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Assessment of antiproliferative activity of *Capparis spinosa L* extract against Ehrlich ascites carcinoma in Swiss albino mice

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ABSTRACT

Background: Medicinal plants have been known as one of the most important therapeutic agents for cancer treatment and prevention. *Capparis spinosa L* is a multipurpose plant that contains different bioactive phytochemicals including phenols and flavonoids.**Aims:** In the present study we explore the invivo anti-tumor activity of *Capparis spinosa L* extract and most potent constituents rutin and hesperidin against Ehrlich ascites carcinoma (EAC). Also study the side effect of treatments on different organs (liver, kidney and heart). **Material and method:** The antitumor effect was assessed by evaluating tumor volume , tumor cell count , survival time and increase in life span of EAC bearing mice. We assessed the effect of *Capparis spinosa L* extract , rutin and hesperidin on the level of malonaldialdhyde (MDA) and Catalase activity. Also, we estimated their effect on Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) , Albumin , urea , creatinine ,Lactate dehydrogenase activity (LDH), Creatine Kinase -MB activity (CK-MB). Apoptosis was assessed by BCL2 and caspase3 activity. **Results :** The *Capparis spinosa L* extract , rutin and hesperidin showed significantly decreased ($p<0.001$) in the volume of the EAC and in the count of EAC cells and increase the life span of EAC bearing mice. The treatment with *Capparis spinosa L* extract , rutin and hesperidin showed significantly decreased ($p<0.001$) the lipid peroxidation marker (MDA) and recovered catalase activity towards normal as compared to positive control. We founded that *Capparis spinosa L* extract , rutin and hesperidin treatment induced apoptosis demonstrated by an increased in Caspase 3 activity and decreased in BCL2 .The treatment of *Capparis spinosa L* extract , rutin and hesperidin significantly reduced the elevated levels of ALT , AST ,LDH ,CK-MB,Urea and Creatinine in positive control as compared with negative control. Also, they showed protection for both liver and kidney histopathologically. **Conclusions:** The present study demonstrated that *Capparis spinosa L* extract , rutin and hesperidin have potent antitumor activity against Ehrlich ascites carcinoma. The anti-tumor mechanism may be mediated by preventing oxidative damage and induction of apoptosis.

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INTRODUCTION

Cancer is fundamentally a disease of tissue growth regulation failure when the genes that regulate cell growth and differentiation are alter. Cancer is the result of DNA aberrations causing deregulation of cell cycle, apoptosis and cell survival [1] . Cancer chemotherapy is associated with severe side effect in the various other organs that are devoid of cancer [2,3,4] .This indicates the need of alternative drugs which are highly effective and less or negligibly toxic to normal cells. The plants and natural products still continue to play important role as complementary and alternative medicine apart from the standard modern chemotherapy in the management of cancer [5].

Medicinal plants have been know as one the most effective and safe therapeutic agents for the treatment of human diseases [6]. There are numerous medicinal plants which possess multiple health-promoting effects [7; 8; 9; 10] . In addition, it is well known that synthetic drugs can cause a wide range of serious adverse effects [11]. Nowadays, medicinal plants are known as an important source of bioactive natural products such as phenols and flavonoids [12,13,14]. Medicinal plants used as folk medicine have strong antitumour activity against the Ehrlich ascites carcinoma (EAC) cell line [15].

Caper (Capparis spinosa L.) is a common member of the genus Capparis (Capparidaceae family) [16] .*Capparis spinosa* consider a multipurpose plant used for the curing of many human ailments as it has pharmacological effects and is utilized in phytomedicine around the world [17]. Phytochemical analysis showed that different parts of C. spinosa are rich sources of bioactive constituents, including polyphenolic compounds, which are responsible for its health-promoting effects. C. spinosa possesses different pharmacological effects including antioxidant, antimicrobial, anticancer and hepatoprotective effects [6] .

Hesperidin is a major dietary flavanone [18]. Hesperidin has been recognized as a potent anti-inflammatory, anti-carcinogenic and anti-oxidantagent according to the data obtained from numerous in vitro and in vivo studies [19].

Rutin is a flavonoid of the flavonol-type that is widespread in the plant kingdom [20] . It has a wide range of pharmacological properties (e.g., antioxidative activity) that have been exploited in human medicine and nutrition. Conventionally, it is used as an antimicrobial, antifungal, and antiallergic agent. Rutin has pharmacological benefits for the treatment of various chronic diseases , such as cancer, diabetes, hypertension, and hypercholesterolemia [21].

2.*Materials and Methods*.

Materials:

Plant material

Capparis spinosa L leaves were collected from Southern Egypt Sinai and a sample of plant was identified by cairo University herbarium , faculty of science , cairo University .

Chemicals

Rutin and Hesperidin were purchased from sigma Aldrich company , Trypan blue dye was purchased from El- Gomhouria Company.

Experimental Animals

Adult female Swiss albino mice weighed (25-30g) purchased from Abo Rawash culture , Giza , Cairo , Egypt . Mice were housed in steel mesh cages (animal house, faculty of science , Zagazig University). The animals were maintained in controlled environment of temperature, humidity and light. The mice had free access to tap water and a commercial pellet .

Tumor cell line

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. This process was repeated every 10 days for keeping the strain available throughout the present study.

Methods:

Plant extraction:

Capparis spinosa L ethanolic extract was prepared according to method [22] .

HPLC analysis :

High performance liquid chromatography (HPLC) was used to identificate the phenolic compounds present in the extracts for *Capparis spinosa L* extract. The analysis of extracts were performed with HPLC (Hewlett Packard Series 1050, USA), the column (Hypersil BDS 5 um C18). Sampling injector by using quaternary HP pump (series 1100), solvent degasser, iso gradient separation was carried out with methanol and acetonitrile as a mobile phase at flow rate of 1 ml/min, temperature was maintained at 35°C. 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 [23].

Toxicity study (LD50):

Determination of the approximate acute median lethal dose (LD 50) was determined according to the method [24].

Experimental design:

In this experiment, the adult female Swiss albino mice were divided into 8 groups (15 mice in each group) as following:

Group (1): Negative Control: This group received received sterile saline solution (0.9 NaCl) day after day for 9 days.

Group (2): Positive Control : This group received Ehrlich ascites carcinoma (EAC), (2.5×10^6 cells/ 0.3 ml/mouse) by (I.P) injection once in the first day.

Group (3): Preventive plant group : This group received plant extract (200 mg/kg b.w.)[25] one day before EAC injection (2.5×10^6 cells/ 0.3 ml/mouse) ,Then next days of EAC inoculation, Mice received plant extract (200 mg/kg b.w.) day after day for 9 days .

Group (4): Therapeutic plant group: This group received plant extract (200 mg/kg b.w.) [25] day after day for 9 days after the injection with Ehrlich ascites carcinoma (EAC), (2.5×10^6 cells/ 0.3mL/mouse) by (I.P) injection once in the first day..

Group (5): Preventive rutin group : This group received Rutin (10 mg/kg b.w.)[26]one day before EAC injection (2.5×10^6 cells/ 0.3 ml/mouse) ,Then next days of EAC inoculation, Mice received rutin (10 mg/kg b.w.) day after day for 9 days.

Group (6): Therapeutic rutin group: This group received Rutin(10 mg/kg b.w.)[26] day after day for 9 days after the injection with Ehrlich ascites carcinoma (EAC), (2.5×10^6 cells/ 0.3mL/mouse) by (I.P) injection once in the first day.

Group(7): Preventive hesperidin group : This group received Hesperidin (50 mg/kg b.w.) [27] one day before EAC injection (2.5×10^6 cells/ 0.3 ml/mouse) ,Then next days of EAC inoculation, Mice received Hesperidin(50 mg/kg b.w.) day after day for 9 days .

Group (8): Therapeutic hesperidin group: This group received Hesperidin (50 mg/kg b.w.) [27] day after day for 9 days after the injection with Ehrlich ascites carcinoma (EAC), (2.5×10^6 cells/ 0.3mL/mouse) by (I.P) injection once in the first day.

Samples collection:

At the end of the experiment; the blood samples were collected. where, blood samples were collected in two types of tubes, first containing ethylene diamine tetra acetic acid (EDTA) to get plasma by centrifugation at 4000 rpm for 20 min and second in empty tubes to get serum by centrifugation at 4000 rpm for 20 min. these two forms were used for various biochemical measurements.

Liver tissues were excised from each mouse and divided into two parts, part of liver tissue from each group was collected in centrifuge tube contains phosphate buffer saline (PBS) pH 7.4 , this part was undergoe biochemical analysis. The second part of liver tissue and kidney tissue were excised and fixed in 10% buffered formaline for histopathological evaluation. The viability of EAC cells was determined by EAC cells were harvested from

each mouse in centrifuge tube containing heparinized saline.

Viability and life span prolongation

The viability of EAC cells was determined by the *Trypan Blue* Exclusion Method [28] . Life span calculation was carried according to method [29] .

Biochemical parameters:

Determination of MDA level

Liver and plasma Malondialdehyde (MDA) were estimated by using a commercial kit derived from Biodiagnostic Company, Egypt [30].

Determination of catalase activity

Plasma and liver CAT were estimated by using a commercial kit derived from Biodiagnostic Company, Egypt [31]

Determination of caspase 3 activity

Caspase 3 activity determination was carried out as indicator for apoptosis , the activity of caspase-3 was determined by the colorimetric caspase-3 kit according to the manufacturer's instructions (R & D Systems, Inc.) [32] .

Determination of BCL2

BCL2 was determined by using a commercial ELISA kit according to the method [33].

Assessment of serum hepatic markers

Serum samples were screened for liver function tests including Albumin [34], Alanine Aminotransferase (ALT)[35] and Aspartate Aminotransferase (AST) [36] .

Assessment of renal functional markers

Serum samples were screened for kidney function tests including urea and creatinine by using a commercial kit derived from Diamond Diagnostic Company, Germany [37]and from Spin react Company, Spain [38]respectively.

Assessment of heart functional markers

Serum Creatine Kinase MB (CK-MB) measure according to method [39] and Lactate dehydrogenase (LDH) activity was measured according to method [40].

Histopathological examination:

A piece of liver and kidney samples were fixed in 10% formalin for histopathological examination. The thin sections were cut and then stained by haematoxylin and eosin and observed under light microscope [41].

Statistical analysis

All statistical analyses were done by a statistical for social science package "SPSS" version 14.0 for Microsoft Windows, SPSS Inc. [42]. Numerical data were expressed as mean ± SE. The levels of markers were analyzed by ANOVA.

3. Results:

Total Capparis spinosa L extract yield

Capparis spinosa L leaves powder (100g) after undergoing extraction, yielded 8 g of ethanolic Capparis spinosa L extract (Thick green paste).

