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Anticancer activity and antioxidant potential of Crocus Sativus .L(saffron) aqueous extract against Ehrlich Ascites Carcinoma cells in Swiss albino mice.

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ABSTRACT

Cancer is considered one of the most common causes of morbidity and mortality worldwide. Many chemopreventive agents have been associated with antiproliferative and apoptotic effects on cancer cells because of their high antioxidant activity. The current study was aimed to evaluate the anti-tumor, antioxidant and anti-inflammatory effects of Saffron aqueous extract and its active components against Ehrlich Ascites tumor (EAC). A total of 120 female mice were randomly divided into eight groups. The antitumor effect was assessed by evaluating tumor volume, tumor cell count , survival time and increase in life span of EAC bearing mice. The effect of Crocus Sativus .L (saffron) aqueous extract , Crocin and Safranal on different parameters (liver enzymes , kidney function , cardiac markers and antioxidant parameters). These results indicated that Saffron , Crocin and Safranal is a promising protective agents against EAC cells. **.** Crocus Sativus L (saffron) aqueous extract (100mg/kg), Crocin(10 mg/kg) and Safranal(1/10 LD_{50}) (0.188 ml/kg) showed significantly decreased ($p<0.0001$) in the volume of the EAC and in the count of EAC cells and increase the life span of EAC bearing mice. Also, showed significantly increased in antioxidant levels, decreased the lipid peroxidation(oxidative stress) as compared to positive control group . We found that The treatment of Crocus Sativus . L (saffron) aqueous extract , Crocin and Safranal significantly reduced the liver enzymes, cardiac markers and reduced elevated levels of Urea, Uric acid and Creatinine in positive control as compared with negative control and also increase the level of albumin as compared with positive control group . our data was confirmed by histopathological examination.

INTRODUCTION

Cancer refers to any one of a large number of diseases characterized by the development of abnormal cells that divide uncontrolled division and have the ability to infiltrate and destroy normal tissue. Cancer often has the ability to spread throughout your body [1] . Breast cancer is the malignant neoplasia with the higest incidence in women worldwide .Chronic oxidative stress and inflammation have been indicated ascamajor mediators during carcinogensis and cancer progression . [2] . Ehrich ascites tumor was chosen as arapidly growing expiremental tumor model where various expiremental designs for anticancervagents can be applied [3]

Medicinal plants contain various phytochemicals that are used for treatment of various diseases. Antioxidants present in the plants play an essential role in protecting the cells and tissues against damage caused by reactive oxygen species .There are numerous medicinal plants which possess multiple health-promoting effects [4]. Nowadays, medicinal plants are known as an important source of bioactive natural products such as phenols and flavonoids [5] . Medicinal plants used as folk medicine have strong antitumour activity against the Ehrlich ascites carcinoma (EAC) cell line [6]

Saffron is a dietary spice derived from the flowers of Crocus sativus - Iridaceae. There are more than 150 different compounds in saffron comprising carbohydrates, polypeptides, lipids, H_2O, minerals and vitamins. Beside these constituents, Saffron has four main bioactive components including crocin ($C_{44}H_{64}O_{24}$), crocetin ($C_{20}H_{24}O_4$), picrocrocin ($C_{16}H_{26}O_7$) and safranal ($C_{10}H_{14}O$). Picrocrocin is another constituent of saffron and has a bitter taste. The aroma and odor of saffron is due to the presence of safranal which is the metabolite of picrocrocin [7]. Saffron as a well-known spice extracted from the flower of Crocus sativus L. has been used in traditional medicine for treating severaldiseases including depression, cardiovascular disease,asthma, insomnia, digestive ailments and some others [8]. The beneficial effects of saffron, Crocus sativus L. stigma, are due to a number of ingredients contained within this spice, including safranal, crocetin and crocins [9]

Crocin(Crocetin di-gentiobiose ester) is the chemical constituent isolated from the Saffron, the dried trifid stigma of the plant Crocus sativus L. . It is found to be effective as antiproliferative, anti-oxidant, learning and memory enhancer, brain neurodegenerative disorder, , biosurfactant, alzheimer disorder [10] .

Safranal (2, 6, 6-trimethyl-1, 3-cyclohexadiene-1-carboxaldehyde), a monoterpene aldehyde, is a main constituent of the essential volatile oil responsible for saffron odor and aroma. [11]. Safranal has shown different pharmacological activities such as reduction of subacute toxicity of diazinon, antianxiety, anticonvulsant, antidepressant, antioxidant and anticancer activities [12].

Materials:

Plant material

Crocus Sativus L (Saffron) is obtained from the flowers (dried, dark red stigmata) of Crocus sativus L. (Iridaceae) from Harraz from cairo , Egypt and a sample of it was identified by cairo University herbarium , faculty of science , cairo University .

Chemicals

Crocin and Safranal were purchased from sigma Aldrich company, Trypan blue dye was purchased from El- Gomhouria Company and (TNF-α) from New test company, USA. All other kits used in this

study were purchased from Biodiagnostic 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) 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 of 0.2 ml of freshly drawn ascites fluid , each inoculum contained approximately 2.5x 10^6 cells. This process was repeated every 10 days for keeping the cell line available throughout the present study[3].

Methods:

Plant extraction:

Crocus Sativus L (saffron) aqueous extract was prepared according to the method [13] .

Toxicity study (LD50):

Approximate **LD_{50}** of aqueous extract of saffron was determined according to method[14]. the

Dose response curve

Dose response curve of crocin in mice was determined according to method[15].

HPLC analysis :

High performance liquid chromatography (HPLC) was used to identify the phenolic, flavonoid and Beta carotene compounds present in the extracts for Crocus Sativus L (Saffron) aqueous extract also HPLC of both , crocin and safranal . Retention time and peak area were used to calculation of phenolic and flavonoid, Beta carotene compounds concentration by the data analysis of HEWLLET packared software [16].

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**: mice injected (i.p) with sterile saline solution (0.9 NaCl) (0.2ml) day after day for 9 days.

Group 2: **Positive Control:** mice injected (i.p) as single dose with EAC cells (2.5 x10^6 cell/ml),

Group 3:. Preventive saffron Group: mice were injected (i.p) with saffron aqueous extract (100mg/kg bw) [17] before EAC cells (2.5 x10^6 cell/ml) injection then treated day by day for 9 days .

Group 4: **Therapeutic saffron Group**: mice injected(i.p) with EAC cells (2.5 x10^6 cell/ml) then Saffron aqueous extract(100mg/kg bw) [17] treatments day by day for 9 days .

Group 5: **preventive crocin Group**

mice were injected(i.p) with Crocin (10mg/kg bw) before EAC cells (2.5 x10^6 cell/ml) injection then treated day by day for 9 days .

Group 6: **Therapeutic crocin Group**

mice injected(i.p) with EAC cells (2.5 x10^6 cell/ml) then crocin(10mg/kg bw) treatments day by day for 9 days.

Group 7: **preventive safranal Group**

mice were injected(i.p) with safranal (1/10 LD_{50}) (0.188 ml / kg) [18] before EAC cells

(2.5 x10^6 cell/ml) injection then treated day by day for 9 days .

Group 8: **Therapeutic safranal Group**

mice injected(i.p) with EAC cells (2.5 x10^6 cell/ml) then Safranal (1/10 LD_{50}) (0.188 ml / kg) [18] treatments day by day for 9 days.

Samples collection:

At the end of the experiment; the blood samples were collected. in empty tubes to get serum by centrifugation at 4000 rpm for 20 min. these Tubes were used for various biochemical measurements.Liver tissues were excised from each mouse , liver tissue from each group was collected for tissue homogenate preparation . The liver, lung and kidney tissues were excised and fixed in 10% buffered formaline for histopathological evaluation.

Viability and life span prolongation

The viability of EAC cells was determined by the Trypan Blue Exclusion the Method [19] . Life span calculation was carried according to the method [20] .

