.09	-68.78	-75.31
	P value	-	0.879	0.000	0.000	0.000	0.000
AST (U/L)	Mean ± SD	85.89+3.40243	80.14+2.15726	58.38+4.10062	138.04+11.40742	95.53+2.22214	85.09+2.01188
	% of change	-	-6.69	-32.03	60.72	-30.79	-38.35
	P value	-	0.02	0.000	0.000	0.000	0.000
TP (g/dl)	Mean ± SD	7.85+0.33747	6.43+0.25408	7.94+0.17764	5.30+0.24495	6.84+0.31693	6.99+0.36652
	% of change	-	-18.08	1.14	-32.48	29.05	31.88
	P value	-	0.000	0.491	0.000	0.000	0.000
ALB (g/dl)	Mean ± SD	3.79+0.912	3.58+0.2974	3.80+0.19437	2.50+0.33665	3.20+0. 16997	3.74+0.11738
	% of change	-	-5.54	0.26	-34.0369	28	49.6
	P value	-	0.047	0.923	0.000	0.000	0.000
Bili T (mg/dl)	Mean ± SD	0.51+0.07803	0.474+0.03836	0.438+0.05808	0.977+0.05677	0.362+0. 05371	0.151+0.03247
	% of change	-	-7.05	-14.11	91.56	-62.94	-84.54
	P value	-	0.148	0.005	0.000	0.000	0.000
Urea (mg/dl)	Mean ± SD	19.40+2.98887	33.20+2.57337	24.50+2.3214	51.40+3.9777	19.20+1. 54919	18.60+1.10555
	% of change	-	71.13	26.28	164.94	-62.64	-63.81
	P value	-	0.000	0.000	0.000	0.000	0.000
Creat (mg/dl)	Mean ± SD	0.438+0.04662	0.492+0.0405	0.486+0.02011	1.063+0.09889	0.801+0. 02685	0.691+0.04175
	% of change	-	12.32	10.95	142.69	-24.64	-34.99
	P value	-	0.025	0.045	0.000	0.000	0.000



Fig(4). Histopathological studies of liver and kidney tissues.
Histopathological studies revealed that liver tissue. (1) Negative control group showed normal hepatic parenchyma; hepatocytes. (2) Positive control liver showed diffuse fibrosis (blue arrows) with mononuclear cells infiltrations (black arrows). (3) DMSO group showed congestion of hepatoportal blood vessels (arrow). (4) Extract group showing dilated sinusoids permeated with leucocytes (arrow). (5) Preventive group showing dilated sinusoids (blue arrow) with atrophied and disorganized hepatocytes (black arrow). (6) Therapeutic group showing hyperplasia of bile duct (blue arrow) with portal tract fibrosis (black arrow).



Kidney histopathological indicated (1) Negative control group showed normal renal parenchyma. (2) Positive control group showing massive infiltrations of mononuclear cells (arrow). (3) DMSO group showed congested blood vessel (arrow). (4) Extract group showing interstitial blood vessel congestion (arrow). (5) Preventive group showing interstitial blood vessel congestion (arrow). (6) Therapeutic group showing vacuolated glomerular tuft (blue arrow) and degenerated renal tubules (black arrow).