Identification of phenolic and flavonoid compounds for capparis spinosa plant ethanolic extracts by using HPLC:

Total phenolic compounds of *C. spinosa* ethanolic extracts were identified by HPLC. Data are illustrated in table (1) showed that twenty five phenolic compounds were identified in plant .Also HPLC results reflected that eleven flavonoid compounds were identified in plant as shown in table (2).

Toxicity study :

As to determine the median lethel dose (LD_{50}) of capparis spinosa ethanolic extract , all doses was found to be safe up to 2000 mg extract/ kg mice, as non of the mice were dead, which suggests that extract may be a safe compounds.While, hesperidin was might safe up to 1000 mg / kg mice and rutin safe up to 200 mg / kg mice.

Effect of plant extract , hesperidin and rutin treatments on tumor growth and survival parameters

Ascites fluid accumulation was observed in the peritoneal cavity of the experimental animals after the inoculation of Ehrlich Ascites Carcinoma cells. The tumor bearing animals showed a marked increase in tumor volume. On treatment with *capparis spinosa* ,rutin and hesperidin there is amarked decrease in the tumour volume EAC by 74.8% and 79.07% ; by 47.09% and 51.7 % ,and by 55.4 % and 64.3% respectively in therapeutic and

preventive group compared to positive control group ($p<0.001$) table (3).

The increased viable cell count of ascites fluid of tumor bearing animals was found to be decreased on treatment with *capparis spinosa* ,rutin and hesperidin by 73.2 % and 75.3% ; by 56.6 % and 57.7% , and by 63.2% and 67.3 % respectively in therapeutic and preventive group compared to positive control group ($p<0.001$) table (4).

Effect of plant, hesperidin and rutin treatments on malondialdehyde (MDA) levels

The mean values of liver MDA were found to be 72.61±0.99 (n mol /g tissue) in the negative control group. These values were very highly increased significantly in the positive control group to 155.90 ±3.85 (nmol/g) by ($p \leq 0.001$).

Treatments by plant , rutin , hesperidin showed a significant reduction of liver MAD levels to 72.02±0.93 and 82.11± 1.46 (nmol/g); 76.87± 0.99 and 82.90± 1.29 (nmol/g) and 88.34±1.30 and 81.80±0.55 (nmol/g) by 53.8% and 47.33% ; by 50.69 % and 46.82%, and by 43.33% and 47.53% (nmol/g) for both preventive and therapeutic groups, respectively; compared to the positive control group ($p \leq 0.001$).The mean values of plasma MDA were found to be 12.57±0.18 (nmol/ml) in the negative control group. These values were very highly increased significantly in the positive control group to 43.33±0.18 (nmol/ml)($p \leq 0.001$). Treatments by plant , rutin , hesperidin indicated a significantly reduction of plasma MAD levels to 12.27±0.14 and 14.29±0.18 (nmol/ml); 18.63±0.18 and 19.70±0.25 (nmol/ml) and 12.42±0.18 and 15.98 ±0.11 (nmol/ml) by 71.68% and 67.02% ; by 57 % and 54.53 %, and by 71.34% and 63.12% (nmol/ml) ($p\leq0.001$) for both preventive and therapeutic groups, respectively; compared to the positive control group table (6).

Effect of plant, hesperidin and rutin treatments on catalase activity

Our results revealed that plasma and liver catalase level were found to be lower in positive control compared to that negative control ($P<0.001$).Treatments with plant, rutin, hesperidin showed a significant increase in liver catalase activities to 9.28± 0.11 and 9.02 ±0.15 (u/g); 8.6 ±0.07 and 8.57± 0.1 (u/g), and 9.29 ± 0.09 and 8.87± 0.11 (u/g) by 63.84% and 59.08%; 51.67 % and 51.15%, and 63.84% and 56.61%, ($p\leq0.001$) in both preventive and therapeutic groups, respectively; compared to the positive control group. It also showed significant increase in plasma catalase activities to 810 ± 11.64 and 806 ± 9.32 (u/L); 798 ± 15.76 and 773 ± 11.08 (u/L), and 811 ± 12.66 and 772 ± 12.34 (u/L) by 56.98% and 56.2%; 54.65% and 49.8%, and 57.17 % and 49.61%, in both preventive and therapeutic groups, respectively; compared to the positive control group ($p\leq0.001$) table (7).

Treatment with plant , hesperidin and rutin lead to apoptosis

Our results revealed that administration of the plant and rutin and hesperidin showed a significantly increase in caspase-3 activity in both therapeutic and preventive groups compared to the positive control group. While, treatment with plant and rutin and hesperidin showed significantly decrease in bcl2 concentration in preventive and therapeutic groups table (8).

Effect of plant , hesperidin and rutin treatments on serum hepatic markers

Our results revealed that a significant increase in AST and ALT activities from 81.6 ± 1.51 (U/L), and 41.95 ± 0.95 (U/L) respectively in negative control group to 346.4 ± 6.94 and to 144.50 ± 10.58 in positive control group but treatments by plant , rutin , hesperidin indicated a significant decreased in ALT levels to 42.64 ± 4.48 and 62.16 ± 5.49 (u/L); 61.81 ± 3.79 and 71.86 ± 7.67 (u/L), and 47.94 ± 7.35 and 66.14 ± 4.53 (u/L) by 70.49% and 56.98%; 57.22% and 50.27%, and 66.82% and 54.23%, in both preventive and therapeutic groups, respectively; compared to the positive control group ($p\leq0.001$).Also decreased AST levels to 77.0 ± 2.72 and 107.4 ± 1.51 (u/L); 85.9 ± 15.76 and 161.3 ± 1.92 (u/L), and 78.3 ± 1.98 and 140.7 ±5.73 (u/L) by 77.77% and 68.99%; 75.2% and 53.43%, and 77.39 % and 59.38%, in both preventive and therapeutic groups, respectively; compared to the positive control group($p\leq0.001$) table (9).

A significant ($p<0.001$) decreased in albumin were observed in positive control group compared to negative control group [1.35 ±0.05 (g/dL) vs. 2.71 ±0.03(g/dL). Meanwhile, treatments by plant , rutin , hesperidin showed a significant increase of albumin levels to

2.64±0.05 and 2.77±0.05(g/dL); 2.65±0.01and 2.43±0.05 (g/dL), and 2.65±0.02 and 2.47±0.1 (g/dL) by 96.29% and 105.19%; 96.29% and 80%, and 97.04% and 82.96%, in both preventive and therapeutic groups, respectively; compared to the positive control group($p \leq 0.001$) table (9).

Effect of plant , hesperidin and rutin treatments on renal functional markers

Our results showed a significant increased in urea and creatinine levels in positive control compared to negative control. The urea and creatinine levels were increased to 27.50 ± 0.34 (mg/dl), and 1.27± 0.08 (mg/dl) in positive control group compared to negative control groups 16.20 ± 0.46 and 0.50 ± 0.02 , respectively. While treatments by plant , rutin , hesperidin showed a very highly significant decrease of urea levels to 19.50 ±0.94 and 19.10 ±0.52 (mg/dL); 17.96 ±0.51 and 18.50 ±0.34 (mg/dL), and 16.86 ±0.24 and 20.00 ±0.66 (mg/dL) by 29.09% and 30.54%; 34.69% and 32.73%, and 38.69% and 27.27%, in both preventive and therapeutic groups, respectively; compared to the positive control group($p \leq 0.001$). Also, treatments by plant, rutin , hesperidin showed a significant decreased in concentration of creatinine to 0.53 ± 0.02 and 0.40 ±0.03 (mg/dL); 0.66 ±0.01 and 0.54 ±0.03 (mg/dL), and 0.55 ±0.03 and 0.53 ±0.02 (mg/dL) by 58.27% and 68.5%; 48.03% and 57.48%, and 56.69% and 58.27%, in both preventive and therapeutic groups, respectively; compared to the positive control group ($p \leq 0.001$) table (10).

Effect of plant , hesperidin and rutin treatments on cardiac functional markers

Our results indicated that a significant ($p \leq 0.001$) increase in CK-MB and LDH activities from 1.81 ±0.17 (U/L), and 3396.3 ±86.49 (U/L) respectively in negative control group to 16.76 ±0.07 and to 4532.2 ±47.96 in positive control group. Meanwhile, treatments by plant , rutin , hesperidin results in a very highly significant decrease in CK –MB to 1.94 ± 0.11 and 2.28 ± 0.13 (U/L); 6.72 ± 0.28 and 9.65 ± 0.46 (U/L), and 1.46± 0.06 and 3355.5 ± 85.60 (U/L)by 88.42% and 86.39%; 59.9% and 42.42%, and 91.29 % and 47.37 %, in both preventive and therapeutic groups, respectively; compared to the positive control group($p \leq 0.001$). Also results in a very highly significant decrease in concentration of LDH to 2997.1 ±139.93 and 2656.4 ±130.45 (U/L); 2656.4 ±130.45 and 3151.2 ± 88.22 (U/L), and 3417.6 ±169.0 and 3355.5 ± 85.60 (U/L) by 33.87% and 41.38%; 30.47% and 25.72%, and 24.59% and 25.96%, in both preventive and therapeutic groups, respectively; compared to the positive control group($p \leq 0.001$) table (11).

Histopathological results

Histopathological studies revealed that liver from control negative group showed normal hepatic parenchyma; hepatocytes, blood sinusoids, and Portal tract . Positive control mice liver showed solid carcinoma of aggregated malignant cells infiltrated with inflammatory cells. Treatment with plant, rutin and hesperidin reduced most of the pathological alterations induced by EAC cells in mice , there is no appearance of solid carcinoma of aggregated malignant cells compared to positive control.

Concerning kidney histopathological study, Kidney from control negative group showed normal renal parenchyma; renal Glomeruli and renal tubules. Positive control mice kidney showed severe glomerular tuft necrosis with poor vascularization .Treatment with plant , rutin and hesperidin for preventive and therapeutic mice groups kidney tissues shows improved kidney tissues changes induced by EAC cells in mice .

4. Discussion

Cancer is an abnormal growth of cells. Although the cancer research has led to a number of effective solutions, the medicines used as treatments have clear limitations and unfortunately cancer is projected as the primary cause of death in the future currently there is a huge scientific and commercial interest in the discovery of new anticancer drugs. Therefore the search for potent, safe and selective anticancer compounds is a crucial aspect of modern cancer research [43] .

Caper (*Capparis spinosa* L.) belongs to the Capparaceae family. *C.spinosa* which was

commonly used as a medicinal plant contained many biologically active chemical groups including, alkaloids, glycosides, tannins, phenolic, flavonoids, triterpenoids steroids, carbohydrates, saponins and a wide range of minerals and trace elements. It exerted many pharmacological effects including antimicrobial, cytotoxic, anti-diabetic, anti-inflammatory, antioxidant effect and many others [44].

Hesperidin(3,5,7-trihydroxy flavanone-7-rhamnoglucoside) is a pharmacologically active bioflavonoid, with good free radical scavenging as well as anti-lipidperoxidation properties in biological membranes [45].It has been reported that they have antiviral, antiallergic, antiplatelet, anti-inflammatory and antioxidant activities as well as antitumor properties [46].