Biochemical parameters:

Determination of TNF- α

TNF- α was determined by using a commercial ELISA kit according to the method [21]

Assessment of serum liver function tests

Serum samples were screened for liver function tests including Albumin according to [22] Alanine Aminotransferase (ALT) according to [23], Aspartate Aminotransferase (AST) according to [24], Alkaline phosphatase (ALP) according to [25].

Assessment of kidney function tests

Serum samples were screened for kidney function tests including urea according to by [26] ,creatinine according to[27] and uric acid according to [28] by using a commercial kit derived from Bio Diagnostic Company

Assessment of heart function tests

Serum Creatine Kinase MB (CK-MB) activity measure according to the method [29] and Lactate dehydrogenase (LDH) activity was measured according to the method . [30]

Determination of antioxidant parameters

TAC,CAT,SOD,GPX and MDA were estimated in liver homogenate by using a commercial kits derived from Biodiagnostic Company, Egypt according to. [31,32,33,34,35]

Histological examination:

Liver, kidney and lung were dissected out and fixed instantaneously in 10% formalin for 24 hours. The specimens were washed in tap water, dehydrated in ascending grades of ethanol, cleared in xylene, embedded in paraffin wax (melting point 55-60 ^oC). Sections of 6mm thickness were prepared and stained with Haematoxylin and eosin [36].

Statistical analysis

All statistical analyses were done by a statistical for social science package "SPSS" version 14.0 for Microsoft Windows, SPSS Inc. [37]. Numerical data were expressed as mean ± SE. The levels of markers were analyzed by ANOVA.

3. Results:

Total Crocus Sativus L (saffron) aqueous extract yield

Crocus Sativus L (saffron) stigma (15g) after undergoing extraction, yielded (3 gram) of aqueous extract (thick red paste) (20%) of crude extract .

Toxicity study :

The acute toxicity was estimated by intraperitoneal injection of the saffron aqueous extract to determination of median lethal dose (LD_{50}),by approximate method , all doses up to 500 mg/kg mice consider be safe and where no mortality was observed which suggests that saffron aqueous extract is consider a safe mixture While, The intraperitoneal LD_{50} value of crocin in female mice is up to 3 g/ Kg . [38]. and The intraperitoneal LD_{50} value of safranal in female mice is 1.88 ml/Kg. [39].

Dose response curve of crocin :

The most effective dose was found to be 10 mg/kg for crocin Fig .(1) as it reduced the number of EAC cells in treated mice group to 62% of EAC cells in positive control mice group as shown in Table .(1)

Identification of phenolic, flavonoid and beta carotene compounds for Crocus Sativus L (saffron) plant aqueous extract by using HPLC:

Total phenolic compounds of Crocus Sativus L (saffron) aqueous extract were identified by HPLC. Data are illustrated in table (2) , figure(2) showed that twenty four phenolic compounds were identified in plant .Also HPLC results reflected that tweleve flavonoid compounds were identified in plant as shown in table (3) figure(3). Also HPLC results reflected that Betacarotene compounds were identified in plant as shown in table (4) figure(4). High performance liquid chromatography (HPLC) was used to separate and identificate crocin and safranal . Safranal and crocin identified by HPLC tables (5), (6) figure(5), (6)

Effect of saffron aqueous extract ,crocin and safranal on volume, viable EAC cell count and life span prolongation results in all studied groups :-

The tumor bearing animals showed a marked increase in tumor volume (4.16±0.724) ml. On treatment with saffron aqueous extract ,crocin and safranal there is amarked decreased by 72.35% in preventive group of saffron aqueous extract ,by 70.9% in therapeutic group of saffron aqueous extract ,by 91.82% in preventive group of crocin, by 74.75% in therapeutic group of crocin , by 59.85%in preventive group of safranal and by 62.98% in therapeutic group of safranal respectively compared to positive control group in table (7) ($p<0.0001$).The mean of EAC cells count in the positive control group was found to be (153.4±7.96102) $x10^6$ cell/ml , which significantly decreased by 58.27% in preventive group of saffron ,by 53.9% in therapeutic group of saffron ,by 79.46% in preventive group of crocin, by 67.73% in therapeutic group of crocin , by 52.02 %in preventive group of safranal and by 57.82% in therapeutic group of safranal respectively compared to positive control group in table (8) .

So that our results demonstrated that different treatments with saffron aqueous extract, crocin and safranal display antitumor groups showed significant increase in the life span prolongation to15 days by 36.36%(T/C ratio= 136.36%)in preventive group of saffron,to 18 days by 63.6% (T/C ratio= 163.6%)in therapeutic group of saffron , to 27 days by 145.45%(T/C ratio= 245.45%)in preventive group of crocin, and to 14 days by 27.27%(T/C ratio= 127.27%)in preventive group of safanal ,to 24 days by 118.18% (T/C ratio= 218.18%)in therapeutic group of crocin, to 14 days by 27.27%(T/C ratio= 127.27%)in preventive group of safanal and to 20 days and by 81.81% (T/C ratio= 181.81%)in therapeutic group of safranal respectively compared to the positive control group in table (9).

Effect of saffron aqueous extract ,crocin and safranal treatments on TNF-α:

The mean values of TNF-α level in EAC were found to be 688±10.43(Pg/ml)in

negative control group.The mean values were highly significantly increased in the positive control group to 2546±78.02 (pg/ml) ($p<0.0001$). the mean values of TNF-α were highly significantly decreased to be 643±24.64 (pg/ml) in preventive group of saffron ,916.9±14.31 (pg/ml) in therapeutic group of saffron ,874.5±33.34 (pg/ml) in preventive group of crocin, 781±14.59 (pg/ml) in therapeutic group of crocin , 542.7±25.36 (pg/ml) in preventive group of safranal and 524.2±13.73 (pg/ml) in therapeutic group of safranal respectively compared to positive control group by ($p\leq0.0001$) .Also, The mean values were highly significantly increased in the positive control group to 147.5±7.213 (pg/ml) by ($p<0.0001$). the mean values of TNF-α were highly significantly decreased to be 98.47±3.171 (pg/ml) in preventive group of saffron , 95.2±2.33 (pg/ml) in therapeutic group of saffron ,62.31±3.384 (pg/ml) in preventive group of crocin, 97.14±1.819 (pg/ml) in therapeutic group of crocin , 98.39±4.206 (pg/ml) in preventive group of safranal and 80.83±3.998 (pg/ml) in therapeutic group of safranal respectively compared to positive control group by ($p\leq0.0001$)in table ( )

Effect of saffron aqueous extract ,crocin and safranal treatments on antioxidant assays

The mean values of TAC in liver homogenate samples were signifiantly decreased to (0.139± 0.01) mM/L in the positive control group compared to the negative control group (0.254± 0.0001) mM/L $p<0.0001$ while the mean TAC level were significantly decrease to(0.25±0.008) mM/L in preventive group of saffron aqueous extract, to(0.363± 0.0001) mM/L in therapeutic group of saffron aqueous extract , to (0.233± 0.003) mM/L in preventive group of crocin , to (0.203± 0.004) mM/L in therapeutic group of crocin , to (0.195±0.0001) mM/L in preventive group of safranal and to (0.191±0.005) mM/L in therapeutic group of safranal ($p<0.0001$).

Also, The mean values of CAT activites in liver homogenate sample were signifiantly decreased to (3.06± 0.056) u/g tissue in the positive control group compared to the negative control group (8.87± 0. 123) u/g tissue .while the mean CAT activity was significantly decrease to(6.64±0.155) u/g tissue in preventive group of saffron aqueous extract, to(4.65± 0.108) u/g tissue in therapeutic group of saffron aqueous extract , to (9.42± 0.087) u/g tissue in preventive group of crocin , to (8.29± 0.186) u/g tissue in therapeutic group of crocin , to (9.25±0.129) u/g tissue in preventive group of safranal and to (9.02±0.09) u/g tissue in therapeutic group of safranal ($p<0.0001$).The mean values of SOD acticity in liver homogenate samples were signifiantly decreased to (165.0±1.136) u/g tissue in the positive control group compared to the negative control group (265.8±5.849) u/g tissue while the mean SOD activity was significantly decrease to(219.2±13.8) u/g tissue in preventive group of saffron aqueous extract, to(304.7± 4.772) u/g tissue in therapeutic group of saffron aqueous extract , to (299.2± 8.583) u/g tissue in preventive group of crocin , to (258.5± 6.673) u/g tissue in therapeutic group of crocin , to (230.1±5.842) u/g tissue in preventive group of safranal and to (229.4±3.115) u/g tissue in therapeutic group of safranal ($p<0.0001$).

The mean values of GPX in liver homogenate samples were signifiantly decreased to (96.9±2.614) u/g tissue in the positive control group compared to the negative control group (416.5±9.637) u/g tissue .while the mean GPX activity was significantly decrease to(800±8.332) u/g tissue in preventive group of saffron aqueous extract, to(507.8± 11.2) u/g tissue in therapeutic group of saffron aqueous extract , to (843.5± 10.57) u/g tissue in preventive group of crocin , to

(327.6± 3.177) u/g tissue in therapeutic group of crocin , to (501.5±11.82) u/g tissue in preventive group of safranal and to (357.6±10.06) u/g tissue in therapeutic group of safranal (p<0.0001).

While The mean values of MDA level in liver homogenate samples were signifiantly decreased to (12.73±0.23) n mol/g tissue in the positive control group compared to the negative control group (5.07±0.173) n mol/g tissue . the mean MDA level were significantly decrease to(8.14±0.394) u/g tissue in preventive group of saffron aqueous extract, to(7.53± 0.179) n mol/g tissue in therapeutic group of saffron aqueous extract , to (4.568± 0.1355) n mol/g tissue in preventive group of crocin , to (9.087± 0.2104) n mol/g tissue in therapeutic group of crocin , to (7.12±0.359) u/g tissue in preventive group of safranal and to (9.46±0.428) n mol/g tissue in therapeutic group of safranal (p<0.0001) in table (7).

Effect of saffron aqueous extract ,crocin and safranal treatments on serum hepatic markers

Our results revealed that The mean of ALT activity in serum samples were signifiantly incresased to (77.86± 1.26) U/ L (245.27%) in the positive control group compared to the negative control group (22.55± 0.27) U/L p<0.0001 while the mean ALT activiity was significantly decrease to(40.78±5.27)U/L 80.84% in preventive group of saffron aqueous extract, to(58.92± 2.36) u/ L 16.12% in therapeutic group of saffron aqueous extract , to (33.32±1.79) U/L 47.76% in preventive group of crocin , to (39.43±4.27)U/L 74.85% in therapeutic group of crocin , to (43.85±1.39) U/L 94.45% in preventive group of safranal and to (40.96±1.01) U/L 81.64% (p<0.0001).The mean values of AST in serum samples were signifiantly incresased to (336.02± 62.89) U/ L (92.01%) in the positive control group compared to the negative control group (190.62± 10.52) U/L.while the mean AST activiity was significantly decrease to(263.91±49.09)U/L 38.44% in preventive group of saffron aqueous extract, to(301.9± 76.46) U/L 58.377% in therapeutic group of saffron aqueous extract , to (247.86± 25.58) U/L 30.02% in preventive group of crocin , to (262.47±23.15)U/L 37.69% in therapeutic group of crocin , to (257.6±27.94) U/L35.13% in preventive group of safranal and to (309.91±37.52) U/L 62.58% (p<0. 001). The mean values of Albumin in serum samples were signifiantly decreased to (2.288± 0.15) U/ L (-40.26%) in the positive control group compared to the negative control group (3.83±0.119) U/L p<0.0001 while the mean ALB level was significantly decrease to(2.77±0.08)U/L -27.67% in preventive group of saffron aqueous extract, to(2.92± 0.11) U/L -23.75% in therapeutic group of saffron aqueous extract , to (3.7± 0.108) U/L -3.93% in preventive group of crocin , to (3.5± 0.17)U/L -8.61% in therapeutic group of crocin , to (3.028±0.18) U/L-21.14% in preventive group of safranal and to (3.62±0.127) U/L 5.48% (p<0. 001).

The mean values of ALP activity in serum samples were signifiantly incresased to (427.82±12.89) U/ L in the positive control group compared to the negative control group (148.9±2.186) U/L p<0.0001 while the mean ALP activiity was significantly decrease to(140.08±0.702)U/L in preventive group of saffron aqueous extract, to(174.34±1.88) U/L in therapeutic group of saffron aqueous extract , to (174.92±2.46) U/L in preventive group of crocin , to (193.92±1.29)U/L in therapeutic group of crocin , to (174.24±8.03) U/L in preventive group of safranal and to (187.32±2.33) U/L in therapeutic group of safranal (p<0.0001). in table (8)

Effect of saffron aqueous extract ,crocin and safranal treatments on renal functional markers

Our results showed the mean values of urea in serum samples were signifiantly incresased to (98.03± 4.74) mg/dL in the positive control group compared to the negative control group (35.87±2.17) mg/dL .while the mean urea level was significantly decrease to(37.92±2.46) mg/dL in preventive group of saffron aqueous extract, to(90.07± 2.09) mg/dL in therapeutic group of saffron aqueous extract , to (80.4± 3. 08) mg/dL n preventive group of crocin , to (77.24± 3.06) mg/dL in therapeutic group of crocin , to (97.08±1.79) mg/dL in preventive group of safranal and to (63.37±2.65) mg/dL in therapeutic group of safranal ($p<0.0001$)

The mean values of creatinine in serum samples were signifiantly incresased to (0.848± 0.038) mg/dL in the positive control group compared to the negative control group (0.349±0.021) mg/dL while the mean creatinine level was significantly decrease to(0.458±0.021) mg/dL in preventive group of saffron aqueous extract, to(0.46± 0.045) mg/dL in therapeutic group of saffron aqueous extract , to (0.351± 0.014) mg/dL n preventive group of crocin , to (0.48± 0.026) mg/dL in therapeutic group of crocin , to (0.533±0.026) mg/dL in preventive group of safranal and to (0.725±0.020) mg/dL in therapeutic group of safranal ($p<0.0001$)

The mean values of Uric acid in serum samples were signifiantly incresased to (5.57± 0.16) mg/dL in the positive control group compared to the negative control group (3.5± 0.19) mg/dL .while the mean uric acid level was significantly decrease to(4.32±0. 12) mg/dL in preventive group of saffron aqueous extract, to(4.07± 0.11) mg/dL in therapeutic group of saffron aqueous extract , to (3.58± 0. 15) mg/dL n preventive group of crocin , to (4.13± 0.09) mg/dL in therapeutic group of crocin , to (5.02±0. 28) mg/dL in preventive group of safranal and to (5.33±0.37) mg/dL in therapeutic group of safranal ($p<0.0001$) in table (9)

Effect of saffron aqueous extract ,crocin and safranal treatments on cardiac functional markers

Our results indicated that The mean values of CKMB activity in serum samples were signifiantly incresased to (21.38± 0.26) U/L in the positive control group compared to the negative control group (4.58± 0.50) U/L .while the mean CKMB activiity was significantly decrease to(13.90±0. 25) U/L in preventive group of saffron aqueous extract, to(17.44± 0.29) U/L in therapeutic group of saffron aqueous extract , to (5.57± 0. 25) U/L in preventive group of crocin , to (8.62± 0.25) U/L in therapeutic group of crocin , to (14.7±0. 11) U/L in preventive group of safranal and to (7.69±0.17) U/L in therapeutic group of safranal ($p<0.0001$)