Rutin, also known as vitamin P or rutoside, has been explored for a number of pharmacological effects including antioxidant, cytoprotective, vasoprotective, anticarcinogenic, neuroprotective and cardioprotective activities. Rutin has been observed for its nutraceutical effect [47].

Phenolic compounds are very important plant constituents because of their scavenging ability due to their hydroxyl groups. Therefore, total phenolic compounds of *C. spinosa* ethanolic extracts were identified by HPLC. Data are illustrated in Table (2) showed that twenty five phenolic compounds were identified in *C. spinosa* ethanolic extract. It can be noticed that e- vanillic are the most abundant phenolic compound being 1232.783 mg / 100g extract. Furthermore, Galic acid acids was found in small amounts being 8.575mg / 100g extract .Also reflected that eleven flavonoid compounds were identified in *C. spinosa* ethanolic extracts. It can be noticed that Hesperidin and Rutin are the most abundant flavonoid compounds in *C. spinosa* extracts being 537.92 mg / 100g and 392.69 mg / 100g extract, respectively. While, 7-OH-ydroxyflavone was found in small amount, 1.1214 mg / 100g extract. Our results are agreement with study revealed that the high performance liquid chromatography of extracted polyphenols are proved the presence of (Gallic acid,Caffeic acid, Coumaric acid , Vanillic acid , Syringic acid, Ferulic acid, Chlorogenic acid, Rutin and Quercetin) in the Capparis spinosa L. leaves extract[48].

Acute toxicity tests are generally the initial step in the assessment and evaluation of the toxic characteristics of a substance. The acute toxicity was estimated by intraperitoneal administration of the compounds *C. spinosa* extract , hesperidin and rutin to determine the median lethal dose (LD50). Our results revealed that, dose up to 2000 mg /kg was considered safe for plant agreement with study [49] suggested that the plant extract is relatively safe in mice .Our results revealed that, hesperidin was considered safe up to 1000 mg / kg mice and rutin safe up to 200 mg / kg mice .Our result in agreement with study [50] indicated the safety of hesperidin in herbal formulations and other study indicated toxicity of rutin [26].

The present study was aimed to evaluating the antitumor potential of *C. spinosa* extract , hesperidin and rutin in EAC bearing mice . It was observed that treatment with plant, hesperidin and rutin increased the life span of EAC bearing mice by reducing the viability of EAC cells and decreasing the tumor volume . The anticancer activity of *Capparis spinosa L.* extract is probably due to presence of flavonoid and phenolic compounds in extract. Furthermore, flavonoids compounds have been shown to possess antimutagenic effect and anticancer properties [51] . N-butanol extract of *Capparis spinosa L.* had anti-tumor activity and significantly inhibited S180 sarcoma in terms of tumor weight [52]. Another study proved that essential oil hydrodistilled from leaves and floral buds of *C. spinosa* were assayed for anticarcinogenic potential on HT-29 human colorectal adenocarcinoma cells [53] .

The anticancer potential of hesperidin is demonstrated by inhibiting cancer cell growth and proliferation via apoptosis induction [54]. Rutin has been extensively studied for anticancer/antineoplastic effects. Rutin is also known to inhibit cancer cell growth by cell cycle arrest and/or apoptosis, along with inhibition of proliferation, angiogenesis, and/or metastasis in colorectal cell lines [55] . Other study suggested that human leukemia HL-60 cells were implanted in a murine model, and rutin (dose 120 mg/kg) caused a significant reduction in tumor size justifying antileukemic potential[56] .

Lipid peroxidation process plays a key role in tumor growth invasiveness [57].MDA, a free oxygen radical product formed during

oxidative degeneration of cancerous tissues and as the end product of lipid peroxidation, is a biomarker of oxidative stress that has been reported to be exhibited at higher levels in cancer tissues than in non-diseased organs [58] . Antioxidants with free radical scavenging activities may have great relevance in the prevention and therapeutics of diseases in which oxidants or free radicals are implicated such as cancer[59].

Our result revealed that MDA concentration increased in EAC positive control group due to that cancer cells induced excessive production of free radicals leading to damage lipids and can induced lipid peroxidation [60]. Our results are in agreement with study [61, 62] which reported that elevation of MDA could be due to cancer cells induced excessive production of free radicals resulted in oxidative stress, which leads to damage of the macromolecules such as lipids which can induce lipid peroxidation (LPO) ,this would cause degeneration of tissues .

Treatment with plant , hesperidin and rutin indicated a significant degree of protection against oxidative damage caused by EAC by decreasing lipid peroxidation in comparison with positive control mice, as it was observed that treatments by plant , rutin , hesperidin showed a significant reduction of plasma and liver MAD levels in preventive and therapeutic groups compared to the positive control group. This finding suggested that treatments may be successful in quenching free radicals, thus inhibiting LPO and protecting against membrane damage from oxidative damage in mice.

Catalase is a hemoprotein and it protects cells from the accumulation of H_2O_2 and able to prevent the tissue from reactive free oxygen and hydroxyl radicals, by catalysing the reduction of $H_2O_2$to form H_2O and O_2 [63]. Catalase protects the tissue from highly reactive hydroxyl radicals by decomposing the hydrogen peroxide. So, reduced levels of catalase may indicate the toxic effects on the tissue. Our result indicated the catalase activity decrease in positive group compared to negative control , catalase activity decreased could be a result of tumor growth and emergence of the malignancy[64]. The administration of plant , rutin , hesperidin restored catalase activity to normal level which may indicate the antioxidant and free radical scavenging properties of treatments.

Previous study demonstrated that an administration of caper extract was found to provide significant protection against DNA damage, decrease malondialdehyde (MDA) levels and increase superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) activities[65]. Hesperidin has antioxidant and anti-inflammatory and anticancer properties. In addition, flavonoids act as a powerful antioxidants providing a remarkable protection against oxidative and free radical damage. It was also reported that hesperidin offers protection by terminating the lipid peroxidation side chain rather than scavenging extracellular non-lipid radicals that initiate lipid peroxidation. Hesperidin decreased MDA level signifying attenuation in lipid peroxidation there by proving its stabilizing power on membranes [66] .On the other study showed that hesperidin treatment reduced increased TBARS levels and induced the antioxidant defense system (increased SOD, CAT enzyme activity and GSH levels), when given together with 2,3,7,8-tetrachlorodibenzo-p-dioxin TCDD [67] .Previous study indicated the lipid peroxidase level was found to be decreased significantly in rutin administered animals as compared to control group animals hence showing its role in detoxification pathway.This result showed that significant increase in GSH, SOD, catalase and protein level was noted in the skin of rutin administered animals than the control group animals [68].

Apoptosis is a process of programmed cell in which biochemical events lead to characteristic cell changes and death. Excessive apoptosis causes atrophy, while an insufficient apoptosis results in uncontrolled cell proliferation leading to cancer. Some factors like caspases promote apoptosis, while some members of the Bcl-2 family of proteins inhibit apoptosis. Caspase-3 is also required for some typical hallmarks of apoptosis [69] .Bcl-2 (B-cell lymphoma 2), encoded in humans by the Bcl-2 gene, is the founding member of the Bcl-2 family of regulator proteins that regulate programmed cell death [70].

Our results showed treatment with plant , rutin , hesperidin cause increase in Caspase-3

activity and decrease bcl-2 level compared with positive control group. Therefore , the results of the present study suggested that treatment with plant , rutin , hesperidin may have induced apoptosis . Previous studies showed the ability of *Capparis spinosa* to induce apoptosis by upregulation of the pro-apoptotic protein, and downregulation of the anti-apoptotic protein BCL-2, which in turn may have induced a reduction in the mitochondrial membrane potential, leading to mitochondrial cytochrome *c* release and subsequent activation of caspase-9 and caspase-3. Therefore, caper plant may have induced apoptosis via the mitochondrial apoptosis pathway [71]. Another study demonstrated the *Capparis spinosa L.* was found to possess antitumor activity, such as antiproliferative and apoptosis-induced effects on HepG2 cells.The regulatory mechanism of HepG2 cell apoptosis involved the increase in Ca^{2+} concentration and ROS levels and downregulation of anti-apoptotic Bcl-2 expression, and upregulation of apoptotic Bax expression[72].

Rutin is also known to inhibit cancer cell growth by cell cycle arrest and/or apoptosis, along with inhibition of proliferation, angiogenesis, and/or metastasis in colorectal cell lines [55]**.** Antineuroblastoma effect of rutin demonstrated by study [73] where, rutin significantly inhibited the growth of LAN-5 cells. The study demonstrated that rutin could decrease BCL2 expression and BCL2/BAX ratio**.**

The anticancer potential of hesperidin is demonstrated by inhibiting cancer cell growth and proliferation via apoptosis induction [54]. Hesperidin induces human colon cancer SNU-C4 cell apoptosis as determined by a decrease in messenger RNA (mRNA) expression of bcl-2 and an increase of bax mRNA levels with an increase of caspase-3 expression and activity [74]**.**

Aminotransferases (AST and ALT) are the first enzymes to be used in diagnosis of liver damage. Since these are normally located in the cytosol, toxicity affecting the liver with subsequent breakdown in membrane of the cells leads to their spillage into plasma while their concentration rises in the blood stream [75]. Liver damage induced by tumor cells generally reflects disturbances in liver cell metabolism, which lead to characteristic changes in serum enzyme activities. The increased levels of AST, ALT, ALK and albumin in serum may be interpreted as a result of liver damage or as changes in membrane permeability indicating the severity of hepatocellular damage by EAC. Serum liver enzymes (ALT, AST) showed significantly increase in EAC bearing mice. The increment in serum enzymatic activities is related to hepatic parenchymal damage since ALT and AST are released from mitochondrial and cytosolic localization and cellular rupture allows the enzyme to escape into the blood [76]**.**

Our results demonstrated that there was a significantly increase in concentration of liver enzymes (AlT&AST) and decreased albumin level in positive control group as compared to negative control group. This increased reflected that hepatotoxicty due to cancer [77]. Treatment with plant , hesperidin and rutin attenuated these increased enzyme activity and recovery towards normal levels. Also, serum albumin showed significant decrease in Ehrlich group and this decrease was improved by treatment. These findings strongly proved the ability of these substances in protecting hepatocyte against membrane fragility and may stabilized the hepatic cellular membrane damage which may decreasing the leakage of enzymes into blood circulation.

Ethanolic root bark extract of *C. spinosa* afford significant dose-dependent protection against CCl4 induced hepatocellular injury. Blood samples from the animals treated with ethanolic root bark extracts showed significant decrease in the levels of serum markers, indicating the protection of hepatic cells [78]**.** Treatment of the paracetamol-induced liver damage in rats with aqueous extract of *Capparis spinosa* decreased alanine amino transferase, aspartate amino transferase activity, total bilirubin and creatinine levels in comparison with non treated group, as well as improving the damaged liver tissues with dose dependent manner [79]

The protective potential of hesperidin against liver fibrosis was evidenced through its ability to significantly suppress serum levels of liver function markers (ALT, AST and total bilirubin) and to significantly increase serum level of albumin, revealing its hepatoprotective nature against CCl4 hepatotoxicity [80]. Rutin is extensively studied

for hepatoprotective activity in experimental animals[81,82] evaluated the protective effect of rutin in carbon tetrachloride (CCl4)-induced liver injuries in rats. Administration of rutin caused a decrement in levels of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and gamma-glutamyl transpeptidase in serum raised due to carbon tetrachloride.

BUN and creatinine are two of the conventional test indices for kidney functions and renal structural integrity. Nephrotoxicity was proved by significant elevations in serum levels of creatinine and BUN as compared to normal control group.The transplantation of EAC into mice group induced increase for both urea and creatinine levels in serum that may be attributed to renal damage as a result of cancer cell invasion [83].

Treatment of EAC bearing mice with plant , hesperidin and rutin showed an improvement of urea and creatinine levels, this level attained nearly to the negative control group. Previous study reported the methanolic extract of *Capparis spinosa* leaves treatment significantly reduced the increased plasma levels of creatinine, urea and uric acid and restored the kidney damage, provoked by cisplatin-treatment [84]. Hesperidin significantly reduced cisplatin-induced elevations in serum creatinine and BUN levels [85] . The elevated serum creatinine and BUN levels induced by cisplatin were significantly restored to their normal levels as in control group by rutin. The rutin protective effect against nephrotoxicity can be attributed to its antioxidant and anti-inflammatory effect on ROS and some cytokines may be involved in the glomerular filtration rate damage [86].

LDH , a cytoplasmic marker enzyme is a known indicator of the cell and tissue damage by toxic compounds [87]. Our result indicate that there was a highly significant increase in LDH activities in positive control group compared to negative control group. This increasing in LDH value resulted from heart tissue damage caused by tumor growth. Creatine kinase- MB (CK- MB), Creatine Kinase (CK) is predominantly found in the cytoplasm; within myocytes, the enzyme occurs in close association with the sarcoplasmic reticulum, mitochondria and myofibrils . CK-MB activity was significantly increase in positive control group compared to negative control group .This increased may be due to the excessive production of free radicals and lipid peroxides that might have caused leakage of cytosolic enzymes and cell membrane damage [88] . Treatment with plant , hesperidin and rutin showed significantly decreased in LDH and CK-MB in the studied groups.

Ethyl acetate extract of *Capparis spinosa L.* had protective effects on the cardiac toxic effect of doxorubicin, and decreased the activity of lactic dehydrogenase (LDH) and creatine kinase (CK). Plant increased the ability of myocardial tissue to scavenge free radicals, inhibited lipid peroxidation, increased recovery activity of antioxidant enzymes, adjusted the energy metabolism of myocardial tissue, inhibited the generation of a large number of ROS in the cells and improved the metabolism of free radicals. *Capparis spinosa L* demonstrated protective effects on doxorubicin-induced myocardial damage[89].The CK-MB and LDH levels in diabetic irradiated rats was significantly decrease in the treated with hesperidin alone or in combination with rosiglitazone when compared to diabetic irradiated rats treated with rosiglitazone alone[90]. Another study reported the protective role of hesperidin against doxorubicin-induced cardiac toxicity and significant reduction in serum LDH and CK after hesperidin treatment [91] . Rutin restored the levels of cardiac marker enzymes along with a reduction in lipid peroxidation. The study suggests the cardioprotective effect is due to the the antioxidant effect of rutin [92].

Histopathological Studies revealed that liver from control negative group showed normal hepatic parenchyma; hepatocytes, blood sinusoids, and Portal tract ,but Positive control mice liver showed solid carcinoma of aggregated malignant cells infiltrated with inflammatory cells. Treatment with plant extract, rutin and hesperidin reduced most of the pathological alterations induced by EAC cells in mice , there is no appearance of solid carcinoma of aggregated malignant cells.As for preventive plant extract group the liver tissue showed normal hepatic parenchyma; hepatocytes, blood sinusoids, and Portal tract, While for the therapeutic plant extract group, Liver showing hepatocytes vacuolation with

prepheral nucleous, the central vein is permeated with leucocytic cells . Our results showed hepatoprotective effect of C. spinosa extract were supported by the study that revealed histopathological studies under light microscope confirms the protective effects of C. spinosa and Quercetin against the t-BHP-induced liver damage [93].

Moreover, treatment with hesperidin livers showed massive hepatocytes vacuolation with prepheral nucleous in therapeutic group and diffuse hepatocytes vacuolations and congested central vein in preventive group. This protective effects of hesperidin on the liver as the result of its antioxidant properties [27].Treatment with rutin in preventive group liver showed congestion of the hepatoportal blood vessel and massive hepatocytes vacuolation with prepheral nucleous in therapeutic group. The protective effects of rutin indicated by study revealed that Quercitin and rutin might potentially be protective agents for 5-FU-induced liver toxicity [94].

Kidney histopathological indicated control negative group showed normal renal parenchyma; renal Glomeruli and renal tubules. Positive control mice kidney showing severe glomerular tuft necrosis with poor vascularization. While, treatment with Capparis spinosa extracts, rutin and hesperidin for preventive and therapeutic mice groups showed improved kidney tissues changes induced by EAC cells in mice .

As for plant extract preventive group the kidney showing normal renal parenchyma; renal Glomeruli and renal tubules and showed congestion of the glomerular capilleries in therapeutic plant extract group. Moreover, treatment with hesperidin kidneys showed normal renal glomeruli and renal tubules in preventive group and congestion of the peritubular blood capilleries in therapeutic group.Also , treatment with rutin kidneys showed normal renal glomeruli and renal tubules in preventive group And in therapeutic group kidneys showed congestion of the interstitial blood vessel .

Our result agreement with study indicate that the hydroalcoholic extract of *C. spinosa* has protective effects against CP-induced nephrotoxicity in mice[95]. Hesperidin treatment showed renoprotective effect against gentamicin and this effect may be due to its antioxidant properties[96]. Nephroprotective effect of rutin indicated by study showed rutin administration attenuated cisplatin-induced alteration changes in the kidney[97] .

References

[1] Perria, F., Longob, F., Giulianoc, M., Sabbatinoe, d., F., Faviaf, G., Ionnab, F., Addeog, R., DellaVittoria, S. G., Di Lorenzoh ,G., and Pisconti, S. (2017) : Critical Reviews in Oncology/Hematology . 111: 166–172.

[2] Pedersen, B., Koktved D.P and Nielsen L.L. (2013): Living with side effects from cancer treatment -a challenge to target information. Scand J Caring Sci 27(3): 715-723.

[3] Iwamoto, T. (2013): Clinical Application of Drug Delivery Systems in Cancer Chemotherapy: Review of the Efficacy and Side Effects of Approved Drugs. Biol Pharm Bull , 36(5): 715-718.

[4] **Zhang, X., Chen ,W.W. and Huang, W.J. (2017)**: Chemotherapy-induced peripheral neuropathy. Biomed Rep , 6: 267-271.

[5] **Greenlee, H., Neugut, A.I. , Falci, L., Hillyer, G.C and Buono, D. (2016)**: Association between complementary and alternative medicine use and breast cancer chemotherapy initiation: the Breast Cancer Quality of Care (BQUAL) study. JAMA Oncol ,2(9): 1170-1176.

[6] Nabavi , S.F., Braidy, N., Orhan, I.E., Badiee, A. , Daglia, M. and Nabavi, S.M. (2016): Rhodiola rosea L. and Alzheimer's disease: from farm to pharmacy. Phytother Res, 30(4): 532–539.

[7] Wink, M. (2012): Medicinal plants: a source of anti-parasitic secondary metabolites. Molecules ,17: 12771–12791.

[8] Hu, Y., Wang, S. and Wu , X. (2013): Chinese herbal medicine-derived compounds for cancer therapy: a focus on hepatocellular

carcinoma. J Ethnopharmacol, 149: 601–612.

[9] Erdem, S.A. , Nabavi, S.F. , Orhan, I.E., Daglia, M. , Izadi, M. , and Nabavi, S.M. (2015): Blessings in disguise: a review of phytochemical composition and antimicrobial activity of plants belonging to the genus Eryngium. DARU J Pharm Sci , 23: 1.

[10] Nabavi, S.M, Marchese A. , Izadi M. , Curti V. , Daglia M. and Nabavi S.F. (2015): Plants belonging to the genus Thymus as antibacterial agents: From farm to pharmacy. Food Chem 173: 339–347.

[11] Gurney, S., Scott, K. , Kacinko, S. , Presley, B. and Logan, B. (2014): Pharmacology, toxicology, and adverse effects of synthetic cannabinoid drugs. Forensic Sci Rev, 26: 54–78.

[12] Ngameni, B., Fotso, G. , and Kamga, J., (2013): 9—Flavonoids and related compounds from the medicinal plants of Africa. Medicinal plant research in Africa. Elsevier: Oxford; 301–350.

[13] Nabavi ,S.F., Russo, G.L., Daglia, M. and Nabavi , S.M. (2015): Role of quercetin as an alternative for obesity treatment: you are what you eat!. Food Chem , 179: 305–310.

[14] Russo, M., Russo, G.L. and Daglia, M.(2016): Understanding genistein in cancer: the "good" and the "bad" effects: a review. Food Chem , 196: 589–600.

[15] Kviecinski, M.R., Felipe, K.B., Schoenfelder ,T., de Lemos Wiese L.P, Rossi ,M.H. , Gonçalez, E., Felicio, J.D., Filho, D.W., and Pedrosa, R.C. (2008): Study of the antitumor potential of Bidens pilosa (Asteraceae) used in Brazilian folk medicine. J Ethnopharmacol , 117:69–75.

[16] Tlili, N.; Elfalleh, W.; Saadaoui, E.; Khaldi, A.; Triki, S. and Nasri, N. (2011): The caper (Capparis L.): Ethnopharmacology, phytochemical and pharmacological properties. Fitoterapia, 82 (2): 93 -101.

[17] Sher, H. and Alyemeni, M. N., (2010) : Ethnobotanical and Pharmaceutical Evaluation of Capparis spinosa L, Validity of Local Folk and Unani System of Medicine. Journal of Medicinal Plants Research, 4 (17):1751-1756.

[18] Roohbakhsh, A., Parhiz, H., Soltani, F., Rezaee, R. and Iranshahi, M. (2014) : Neuropharmacological properties and pharmacokinetics of the citrusflavonoids hesperidin and hesperetin-A mini-review. Life Sci. 113 (1), 1–6.

[19] Roohbakhsh, A., Parhiz, H., Soltani, F., Rezaee, R. and Iranshahi, M., (2015): Molecular mechanisms behind the biological effects of hesperidin and hesperetin for theprevention of cancer and cardiovascular diseases. Life Sci., 124 : 64–74.