The mean values of LDH activity in serum samples were signifiantly incresased to (4748± 254.98) U/L in the positive control group compared to the negative control group (2158.4± 9.6) U/L .while the mean LDH activiity was significantly decrease to(2181±12.22) U/L in preventive group of saffron aqueous extract, to(2286.4± 12.64) U/L in therapeutic group of saffron aqueous extract , to (2178.6± 7.40) U/L in preventive group of crocin , to (2348.1± 21.74) U/L in therapeutic group of crocin , to (2902.2±84.5) U/L in preventive group of safranal and to (2538.1±34.09) U/L in therapeutic group of safranal ($p<0.0001$). in table (10)

Histological results

Histological studies revealed that liver and kidney differ from negative control group compared with Positive control mice . Treatment with saffron aqueous

extract ,crocin and safranal reduced most of the pathological alterations induced by EAC cells in mice figures (7), (8)

4. Discussion

Cancer is the second largest cause of death worldwide [40]. Although great advancements have been made in the treatment and control of cancer progression, significant deficiencies and room for improvement remains. A number of undesired side effects sometimes occur during chemotherapy. Natural therapies, such as the use of plant-derived products in cancer treatment, may reduce adverse side effects. There are many natural products including phytochemicals and dietary compounds from vegetables, plants, spices and herbs that have been used for the treatment of cancer throughout history due to their safety, low toxicity, and general availability [41].. Medicinal plants are important sources of biologically active natural products which due to their curative properties have been studied for many years . [42]. Natural products have long been used to prevent and treat diseases including cancers and might be good candidates for the development of anti-cancer drugs. [43].. Saffron, acommonly-used spice and food additive, is known for its anti-cancer and anti-tumor properties.

The present study was aimed to evaluating the antitumor potential of saffron aqueous extract ,crocin and safranal in EAC bearing mice . It was observed that treatment with them increased the life span of EAC bearing mice by reducing the viability of EAC cells and decreasing the tumor volume . The anticancer activity of saffron aqueous extract is probably due to presence of B- carotene flavonoid and phenolic compounds in extract. Furthermore, flavonoids compounds have been shown to possess antimutagenic effect and anticancer properties [44]..Phenolic compounds such as phenolic acids and flavonoids are reported to be involved in various biochemical activities like antioxidant, antimicrobial, antithrombotic, antiartherogenic, antiinflammatory, anticarcinogenic and antimutagenic [45]. Superoxide dismutase (SOD) is the only enzyme that disrupts superoxide radicals and is presented in all cells with high amounts {47}. It protects the cells against superoxide and hydrogen peroxide mediated lipid peroxidation. The malignant cells of different cancer types exhibit heterogeneity in the levels of oxidative stress associated with various expressive levels of SOD and other antioxidant enzymes [46]. Superoxide dismutase (SOD) and catalase are involved in the clearance of superoxide and hydrogen peroxide radicals [47].

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 [48].

Catalase protects the tissue from highly reactive hydroxyl radicals by decomposing the hydrogen peroxide. 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 [49].. The administration of saffron aqueous extract 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 saffron aqueous 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. [50]. There is a possibility that saffron extract acts as free radical scavenger which leads to the activation of antioxidative enzymes.Saffron components were shown to down-regulate several pro-

inflammatory cytokines' expression. The effects of crocin and crocetin against oxidative stress include reduction of malondialdehyde level, improving the levels of glutathione and anti-oxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase, as well as reducing lipid peroxidation. [51].Lipid peroxidation process plays a key role in tumor growth invasiveness [52].In the present study, SOD activity was decreased in liver homogenate in EAC – bearing mice when compared with the normal control animals. It may be due to the increase in circulating lipid peroxide. This can result in accumulation of superoxide anions, a highly diffusible and potent oxidizing radical capable of traversing membranes causing deleterious effects at sites far from the tumors [53]. It has been established that SOD activity is inhibited in cancer [54].. The lowering activity of SOD as a result of tumor growth was reported [55, 56,57].The decrease in hepatic SOD activity was also reported in EAC bearing mice [58,59]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[60] . 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 [61]

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 [62] Our results are in agreement with study [63] 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 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 showed a significant reduction of liver MDA 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.

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

[64] . 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, and ALP 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, ALP) 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 [65]

Our results demonstrated that there was a significantly increase in concentration of liver enzymes (AlT, AST&ALP) and decreased albumin level in positive control group as compared to negative control group. This increased reflected that

hepatotoxicty due to cancer [66] . Treatment with Saffron aqueous extract 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.

BUN, uric acid and creatinine are the conventional tests indices for kidney functions and renal structural integrity. Nephrotoxicity was proved by significant elevations in serum levels of creatinine , uric acid and BUN as compared to normal control group.The transplantation of EAC into mice group induced increase for urea , uric acid and creatinine levels in serum that may be attributed to renal damage as a result of cancer cell invasion [67] .Treatment of EAC bearing mice with Saffron aqueous extract , showed an improvement of urea, uric acid and creatinine levels, this level attained nearly to the negative control group.

LDH , a cytoplasmic marker enzyme is a known indicator of the cell and tissue damage by toxic compound[68]

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. 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 [69].Treatment with saffron aqueous extract showed significantly decreased in LDH and CK-MB in the studied groups. Saffron aqueous extract had protective effects on the cardiac toxic effect of doxorubicin, and decreased the activity of lactic dehydrogenase (LDH) and creatine kinase (CK).

Apoptosis or programmed cell death is a widely important mechanism that contributes to cell growth reduction. This process occurs through two main pathways (intrinsic and extrinsic). The intrinsic pathway initiates with intracellular signals and causes changes in the inner membrane of mitochondria. These changes lead to cytochrome C release, formation of apoptosome complex and caspase cascade activation. On the other hand, the extrinsic pathway involves trans-membrane receptormediated interactions which are mostly members of the tumour necrosis factor (TNF) receptor and transmit death signals from the cell surface to the intracellular signalling pathways. These signals and intrinsic pathway signals activate caspase cascade and lead to apoptosis cell death [70]

Our results showed treatment with plant cause decrease TNF- alpha level compared with positive control group. Therefore , the results of the present study suggested that treatment with plant may have induced apoptosis .

Previous studies showed the Crocus sativus induce apoptosis in human cancer cell lines [71].Crocus sativus has been used to treat several medical conditions, such as gastrointestinal disorders, urological infections, as well as in treating malignancies *Crocus sativus* contains components like safranal, alpha crocin or crocin is the major component. Additionally *Crocus sativus* also contains amino acids, flavoniods and other chemical compounds (Among these, crocin is the most important since it is the major component in *Crocus sativus* and has shown significant biological activities [72]

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, crocin and safranal 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 .

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 Saffron,crocin] and safranal for preventive and therapeutic mice groups showed improved kidney tissues changes induced by EAC cells in mice [73].

Conclusions: The present study demonstrated that Crocus Sativus L (saffron) aqueous extract , Crocin and Safranal showed strong effect of antitumor activity against Ehrlich ascites carcinoma. The anti-tumor mechanism may be mediated by preventing oxidative damage.

References

[1] Saad, E. A., & Waly, H. M. (2019). Encapsulation of a new quinoxaline derivative in PLGA alters the pattern of its anticancer potency and induces apoptosis. *Cancer chemotherapy and pharmacology*, *83*(4), 649-658.

[2] Panis, C., Victorino, V. J., Herrera, A. C. S. A., Freitas, L. F., De Rossi, T., Campos, F. C., ... & Cecchini, A. L. (2012). Differential oxidative status and immune characterization of the early and advanced stages of human breast cancer. *Breast cancer research and treatment*, *133*(3), 881-888.