[20] Hosseinzadeh, H. and Nassiri-Asl, M. (2014): Review of the pro-tective effects of rutin on the metabolic function as an important dietary flavonoid. J Endocrinol Invest; 37:783-8.

[21] Sharma, S., Ali, A., Ali, J., Sahni, J.K. and Baboota, S. (2013): Rutin: therapeutic potential and recent advances in drug de-livery. Expert Opin Investig Drugs; 22:1063-79.

[22] Contini, M.; Baccelloni, S.; Massan, T.R. and Amelli, G. (2008): Extraction of natural antioxidants from hazelnutcc corylusavellanal shell and skin wastes by long maceration at room temperature. Food chem., 110, 659-669.

[23] Goupy, P.; Hugues, M.; Biovin, P. and Amiot, M.J. (1999): Antioxidant composition and activity of barley (Hordeum

vulgare) and malt extracts nd of isolated phenolic compounds. J.Sci. Food Agric., 79:1625-1634.

[24] Meier, J. and Theakston, R.D.G. (1986): Approximate LD50 determination of snake venoms using eight to ten experimental animals. Toxicon., 24(4): 395-401.

[25] Ji, Y.B. , Dong, F., Ma, D.B., Miao, J., Jin, L.N., Liu, Z.F.1 and Zhang, L.W. (2012): Optimizing the Extraction of Anti-tumor Polysaccharides from the Fruit of Capparis spionosa L. by Response Surface Methodology. Molecules , 17: 7323-7335.

[26] Patil, S.L., Somashekarappa, H., and Rajashekhar, K., (2012):EVALUATION OF THE RADIOPROTECTIVE ACTION OF RUTIN IN MICE EXPOSED TO GAMMA-RADIATION. International Journal of Biological & Pharmaceutical Research; 3(1): 12-18.

[27] Khedr, N. F. and Khalil, R.M. (2015): Effect of hesperidin on mice bearing Ehrlich solid carcinoma maintained on doxorubicin. Tumor Biol. ,36:9267–9275.

[28] Mazumdar, U.K.; Gupta, M.; Maiti, S. and Mukherjee, D. (1997): Antitumour activity of Hygrophila spinosa on Ehrlich ascites carcinoma and sarcoma-180 induced mice. Jndian J. Exp. Biol. 35: 473-477.

[29]Meliman, W.F.; Dairs, E.V.; Glover, F.L. and Rake, G.W. (1997): The submerged culture of mammalian cells. The Spinner Culture . J. Immunol., 79: 422-428.

[30] Satoh, K. (**1978):** Serum Lipid Peroxide in cerebrovascular disorders determined by a new colorimetric method. Clinic. Chimic. Acta., 90:37-43.

[31] Aebi, H. (1984): Catalase *in vitro.* Method Enzymol. 105:121-126.

[32] Casciola-Rosen, L.; Nicholson, D.W.; Chong, T.; Rowan, K.R.; Thornberry, N.A.; Miller, D.K. and Rosen, A. (1996): Apopain/CPP32 cleaves proteins that are essential for cellular repair: a fundamental principle of apoptotic death. J. Exp. Med., 183, 1957-1964.

[33] Reed, J.C. ,Miyashita, T. , Takayama, S., Wang, H.G. , Sato ,T ., Krajewski, S., Imesempe, C. , Bodrug, S., Kitada, S., and Hanada , M. (1996): BCL2 family proteins : regulator of cell death involved in the pathogenesis of cancer and resistance to therapy . J . Cell Biochem.: 60 : 23-32.

[34] Webster, D. (1974): Clin Chem. Acta., 53, 109-115.

[35] Young , D.S. **(1990):** Effect of drug on clinical laboratory tests. Third Edition:3;6-12.

[36] Karmen, A. (1955): J. Clin. Invest., 34,126.

[37] Patton, C.J. and Crouch, S.R. (1977): Determination of blood urea. Anal. Chem., |49, 464-469.

[38] Murray, R. (1984): Creatinine. Kaplan A *et al.* Clin Chem the C.V. Mosby Co. St Louis. Toronto. Princeton; 1261-1266 and 418.

[39] Wu, A.H.B. and Bowers, C.N. (1982): Evaluation and comparison of immunoinhibition and immunoprecipitation methods for differentiating MB from BB and macio forms of creatine kinase isoenzymes in patients and healthy individuals. Clin Chem.; 28:2017-2021.

[40] Bergmeyer, H.U. (1963): Methods of enzymatic analysis. New York: Academic Press; 1-1063.

[41] Culling, C. F. A. (1983): Handbook of histopathological and histochemical technique, 3rd edition .Butter Worth London , Boston: 214.

[42] Levesque, R. (2007): SPSS., Programming and Data Management: A Guide for SPSS and SAS Users, Fourth Edition, SPSS Inc., Chicago Ill.

[43] Vani, N.D., Jung, H.K., Ki-Cheol, H., Eun ,G.Y., Hyunah, C., Ae, N.P., Ghilsoo, N., and Kyung I.C. (2010): Novel 6-N-

arylcarboxamidopyrazolo[4,3-d]pyrimidin-7-on derivatives as potential anti-cancer agents. Bioorganic & Medicinal Chemistry Letters. 20, 1630–1633.

[44] Rahnavard, R. and Razavi, N. (2016): A review on the medical effects of *Capparis spinosa* L. Adv Herb Med; 2(1): 44-53.

[45] Pari,L. , Karthikeyan,A., Karthika,P., and Rathinam,A. (2015): Protective effects of hesperidin on oxidative stress,dyslipidaemia and histological changes in iron-inducedhepatic and renal toxicity in rats . Toxicology Reports 2 : 46–55.

[46] Barnes, S., Petti, S., Scully, C., Mortensen, A., Kulling, S.E., Schwartz, H., Rowland, I., Ruefer, C.E., Rimbach, G., Cassidy, A., Magee, P., Millar, J., Hall, W.L., Kramer Birkved, F., Sorensen, I.K., and Sontag, G. (2008): Nutritional genomics, polyphenols, diets, and their impact on dietetics. J. Am. Diet. Assoc. 108:1888–1895.

[47] Ganeshpurkar, A., and Saluja, A.K. (2017): The Pharmacological Potential of Rutin. Saudi Pharmaceutical Journal . 25 (2) : 149-164.

[48] Shamam, K. O. , Raid, M. H. A. and Sajid, H. G. (2014): Study The Role of Polyphenolic Extract of Capparis Spinosa L. Leaves As a Hypoglycemic agent. International Journal of Scientific & Engineering Research, Volume 5 (5) : 1561-1575.

[49] Liu , H.J., Yang, T., Cheng , X.M. and Wang , C.H. (2014): Comparative evaluation of anti-inflammatory and analgesic activities of various medicinal parts of capparis spinosa: a consideration of ecological environment and resource conservation. Indian Journal of Pharmaceutical Science & Research, 4 (1): 53-59 .

[50] Zanwar , A.A. , Badole , S.L. , Shende ,P.S. , Hegde, M.V. , Bodhankar, S.L. (2014): Polyphenol in human health and disease . ACADEMIC PRESS ; 2 : 989- 992.

[51] Kumari, P. and Jesudas, .(2014). Anticancer Activity Of Crude Extract Of Eucalyptus On MCF-7 Cell Line. L.Int.J.Bioassays. 3 (01): 1699-1707.

[52] Li , W.L., Yu, L., and Ji, Y.B. (2014): Study on chemical constituents of n-butanol extract of Capparis spinosa L. Asian J Chem, 26 (11): 3435-3437 .

[53] Kulisic-Bilusic, T., Schmöller, I., Schnäbele, K., Siracusa, L., and Ruberto, G. (2012): The anticarcinogenic potential of essential oil and aqueous infusion from caper (Capparis spinosa L.) Food Chem 132: 261–267.

[54] Roohbakhsh A., Parhiz H., Soltani F., Rezaee R. and Iranshahi M.(2015): Molecular mechanisms behind the biological effects of hesperidin and hesperetin for the prevention of cancer and cardiovascular diseases. Life Sci.;124 :64–74.

[55] Arau,´ j.o., Gonc, J.R., alves, P., and Martel, F. (2011): Chemopreventive effect of dietary polyphenols in colorectal cancer cell lines. Nutr. Res. 31 (2): 77–87.

[56] Lin, J.P., Yang, J.S., Lin, J.J., Lai, K.C., Lu, H.F., Ma, C.Y., Sai-Chuen Wu, R., Wu, K.C., Chueh, F.S., Gibson Wood, W., and Chung, J.G. (2012): Rutin inhibits human leukemia tumor growth in a murine xenograft model in vivo. Environ. Toxicol. 27 (8): 480–484.

[57] Chakraborty, S.O.M. Prakash,S., Dasgupta, A., Mandal, N. and Das, H.N. (2009): Correlation between

lipid peroxidation-induced TBARS level and disease severity in obsessive–compulsive disorder Neuro- Psychopharmacology and Biological Psychiatry 33(2), 363-366.

[58] ALAM ,B., MAJUMDER,R., AKTER, S. and LEE, S. (2015): Piper betle extracts exhibit antitumor activity by augmenting antioxidant potential. ONCOLOGY LETTERS 9: 863-868.

[59] Mayo Clinic. (2007). Antioxidants: Preventing Diseases, Naturally. Science Daily.

[60] Foud, F.M. (2005): Anti-tumor activity of tetrodotoxin extracted from the Masked Puffer fish Arothron diadematus. Egyptian Journal of Biology; 7:10-22.

[61] Hegazi A., Al Tahtawy R.H., Abd Allah, F., Abdou, A.M. (2014): Antitumor and Antioxidant Activity of Honey in Mice Bearing Ehrlich Ascites Carcinoma. Academic Journal of Cancer Research; 7: 208-214.

[62] Abd El Dayem, S., Foda, F., Helal, M., Zaazaa, A. (2010): The role of catechin against doxorubicin induced cardiotoxicity in Ehrlich Ascites Carcinoma Cells (EAC) bearing mice. Journal of American Science; 6(4).

[63] Perumal, P., Rajesh, V., Sekar, V., Gandhimathi , S., Sampathkumar, R., Jayaseelan, S., and Sandeep, P. (2010): Antitumor And Antioxidant Activity Of Artemisia nilagirica (Clarke) Against Ehrlich's Ascites Carcinoma In Swiss Albino Mice. Int .J .of Pharmacol ; ISSN: 1531-2976.

[64] Abou El Fatoh, M.F., Kamel, M.A., Hussein, M.A., and Nora, E.A. (2014): Anticarcinogenic and Antioxidant Effects of Propolis Aqueous extract against Ehrlich Ascites Carcinoma (EAC) Cells bearing Mice. International Journal of Pharma Sciences; 4: 606-610.