[3] Salem, F. S., Badr, M. O., & Neamat-Allah, A. N. (2011). Biochemical and pathological studies on the effects of levamisole and chlorambucil on Ehrlich ascites carcinoma-bearing mice. *Vet Ital*, *47*(1), 89e95.

[4] **Erdem, S. A., Nabavi, S. F., Orhan, I. E., Daglia, M., Izadi, M., & Nabavi, S. M. (2015).** Blessings in disguise: a review of phytochemical composition and antimicrobial activity of plants belonging to the genus Eryngium. *DARU Journal of Pharmaceutical Sciences*, *23*(1), 53.

[5] **Russo, M., Russo, G. L., Daglia, M., Kasi, P. D., Ravi, S., Nabavi, S. F., & Nabavi, S. M. (2016).** Understanding genistein in cancer: The "good" and the "bad" effects: A review. *Food chemistry*, *196*, 589-600.

[6] Kviecinski, M. R., Felipe, K. B., Schoenfelder, T., de Lemos Wiese, L. P., Rossi, M. H., Gonçalez, E., ... & Pedrosa, R. C. (2008). Study of the antitumor potential of Bidens pilosa (Asteraceae) used in Brazilian folk medicine. *Journal of ethnopharmacology*, *117*(1), 69-75.

[7] Bathaie, S. Z., & Mousavi, S. Z.

(2010) New applications and mechanisms of action of saffron and its important ingredients. *Critical reviews in food science and nutrition*, *50*(8), 761-786..

[8] Mousavi, B., Bathaie, S. Z., Fadai, F., Ashtari, Z., Beigi, N. A., Farhang, S., ... & Heidarzadeh, H. (2015). Safety evaluation of saffron stigma (Crocus sativus L.) aqueous extract and crocin in patients with schizophrenia. *AVICENNA JOURNAL OF*

PHYTOMEDICINE, *5*(5), 413-419.

[9] Gohari, A. R., Saeidnia, S., & Mahmoodabadi, M. K. (2013). An overview on saffron, phytochemicals, and medicinal properties. *Pharmacognosy reviews*, *7*(13), 61.

[10] . Singla, R. K., & Bhat, G. V. (2011). Crocin: an overview. *Indo Global Journal of Pharmaceutical Sciences*, *1*(4), 281-286.

[11] Maggi, L., Sánchez, A. M., Carmona, M., Kanakis, C. D., Anastasaki, E., Tarantilis, P. A., ... & Alonso, G. L. (2011). Rapid determination of safranal in the quality control of saffron spice (Crocus sativus L.). *Food Chemistry*, *127*(1), 369-373.

[12] Hosseinzadeh, H., & Ghenaati, J. (2006). Evaluation of the antitussive effect of stigma and petals of saffron (Crocus sativus) and its components, safranal and crocin in guinea pigs. *Fitoterapia*, *77*(6), 446-448.

[13] Premkumar, K., Thirunavukkarasu, C., Abraham, S. K., Santhiya, S. T., & Ramesh, A. (2006). Protective effect of saffron (Crocus sativus L.) aqueous extract against genetic damage induced by anti-tumor agents in mice. *Human & experimental toxicology*, *25*(2), 79-84.

[14] Meier, J., and Theakston, RDG(1985).., Approximate LD50 determinations of snake venoms using eight to ten experimental animals. Toxicon. 24(4): 395-401.

[15] Crump K. S., Hod D. G., Langley C. H., and Peto R. (1976)CANCER RESEARCH 36, 2973-2979

[16] 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. .

[17] Salomi, M. J., Nair, S. C., & Panikkar, K. R. (1991). Inhibitory effects of Nigella sativa and saffron (Crocus sativus) on chemical carcinogenesis in mice.

[18] Hosseinzadeh, H., & Noraei, N. B. (2009). Anxiolytic and hypnotic effect of Crocus sativus aqueous extract and its constituents, crocin and safranal, in mice. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, *23*(6), 768-774.

[19] Mcliman, 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

[20] 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.

[21] Chen, C.N.; Weng, M. S.; WU. C-L.; Lin J. K. (2004): Comparison of radical scavenging activity, cytotoxic effect and apoptosis induction in human melanoma

cells by Taiwanese propolis from different sources . *Evidence – based complement Alternate Med*, 1: 175 – 185.

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

[24] Karmen A, Wroblewski F, and Ladue JS (1955). Transaminase activity in human blood. J. Clin. Invest.; 34:126-31.

[25] Belfield, A. and Goldberg,D.(1971) : colorimetric determination of alkaline phosphatase activity . enzyme , ,12:561-566.

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

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

[28] Barham, D., & Trinder, P. (1972). An improved colour reagent for the determination of blood glucose by the oxidase system. *Analyst*, *97*(1151), 142-145.

[29] Barham, D., Trinder, P ., (1972) : Analyst, 97 , 142 .

[30] 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.

[31]. **Koracevic, D., et al.(2001).**Method for the measurement of antioxidant activity in human fluids. Journal of clinical pathology.. 54(5): 356-361

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

[24] Nishikimi, M., et al. (1972) The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochemical and Biophysical Research Communications.. 46(2): 849-854.

[33] Paglia, DE., and Valentine, W N. (1967) Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. The Journal of Laboratory and Clinical Medicine. 70(1): 158-169. 26.

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

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

[36] Drury, R.A. and Wallington, E.A. (1980) arleton's Histological Technique. 5th Edition, Oxford University Press, New York.

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

[38] Hosseinzadeh, H., Shariaty, V. M., Sameni, A. K., & Vahabzadeh, M. (2010). Acute and sub-acute toxicity of crocin, a constituent of Crocus sativus L.(saffron), in mice and rats. *Pharmacologyonline*, *2*(8), 943-995.

[39] Hosseinzadeh, H., Shakib, S. S., Sameni, A. K., & Taghiabadi, E. (2013). Acute and subacute toxicity of safranal, a constituent of saffron, in mice and rats. *Iranian journal of*

pharmaceutical research: IJPR, 12(1), 93.

[40] Desai AG, Qazi GN, Ganju RK, El-Tamer M, Singh J, Saxena AK, Bedi YS, Taneja SC, Bhat HK(2007). Medicinal plants and cancer chemoprevention. Curr Drug Metab.;9:581.

[41] Elkady AI, Osama AA, Baeshen NA, Rahmy TR. (2012) Differential Control of Growth, Apoptotic activity, and gene expression in human breast cancer cells by extracts derived from medicinal herbs Zingiber officinale. J Biomed Biotechnol.;2012:6143

[42] Ahmed N, Mahmood A, Ashraf A, Bano A, Tahir SS, Mahmood A. (2015) Ethnopharmacological relevance of indigenous medicinal plants from district Bahawalnagar, Punjab, Pakistan. J Ethnopharmacol.;175:109–23

[43] Liu RH: (2004)Potential synergy of phytochemicals in cancer prevention: Mechanism of action. J Nutrition, 134:3479S–3485S

[44] 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

[45] Alpinar K, Ozyurek M, Kolak U, Guclu K, Aras C, Altun M, Celik SE, Berker KI, Bektasoglu B, Apak R.(2009) Antioxidant Capacities of some food plants wildly grown in ayvalik of turkey. Food Sci Tech Res.;15:59–64

[46] Beutler, E. and Gelbart, T. (1985): Plasma glutathione in health and inpatient with malignant disease. J. Lab. and Clin. Med, 105: 581 – 584.

[47] Padmavathi, R.; Santhilnathan, P.; Chodon, D. and Sakthisekaran, D. (2006): Therapeutic effect of Paclitaxel and propolis on lipid peroxidation and antioxidant system in 7, 12 dimethylbenz (a) anthracene – induced breast cancer in female Sprague dawley rats. Life Sci, 78: 2820 – 2825

[48]. **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

[49]. **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.

[50] Abd El-Azime A. Sh1 ., Sherif N.H.2 and Eltahawy N. A. (2014). Efficacy of Aqueous Extract of Saffron (Crocus sativus L.) in Modulating Radiation-Induced Brain and Eye Retina Damage in Rats The Egyptian Journal of Hospital Medicine Vol. 54, Page 101–108

[51] Rezaee-Khorasany, A.; Razavi, B.M.; Taghiabadi, E.; Tabatabaei Yazdi, A.; Hosseinzadeh, H. . (2019)Effect of saffron (stigma of Crocus sativus L.) aqueous extract on ethanol toxicity in rats: A biochemical, histopathological and molecular study. J. Ethnopharmacol, 237, 286–299. [CrossRef] [PubMed]

[52] 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.

[53] Oberley, I. W. and Buettner, G. R. (1979): Role of superoxide dismutase in cancer. A review. Cancer, 39: 1141 – 1149

[54] Oberly, T. D. and Oberley, L. W. (1997): Antioxidant enzyme levels in cancer. Histol Histopathojol, 12: 525 – 535

[55] Haldar, P. K.; Bhattacharya, S.; Kar, B.; Bala, A.; Mazumder, U. K. (2010b): Chemopreventive role of Indigofera aspalathoides in 20- methylcholanthrene induced carcinogenesis in mouse. Toxicological and Environmental Chemistry, 92:1749–1763

[56] Dolai, N.; Karmakar, I.; Kumar, R. B. S, Kar, B.; Bala, A . and Haldar , P. K. (2012): Evaluation of antitumor activity and in vivo antioxidant status of Anthocephalus cadamba on Ehrlich ascites carcinoma treated mice . Journal of Ethnopharmacology, 142: 865– 870.

[57] Nahar, L.; Zahan, R.; Mosaddik, A.; Islam, S.; Haque, A.; Fazal, A. and Jesmin, M. (2012): Antioxidant and antitumor activity of chloroform extract of Alangium salvifolium flower Phytopharmacology, 2(1) :123-134.

[58] Gupta, M.; Mazumder, U. K.; Kumar, R. S.; Sivakumar, T. and Vamis, M. L. M. (2004a): Antitumor activity and antioxidant status of Caesalpinia bunducella against Ehrlich ascites carcinoma in Swis albino mice. J. pharmacol. Sci, 94: 177 – 184.

[59] Halder, P.K.; Kar, B.; Bala, A.; Bhattacharya, S. and Mazunder, U. K. (2010a): Antitumor activity of Sansevieria roxburghianarhizome against Ehrlich ascites carcinoma in mice . pharm Biol, 48 : 1337 – 1343.

[60] 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.

.

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

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

[63] 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

[64] 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

[65] 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

[66] 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 Cancer Detection and Prevention (29): 470–478.

[67] 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.

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

[69] 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.

[70] Elmore S.(2007):Apoptosis: a review of programmed cell death. Toxicol Pathol

; 4: 495–516

[71]Hamid bakshi, Smitha S, Manik S, et al (2008).

Assessment of cytotoxic and apoptogenic activity of *Crocus sativus* on human cancer cell lines. *Ind J applied life Sci*, 4, 34-8 tumor-bearing mice by curcumin.

[72]Winterhalter P, Straubinger M (2000).

Saffron-renewed interest

in an ancient spice. *Food Rev Int*, 16, 39-59.

[73] Deways, W. D. (1982): Pathophysiological of cancer cachexia: Current understanding and areas for future research. Cancer Res, 42: 721s –726s

Table (1) (dose response curve of crocin)

Doses	**Count x10^6 cell/ml**
2.5 mg/kg	**126**
5 mg/kg	**113**
7.5 mg/kg	**95**
10 mg/kg	**75**
20 mg/kg	**128**
Positive control EAC	**196**

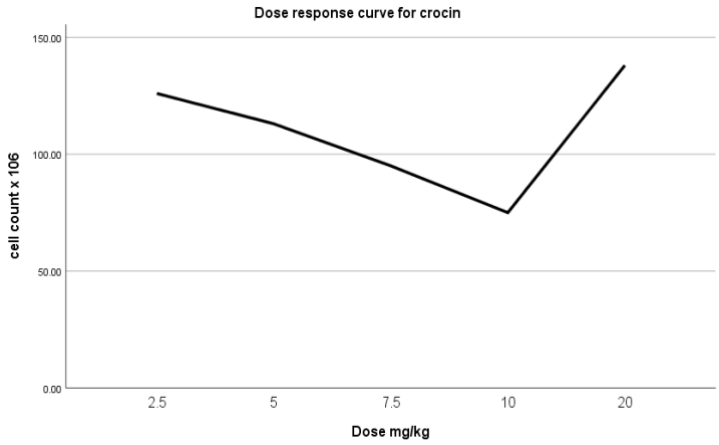


Fig (1) : Dose response curve of crocin

Table (2):Fractions of phenolic compounds for saffron aqueous extract (mg/100 g DW).

Peak No.	Identified compounds	Retention time (min)	100 g)/Conc. (mg
1	Pyrogallol	6.918	40.579
2	Gallic	7.023	0.59053
3	4- amino-benzoic	8.205	1.8172
4	Catechein	8.738	6.239
5	Catechol	8.973	2.962
6	Epicateachein	9.574	13.24
7	p-oH-benzoic	9.804	0.9003
8	Caffeine	9.957	3.024
9	Chlorogenic	10.073	3.419
10	Vanillic	10.142	6.676
11	Caffeic	10.349	1.032
12	p-coumaric	11.569	1.119
13	Ferulic	11.863	2.971
14	E-vanillic	12.299	88.793
15	A coumaric	13.221	0.636
16	Benzoic	13.286	4.263
17	Ellagic	13.466	63.350
18	3,4,5-methoxy-cinnamic	14.163	4.620
19	Coumarin	14.462	1.197
20	Cinnamc	15.260	0.098
21	Salycilic	16.320	3.803

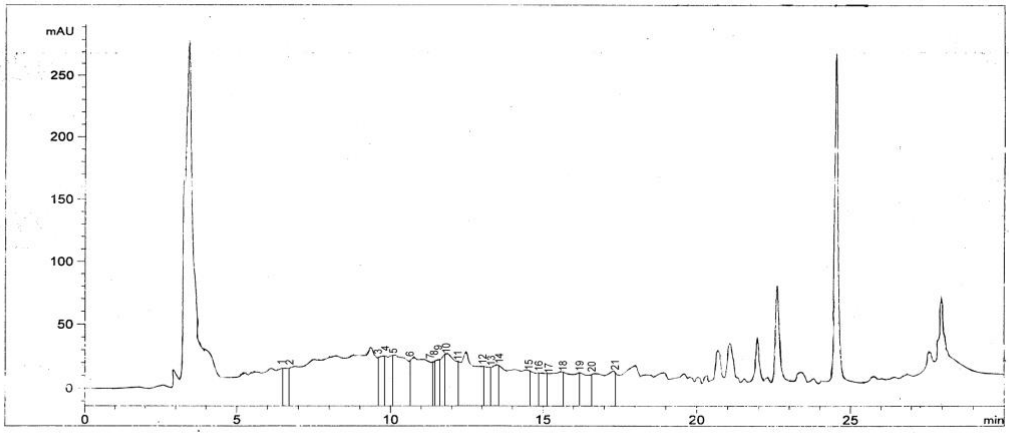


Fig (2): HPLC chromatogram of phelonic compounds for saffron aqueous extracts.

Table (3): Fractions of flavonoid compounds for saffron aqueous extracts (mg/ 100 g DW).