[65] Turgut NH, Kara H, Arslanbaş E, Mert DG, Tepe B, Güngör H. (2015): Effect of Capparis spinosa L. on cognitive impairment induced by D-galactosein mice via inhibition of oxidative stress. Turk J Med Sci 45: 1127–1136.

[66] Balakrishnan,A. and Menon, V.P. (2007): Effect of hesperidin on matrix metalloproteinases and antioxidant status during nicotine-induced toxicityToxicology 238 : 90–98.

[67] Bentli, R. , Ciftci, O. , Cetin, A. , Unlu, M. , Basak, N. and C¸ay, M.(2013): Oral administration of hesperidin, a citrus flavonone, in rats counteracts the oxidative stress, the inflammatory cytokine production, and the hepatotoxicity induced by the ingestion of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Eur. Cytokine Netw.; 24(2): 91-6.

[68] Savita, D . (2014): Anticancer Effect of Rutin Isolated from the Methanolic Extract of Triticum aestivum Straw in Mice. Med. Sci., 2: 153-160.

[69] Kalimuthu, S. and Se-Kwon, K. (2013): Cell Survival and Apoptosis Signaling as Therapeutic Target for Cancer: Marine Bioactive Compounds. Int J Mol Sci; 14: 2334-54.

[70] Czabotar, P.E. , Lessene, G. , Strasser, A. , Adams , J.M. (2014): Control of apoptosis by the BCL-2 protein family: implications for physiology and therapy. Nature Reviews Molecular Cell Biology; 15: 49-63.

[71] JI, Y.B. and YU, L. (2015): In vitro analysis of the role of the mitochondrial apoptosis pathway in CSBE therapy against human gastric cancer. EXPERIMENTAL AND THERAPEUTIC MEDICINE, 10: 2403-2409.

[72] Yu, L. , Yang ,J. , Wang, X. , Jiang, B. , Sun, Y. and Ji, Y. (2017): Antioxidant and antitumor activities of Capparis spinosa L. and the related mechanisms. Oncol Rep., 37(1):357-367.

[73] Chen, C.Y., Li, H., Yuan, Y.N., Dai, H.Q., Yang, B., (2013): Antioxidant activity and components of a traditional Chinese medicine formula consisting of Crataegus pinnatifida and Salvia miltiorrhiza. BMC Complement Altern. Med. 13, 99.

[74] Park, H.J,. Kim, M.J., Ha, E., and Chung, J.H. (2008): Apoptotic effect of hesperidin through caspase3 activation in human colon cancer cells, SNU-C4. Phytomedicine;15(1-2):147–51.

[75] Jagadeesan, G., and Kavitha, A.V. (2006): Recovery of phosphatase and transaminase activity of mercury intoxicated Mus musculus (Linn.) liver tissue by Tribulus terrestris (Linn.) (Zygophyllaceae) extract. Trop Biomed. 23(1):45-51.

[76] Gressner, O.A.; Weiskirchen, R. and Gressner, A.M. (2007): Biomarkers of liver fibrosis: Clinical translation of molecular pathogenesis or based on liver-dependent malfunction tests. Clin Chim Acta 381(2): 107–113.

[77] Pal, S., Sankar, B., Tathagata, C., Goutam, K. D., Tanya, D., and Gaurisankar, S. (2005): Amelioration of immune cell number depletion and potentiation of depressed detoxification system of tumor-bearing mice by curcumin. Cancer Detection and Prevention (29): 470–478.

[78] Aghel N, Rashidi I and Mombeini A.(2007): Hepatoprotective activity of Capparis spinosa root bark against CCl4 induced hepatic damage in mice. Iranian Journal of Pharmaceutical Research, 6(4): 285-290.

[79] Alnuaimy R.J.M. and Al-Khan H.I.A.(2012): Effect of aqueous extract of Capparis spinosa on biochemical and histological changes in paracetamol-induced liver damage in rats. Iraqi Journal of Vet Sciences, 26(1): 1-10.

[80]ABDEL-STTAR1,A.R. , KHALAF,M.M , ABOYOUSSEF,A.M. and ABOSAIF, A.A. (2017): AMELIORATIVE EFFECT OF HESPERIDIN ON CARBON TETRACHLORIDE INDUCED LIVER FIBROSIS IN RATS. Int J Pharm Pharm Sci, 9(7): 45-51

[81] Khan, R.A., Khan, M.R., Sahreen, S., (2012a): CCl4-induced hepatotoxicity: protective effect of rutin on p53, CYP2E1 and the antioxidative status in rat. BMC Complement. Altern. Med. 12, 178.

[82] Khan, R.A., Khan, M.R., Sahreen, S., (2012b): Protective effects of rutin against potassium bromate induced nephrotoxicity in rats. BMC Complement. Altern. Med. 12, 204.

[83] Hashem, M.A.; Mohamed, H.M. and Magda, S.H. (2004): Clinicopathological, pathological and biophysical studies on the effect of electromagnetic field on the Ehrlich tumour cells implanted in mice. Egypt J. Comp pathol. Clin pathol., 17 (2): 117-147.

[84] Tlili, N., Feriani, A., Saadoui, E., Nasri, N., and Khaldi, A. (2017): Capparis spinosa leaves extract: source of bioantioxidants with nephroprotective and hepatoprotective effects. Biomed. Pharmacother. 87, 171–179.

[85] Jain DP and Somani RS.(2015): Antioxidant Potential of Hesperidin Protects Gentamicin Induced Nephrotoxicity in Experimental Rats. Austin J Pharmacol Ther; 3(2).1071 .

[86] Alhoshani, A.R., Hafez,M.M. , Husain,S. , Al-sheikh, A.M. , Alotaibi,M.R. , Al Rejaie,S.S. , Alshammari,M.A. , Almutairi, M.M. and Al-Shabanah,O.A. (2017): Protective effect of rutin supplementation against cisplatin-induced Nephrotoxicity in rats. BMC Nephrology ; 18:194

[87] Turna . (2004) : Lactate dehydrogenase level predict pulmonary morbidity after lung resection for non-small lung cancer . Eur J Cardiothorac Surg , 26 : 483.

[88] Maghamiour N and Safaie N (2014): High Creatine Kinase (CK)-MB and Lactate Dehydrogenase in the Absence of Myocardial Injury or Infarction: A Case Report, J Cardiovasc Thorac Res.; 6: 69–70.

[89] Yu L. , Yang J. , Wang X. , Jiang B. , Sun Y. and Ji Y. (2017): Antioxidant and antitumor activities of Capparis spinosa L. and the related mechanisms. Oncol Rep., 37(1):357-367.

[90] Morcos N.Y.1 ; Osman S.A.2 ; Arbid M.S.3 ; El-Eraky W.I.3 ; Abdel-Ghaffar A.B.1 and Mohamed M.Kh. (2012): Hesperidin and low dose gamma irradiation alleviate rosiglitazone -induced cardiotoxicity in type 2 diabetic rats. J. Rad. Res. Appl. Sci., Vol. 5, No. 4, pp. 799 – 822.

[91]Abdel-Raheem, I.T. and Abdel-Ghany, A.A. (2009): Hesperidin alleviates doxorubicin-induced cardiotoxicity in rats. Journal of the Egyptian Nat. Cancer Inst., 21(2): 175-184.

[92] Chan, E., Hegde, A., Chen, X., (2009): Effect of rutin on warfarin anticoagulation and pharmacokinetics of warfarin enantiomers in rats. J. Pharm. Pharmacol. 61 (4), 451–458.

[93] Kalantari ,H. Foruozandeh ,H. Khodayar ,M.J. Siahpoosh ,A. Saki ,N. Kheradmand,P. (2017): Antioxidant and hepatoprotective effects of Capparis spinosa L. fractions and Quercetin on tert-butyl hydroperoxide- induced acute liver damage in mice. Journal of Traditional and Complementary Medicine 3 ; 1-8

[94] Gelen,V. Sengul, E. , Gedikli,S. , Atila,G. , Uslu, H. and Makav,M. (2017): The protective effect of rutin and quercetin on 5-FU-induced hepatotoxicity in rats Asian Pac J Trop Biomed; 7(7): 647–653.

[95] Kalantar, M. , Goudarzi, M . , Khodayar,M.J. , Babaei,J. , Foruozandeh,H., Bakhtiari,N., and Alidadi, H. (2016): Protective Effects of the Hydroalcoholic Extract of Capparis spinosa L. Against Cyclophosphamide-Induced Nephrotoxicity in Mice. Jundishapur J Nat Pharm Prod. 2016 November; 11(4):e37240.

[96] Jain, D.P. and Somani, R.S. (2015): Antioxidant Potential of Hesperidin Protects Gentamicin Induced Nephrotoxicity in Experimental Rats. Austin J Pharmacol Ther.; 3(2):1071.

[97] Alhoshani, A.R., Hafez,M.M. , Husain,S. , Al-sheikh, A.M. , Alotaibi,M.R. , Al Rejaie,S.S. , Alshammari,M.A. , Almutairi, M.M. and Al-Shabanah,O.A. (2017): Protective effect of rutin supplementation against cisplatin-induced Nephrotoxicity in rats. BMC Nephrology ; 18:194.

Table (1): Fractions of phenolic compounds for *capparis spinosa L* ethanolic extract by HPLC.

Peak No.	Identified compounds	Retention time (min)	Conc. (mg/100 g)
1	Galic acid	7.377	8.575
2	Pyrogallol	7.495	211.978
3	4-Amino-bebzoic	8.221	12.151
4	3-OH-Tyrosol	8.613	10.677
5	Protocatchuic	8.737	90.132
6	Catechein	8.883	96.842
7	Chlorogenic acid	9.486	132.638
8	Catechol	9.681	168.231
9	Epicatechein	9.912	79.869
10	Caffeine	10.141	46.262
11	P-OH-benzoic	10.286	20.758
12	Caffeic	10.565	65.282
13	Vanillic acid	10.680	108.247
14	P-Coumaric	12.031	41.215
15	Ferulic	12.316	169.089
16	Iso- ferulic	12.717	255.237
17	Reversetrol	13.113	29.723
18	e- vanillic	13.340	1232.783
19	Ellagic	13.412	294.416
20	Alpha- Coumaric	13.623	49.114
21	Benzoic	13.828	211.747
22	3,4,5-methoxy-cinnamic	14.254	38.158
23	Coumarin	14.341	38.981
24	Salicylic	14.483	355.066
25	Cinnamic	15.523	20.796

Table (2):Fractions of flavonoid compounds for *capparis spinosa* plant ethanolic extract by HPLC

Peak No.	Identified compounds	Retention time(min)	Conc. (mg / 100 g DW)
1	Luteolin	12.481	374.93
2	Narengin	12.579	197.15
3	Rutin	12.684	392.69
4	Hisperidin	12.830	537.92
5	Rosmarinic	13.049	23.094
6	Quercetrin	13.602	110.3
7	Quercetin	15.017	60.5
8	Hisperetin	15.621	71.41
9	Kampferol	16.287	11.34
10	Apigenin	16.501	8.8086
11	7-OH-ydroxyflavone	17.363	1.1214