Peak No.	Identified compounds	Retention time (min)	100 g DW)/Conc. (mg
1	Acacetin	1.49	0.226
2	Acacetin neo.rutinoside	1.94	0. 67
3	Kamferol	2.593	5.714
4	Hespritin	2. 85	18.081
5	Narengenin	3.147	4.226
6	Rutin	3.405	3.45
7	Naringin	4.948	2.662
8	Safranal	5.1	8.301
9	Apigenin	5.468	2.742
10	Querctin	7.463	0.784
11	Luteo.6-arbinose8-glucose	9.463	1.148
12	Luteo.6- glucose 8-arabinose	10.683	3.567

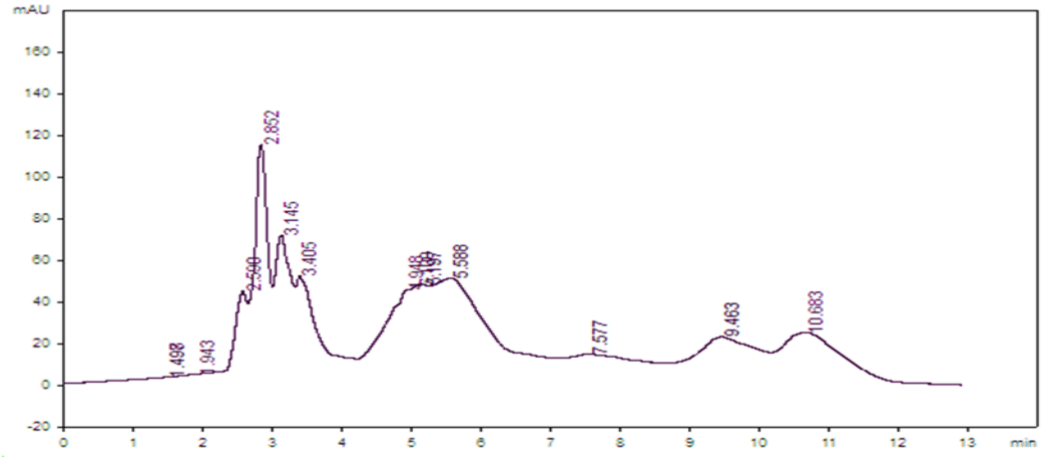


Fig (3): HPLC chromatogram of flavonoid compounds for saffron aqueous extracts.

Table (4): Fractions of betacarotene compounds for saffron aqueous extracts (mg/ 100 g DW).

Peak No.	Identified compounds	Retention time (min)	Conc. (mg / 100 g DW)
1	Rhamnetin	2.37	1.09
2	Kamferol	2.633	5.714
3	Quercetin-3-o-glucoside	2.97	3.45
4	Rutin	3.048	18.081
5	Corcin	3.277	27.603
6	Apigenin	5.468	49.742
7	Querctin	7.463	0.784
8	B carotene	10.007	21.205

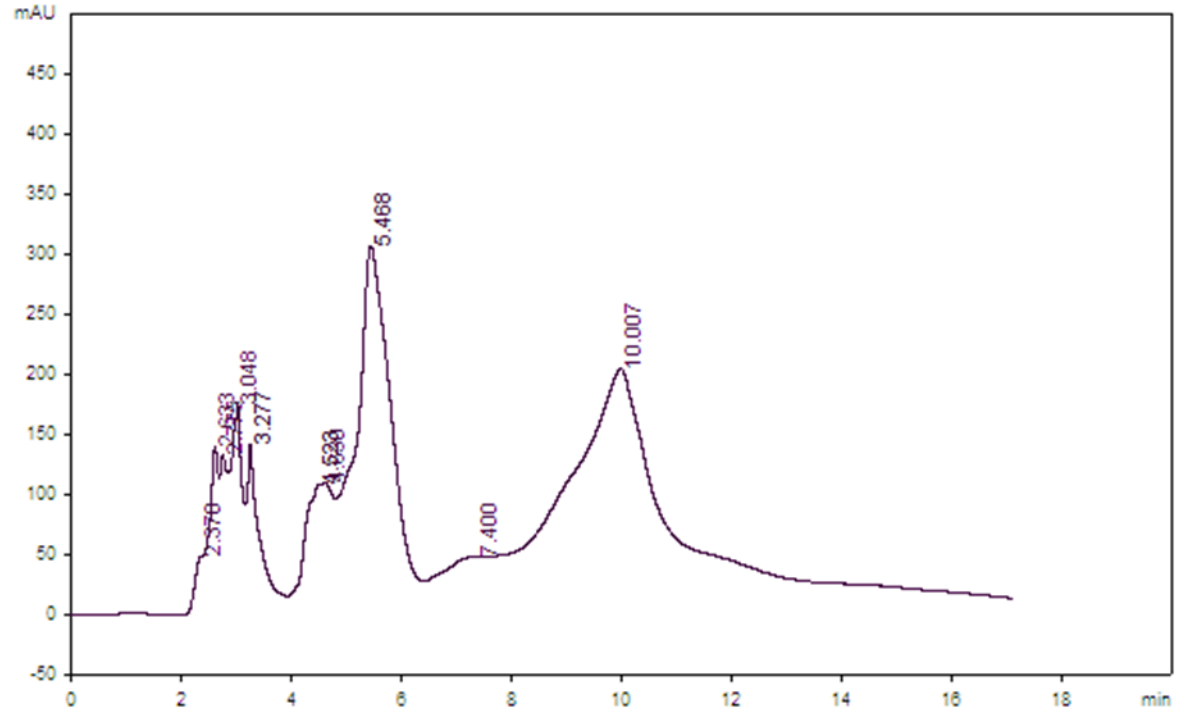


Fig (4): HPLC chromatogram of Betacarotene compounds for saffron aqueous extracts.

Table (5): HPLC of safranal

Peak No.	Identified compounds	Retention time (min)	100 g /Conc. (mg DW)
1	Safranal	5.03	13. 08

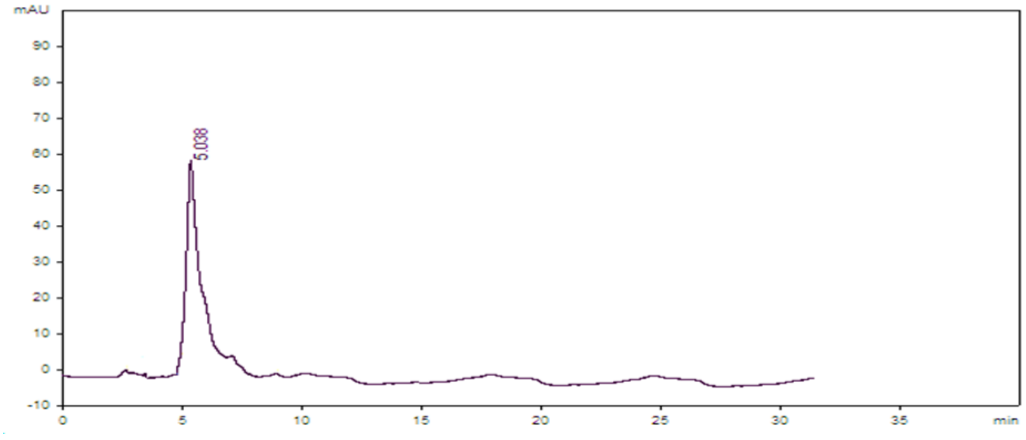


Figure (5) : HPLC of safranal

Table (6): HPLC of crocin

Peak No.	Identified compounds	Retention time (min)	100 g /Conc. (mg DW)
1	Crocin	3.478	20. 09