Table (3): EAC volume (ml) in different studied groups

Groups	Group(2) Positive Control	Group (3) Preventive Capparis spinosa L extract	Group(4) Therapeutic Capparis spinosa extract	Group(5) Preventive rutin	Group(6) Therapeutic rutin	Group(7) Preventive hesperidin	Group (8) Therapeutic hesperidin
EAC volume(ml)	5.16 ± 0.16	1.08 ± 0.08	1.3± 0.09	2.49 ± 0.05	2.73 ± 0.06	1.84 ± 0.06	2.38 ± 0.09
%Change		79.07	74.8	51.7	47.09	64.3	55.4
P value		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Table (4): EAC count (x10^{6}cells/ml) in different studied groups

Groups	Group(2) Positive Control	Group (3) Preventive Capparis spinosa L extract	Group(4) Therapeutic Capparis spinosa extract	Group(5) Preventive rutin	Group(6) Therapeutic rutin	Group(7) Preventive hesperidin	Group (8) Therapeutic hesperidin
EAC count (x10^{6}cells/ml)	149.8 ± 2.80	37 ±0.93	40.2 ±0.96	63.3 ± 0.77	65 ± 0.66	48.9 ± 0.64	55.1 ±0.54
%Change		75.3	73.2	57.7	56.6	67.3	63.2
P value		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Table (5) :The effect of different treatments on Life Span Prolongation

Groups	Group(2) Positive Control	Group (3) Preventive Capparis spinosa L extract	Group(4) Therapeutic Capparis spinosa extract	Group(5) Preventive rutin	Group(6) Therapeutic rutin	Group(7) Preventive Hesperidin	Group (8) Therapeutic hesperidin
Survival days	9	20	19	18	16	15	16
life span T/C%	---	222.22	211.11	200	177.77	166.66	177.77
Increase in life span %	---	122.2	111.11	100	77.77	66.66	77.77

Table (6) : Effect of treatments on plasma and liver MDA level

Groups	Group(1) Negative Control	Group(2) Positive Control	Group (3) Preventive Capparis spinosa extract	Group(4) Therapeutic Capparis spinosa extract	Group(5) Preventive rutin	Group(6) Therapeutic rutin	Group(7) Preventive Hesperidin	Group (8) Therapeutic Hesperidin
PlasmaMDA (n mol /ml)	12.57± 0.18	43.33 ± 0.18	12.27 ± 0.14	14.29 ±0.18	18.63 ± 0.18	19.70 ± 0.25	12.42 ±0.18	15.98 ±0.11
%Change			-71.68	-67.02	-57	-54.53	-71.34	-63.12
P value		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Liver MDA (n mol /gtissue)	72.61± 0.99	155.90 ±3.85	72.02 ± 0.93	82.11 ± 1.46	76.87 ±0.99	82.90 ± 1.29	88.34 ±1.30	81.80 ±0.55
%Change			-53.8	-47.33	-50.69	-46.82	-43.33	-47.53
P value		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Table (7): Effect of treatments on plasma and liver catalase activity

Groups	Group(1) Negative Control	Group(2) Positive Control	Group (3) Preventive Capparis spinosa L extract	Group(4) Therapeutic Capparis spinosa extract	Group(5) Preventive rutin	Group(6) Therapeutic rutin	Group(7) Preventive Hesperidin	Group (8) Therapeutic Hesperidin
Plasma catalase (U/ L)	813 ±11.08	516 ± 11.54	810 ± 11.64	806 ± 9.32	798 ± 15.76	773 ± 11.08	811±12.66	772 ± 12.34
%Change			56.98	56.2	54.65	49.8	57.17	49.61
P value		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Liver catalase (U/g tissue)	9.76± 0.09	5.67 ± 0.29	9.28 ± 0.11	9.02 ± 0.15	8.60 ± 0.07	8.57 ±0.10	9.29 ±0.09	8.87 ±0.11
%Change			63.84	59.08	51.67	51.15	63.84	56.61
P value		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Table (8): Collective table of apoptosis parameters among different studied groups

Groups	Group(1) negative Control	Group(2) Positive Control	Group (3) Preventive Capparis spinosa L extract	Group(4) Therapeutic Capparis spinosa extract	Group(5) Preventive rutin	Group(6) Therapeutic rutin	Group(7) Preventive hesperidin	Group (8) Therapeutic hesperidin
Caspase-3 (ng/ml)	3.44 ± 0.05	2.006 ± 0.19	4.05 ±0.23	4.31 ±0.15	4.47 ±0.39	4.25 ±0.36	4.05 ±0.19	2.63 ±0.14
%Change			102.5	115.5	123.5	112.5	102.5	34
P value		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.05
Bcl2 (ng/ml)	2.57 ± 0.05	3.71 ± 0.05	2.47 ±0.09	2.73 ± 0.1	2.47 ± 0.08	2.55 ±0.12	2.88 ± 0.06	2.85 ± 0.04
%Change			33.4	26.4	33.4	31.2	22.3	23.2
P value		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Table(9):Collective table of serum hepatic markers among different studied groups

Groups	Group(1) Negative Control	Group(2) Positive Control	Group (3) Preventive Capparis spinosa L extract	Group(4) Therapeutic Capparis spinosa extract	Group(5) Preventive rutin	Group(6) Therapeutic rutin	Group(7) Preventive hesperidin	Group (8) Therapeutic hesperidin
ALT (U/L)	41.95 ± 0.35	144.50 ± 3.54	42.64 ± 1.4	62.16 ± 1.7	61.81 ± 1.2	71.86 ± 2.4	47.94 ± 2.35	66.14± 1.4
%Change			-70.49	-56.98	-57.22	-50.27	-66.82	-54.23
P value		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
AST(U/L)	81.6 ± 1.51	346.4 ±6.94	77.0 ± 2.72	107.4 ± 1.51	85.9 ± 15.76	161.3 ± 1.92	78.3 ±1.98	140.7 ±5.73
%Change			-77.77	-68.99	-75.2	-53.43	-77.39	-59.38
P value		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Albumin (g/dl)	2.71± 0.03	1.35 ± 0.05	2.64 ±0.05	2.77 ±0.05	2.65 ±0.01	2.43 ±0.05	2.65 ±0.02	2.47 ± 0.10
%Change			96.29	105.18	96.29	80	97.04	82.96
P value		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Table (10): Collective table of renal functional markers among different studied groups

Mice groups	Group(1) Negative Control	Group(2) Positive Control	Group (3) Preventive Capparis spinosa L extract	Group(4) Therapeutic Capparis spinosa extract	Group(5) Preventive rutin	Group(6) Therapeutic rutin	Group(7) Preventive Hesperidin	Group (8) Therapeutic hesperidin
Creatinine (mg/dl)	0.50 ± 0.02	1.27± 0.08	0.53 ± 0.02	0.40 ±0.03	0.66 ±0.01	0.54 ±0.03	0.55 ±0.03	0.53 ±0.02
%Change			-58.27	-68.5	-48.03	-57.48	-56.69	-58.27
P value		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Urea (mg/dl)	16.20 ± 0.46	27.50± 0.34	19.50 ±0.94	19.10 ±0.52	17.96 ±0.51	18.50 ±0.34	16.86 ±0.2	20.00 ±0.66
%Change			-29.09	-30.54	-34.69	-32.73	-38.69	-27.27
P value		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Table (11): Collective table of cardiac functional markers among different studied groups:

Mice Groups	Group(1) Negative Control	Group(2) Positive Control	Group (3) Preventive Capparis spinosa L extract	Group(4) Therapeutic Capparis spinosa extract	Group(5) Preventive rutin	Group(6) Therapeutic rutin	Group(7) Preventive Hesperidin	Group (8) Therapeutic hesperidin
CK – MB (U/L)	1.81 ± 0.17	16.76 ± 0.07	1.94 ± 0.11	2.28 ± 0.13	6.72 ± 0.28	9.65 ± 0.46	1.46 ± 0.06	8.82 ± 0.58
%Change			-88.42	-86.39	-59.9	-42.42	-91.29	-47.37
P value		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
LDH (U/L)	3396.3 ±86.49	4532.2 ±47.96	2997.1 ±139.93	2656.4 ±130.45	3151.2 ± 88.22	3366.3 ±140.73	3417.6 ±169.0	3355.5 ±85.60
%Change			-33.87	-41.38	-30.47	-25.72	-24.59	-25.96
P value		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

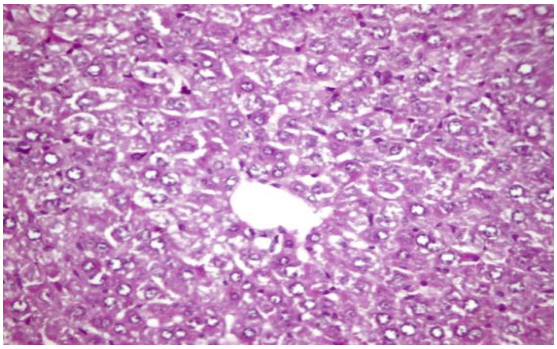

Fig (1): Photomicrograph of liver section from control negative group showing normal central vein, hepatocytes, and blood sinusoids, (H&E X 400)

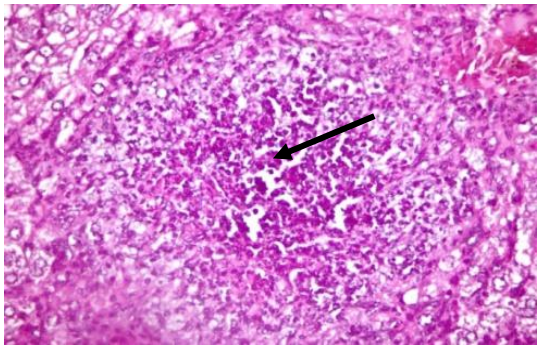

Fig (2): Photomicrograph of liver section from positive control group showing solid carcinoma of aggregated malignant cells infiltrated with inflammatory cells, (arrow), (H&E X 400).

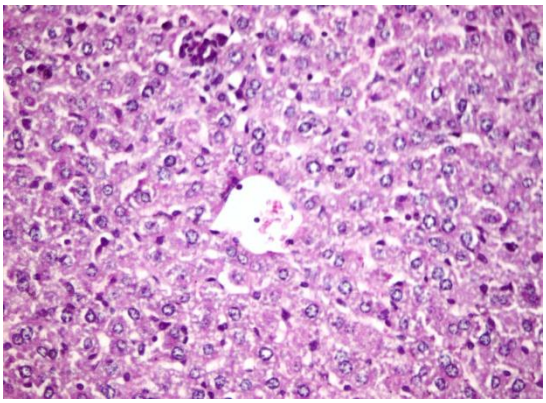

Fig (3):Photomicrograph of liver section from preventive plant group showing normal hepatic parenchyma; hepatocytes, blood sinusoids, and Portal tract (H&E X 400).

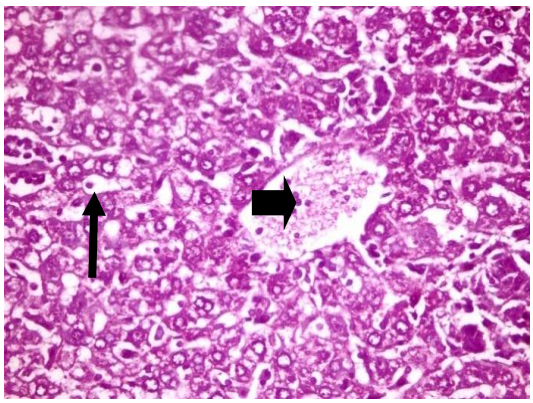

Fig (4): Photomicrograph of liver section from therapeutic plant group showing massive hepatocytes vacuolation with prepheral nucleous (arrows), the central vein is permeated with leucocytic cells (arrow head) (H&E X 400).

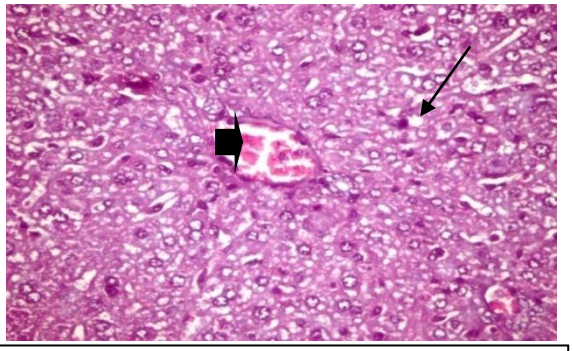

Fig (5): Photomicrograph of liver section from preventive hesperidine group showing diffuse hepatocytes vacuolations (arrows) and congested central vein (arrow head), (H&E X 400).

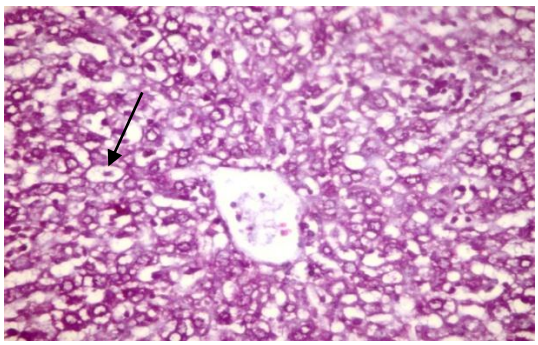

Fig (6): Photomicrograph of liver section from therapeutic hesperidine group showing massive hepatocytes vacuolation with prepheral nucleous (arrows), (H&E X 400).

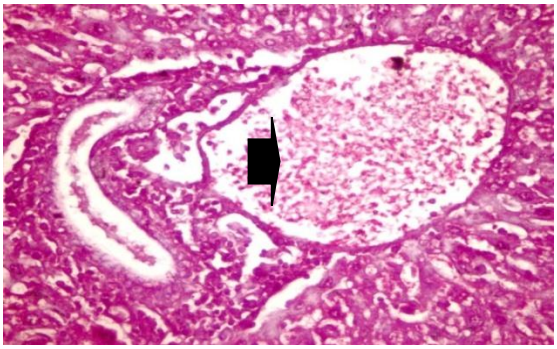

Fig (7): Photomicrograph of liver section from preventive rutin Live group showing congestion of the hepatoportal blood vessel (arrow head) (H&E X400).

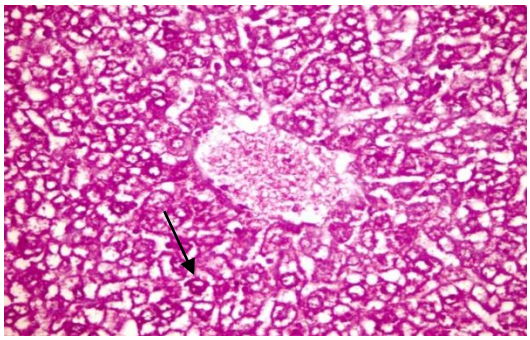

Fig (8): Photomicrograph of liver section from therapeutic rutin group showing massive hepatocytes vacuolation with prepheral nucleous (arrows), (H&E X 400).

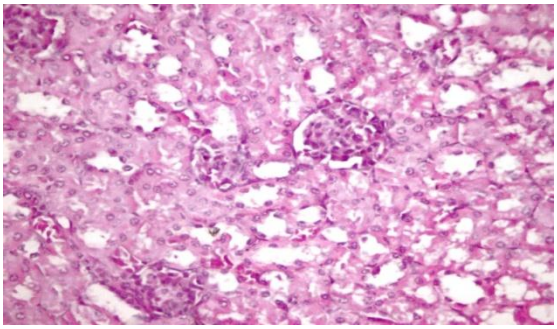

Fig (9):Photomicrograph of kidneys from control negative group showing normal renal parenchyma; Renal Glomeruli and renal tubules (H&E X 400).

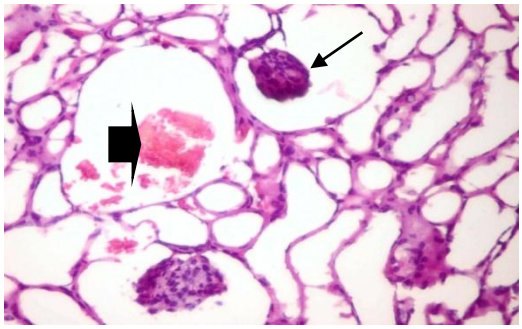

Fig (10): Photomicrograph of kidneys from control positive group showing severe glomerular tuft necrosis (arrow) with poor vascularization (arrow head), (H&E X 400).

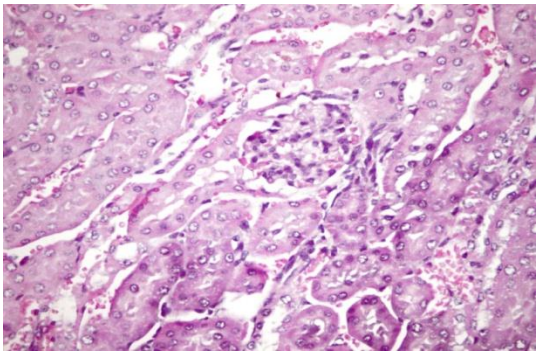

Fig (11): Photomicrograph of kidneys section from preventive plant group showing normal renal parenchyma; Renal Glomeruli and renal tubules (H&E X 400).

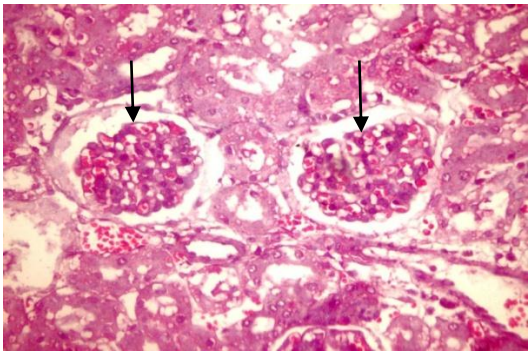

Fig (12):Photomicrograph of kidneys section from therapeutic plant group showing congestion of the glomerular capilleries (arrow) (H&E X 400).

Biochemistry letters, 13 (5)2018, Pages: (54-78)

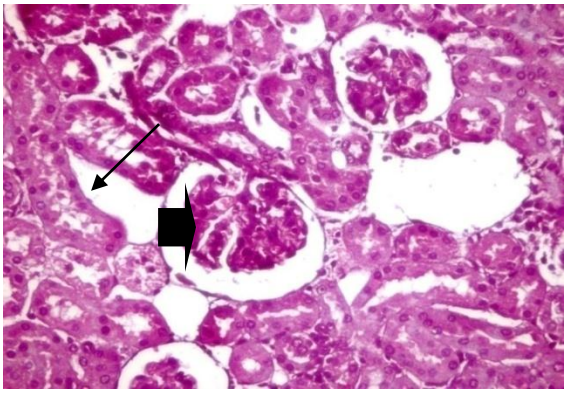

Fig (13):Photomicrograph of kidneys section from preventive rutin group showing normal renal glomeruli (arrow head) and renal tubules (arrows), (H&E X 400).

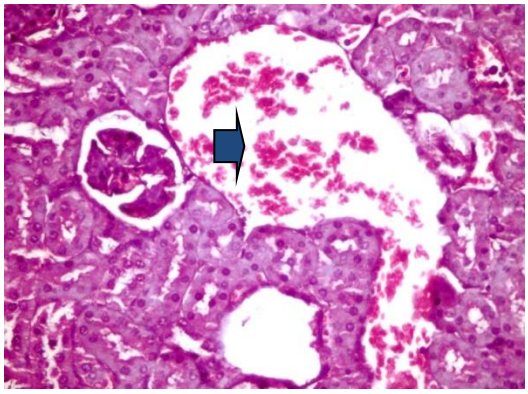

Fig (14) :Photomicrograph of kidneys section from therapeutic rutin group showing congestion of the interstitial blood vessel (arrow head), (H&E)

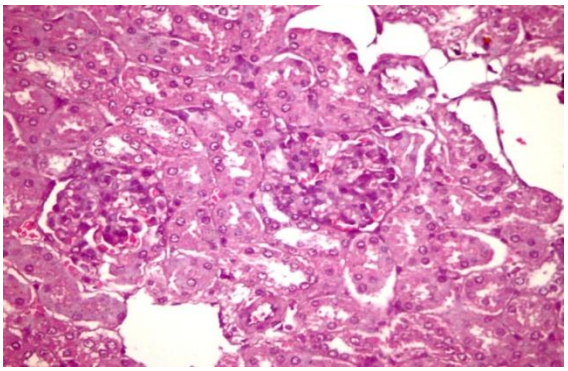

Fig (15): Photomicrograph of kidneys section from preventive hesperidin group showing normal renal glomeruli and renal tubules (H&E X 400).

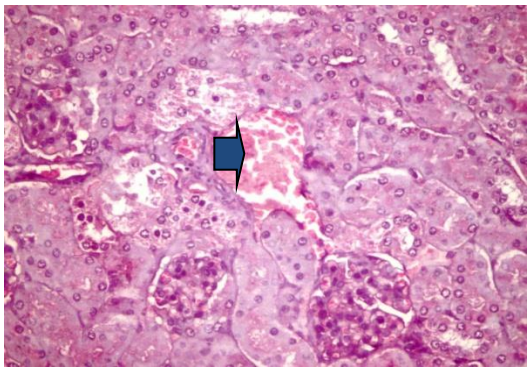

Fig (16): Photomicrograph of kidneys section from therapeutic hesperidin group showing congestion of the peritubular blood capilleries (arrow head), (H&E X 400).