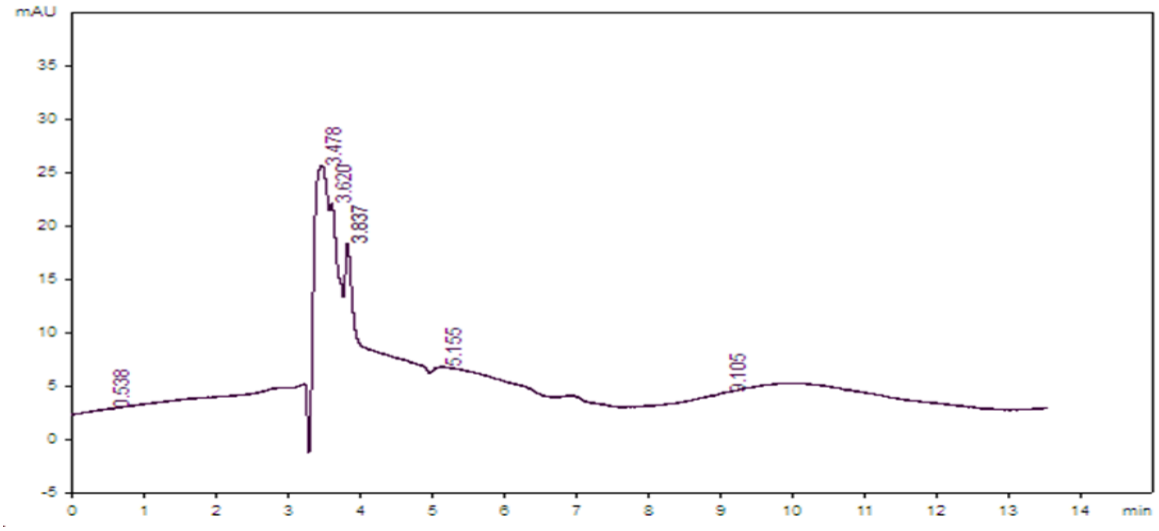


Figure (6) : HPLC of crocin

Table (7): the effect of different treatments on EAC volume (ml) in different studied groups

Groups	Group(2) Positive Control	Group (3) Preventive saffron aqueous extract	Group(4) Therapeutic saffron aqueous extract	Group (5) Preventive crocin	Group(6) Therapeutic crocin	Group (7) Preventive safranal	Group(8) Therapeutic Safranal	P value <0.0001
EAC Volume mean(ml) ±SEM	4.16±0.11	1.15****±0.04	1.12****±0.08	0.34****±0.11	1.05****±0.09	1.67****±0.22	1.54****±0.08	
%Change	……	-72.35%	-70.9%	-91.82%	-74.75%	-59.85%	-62.98%	

Table (8): the effect of different treatments on EAC count (x10^{6}cells/ml) in different studied groups

Groups	Group(2) Positive Control	Group (3) Preventive saffron aqueous extract	Group(4) Therapeutic saffron aqueous extract	Group (5) Preventive crocin	Group(6) Therapeutic crocin	Group (7) Preventive safranal	Group(8) Therapeutic Safranal	P value
EACcount mean (x106cells/ml) ±SEM	153.4±2.51	64****±2.59	70.7****±3.725	31.5****±2.35	49.5****±2.717	73.6****±1.99555	64.7****±3.77	<0.0001
%Change	…….	-58.27%	-53.91%	-79.46%	-67.73%	-52.02%	-57.82%	

Table (9) :The effect of different treatments on Life Span Prolongation

Groups	Group(2) Positive Control	Group (3) Preventive Saffron Aqueous extract	Group(4) Therapeutic Saffron aqueous extract	Group (5) Preventive crocin	Group(6) Therapeutic crocin	Group (7) Preventive safranal	Group(8) Therapeutic Safranal	P value
Survival days	11days	15 days	18 days	27days	24days	14days	20days	<0.0001
life span T/C%	…….	136.36%	163.6%	245.45%	218.18%	127.2%	181.81%	
Increase in life span %	……..	36.36%	63.6%	145%	118.18%	27.27%	81%	

Table (10):): the effect of different treatments on TNF_α in liver tissue and EAC tissue among different studied groups:

Mice Groups	Group(1) Negative Control	Group(2) Positive Control	Group (3) Preventive saffron aqueous extract	Group(4) Therapeutic Saffron aqueous extract	Group (5) Preventive crocin	Group(6) Therapeutic crocin	Group (7) Preventive safranal	Group(8) Therapeutic safranal	P value
TNF-α in EAC mean ± SEM (pg/ml)	……..	147.5****±7.213	98.47****±3.171	95.2****±2.333	62.31****±3.38	97.14****±1.81	98.39****±4.20	80.83****±3.9	<0.0001
% Change	……..	…….	-35.5%	-33.2%	-34.1%	-57.7%	-45.2%	-33.2%	
TNF-α in liver mean ± SEM pg/ml	688±10.43	2546****±78.02	643****±24.64	916.9****±14.31	874.5****±33.34	781****±14.59	±****542.725.36	524.2****±13.73	
%Change	……	270%	-6.5%	33.2%	27.1%	13.5%	-21.1%	-23.8%	

Table (11): Collective table of antioxidant parametersb among different studied groups:

Mice Groups	Group(1) Negative Control	Group(2) Positive Control	Group (3) Preventive saffron aqueous extract	Group(4) Therapeutic Saffron aqueous extract	Group (5) Preventive crocin	Group(6) Therapeutic crocin	Group (7) Preventive safranal	Group(8) Therapeutic safranal	P value
Liver MDA (nmol /gtissue)	0.173±5.07	0.23****±12.73	0.39****±8.14	0.179****±7.53	0.13±4.56	0.210±9.087	0.359±7.12	0.428±9.46	<0.0001

%Change	……	151%	60%	48%	-9%	79%	40%	86%	
Liver (TAC) (mM/L)	0.254±0.00	****0.139 ±0.0001	****0.25 ±0.008	****0.363 ±0.0001	****0.233 0.003±	****0.203 0.004±	****0.195 0.0001±	****0.191 0.005±	
%Change	…….	-45%	-1.5%	42%	-8%	-20%	-23%	-24%	
Liver cat (u/g tissue)	8.87 0.1239±	****3.06 0.056±	****6.64 ±0.1551	****4.65 0.1088±	****8.29 0.1865±	****9.42 0.08794±	****9.02 0.09±	****9.25 0.1293±	
%Change	…….	-65.9%	-25%	-47.7%	-6.8%	-6.8%	2%	4%	
Liver SOD) (u/g tissue)	265.8±5.849	165.0****±1.136	219.2****±13.8	304.7****±4.7	299.2****±8.583	258.5****±6.673	230.1****±5.842	229.4****±3.115	
%Change	…….	-37.9%	14.6%	-17.5%	-2.7%	12.56%	-1.37%	-13.4%	
Liver GPX) (u/g tissue	416.5 9.637±	****96.9 2.614±	****800 8.332±	****507.8 11.21±	****843.5 10.57±	****327.6 3.177±	****501.5 11.82±	****357.6 10.06±	
%Change	…….	-76%	92%	21.9%	102%	-21%	20%	-14%	

Table(12): Collective table of liver function tests among different studied groups:

Mice Groups	**Group(1) Negative Control**	**Group(2) Positive Control**	**Group (3) Preventive saffron aqueous extract**	**Group(4) Therapeutic Saffron aqueous extract**	**Group (5) Preventive crocin**	**Group(6) Therapeutic crocin**	**Group (7) Preventive safranal**	**Group(8) Therapeutic Safranal**	
ALT (U/L) mean ± SE	22.55± 0.27	77.86**** ±1.26	40.78**** ±5.27	58.92**** ±2.36	33.32**** ±1.79	39.43**** ±4.27	43.85*** *±1.39	40.96**** ±1.01	**P value**
%Change	…….	24.5%	16.12%	80.84%	74.85%	47.76%	81.64%	94.45%	<0.0001
AST(U/L)	190.62	366.02***	263.91***	301.9****	247.86** **±25.58	262.47** **±23.15	257.6*** *±27.94	309.91** **±37.52	

mean ± SE	±10.52	*±62.89	*±49.09	±76.46					
%Change	……	92.01%	38.448%	58.377 %	30.02%	37.69%	35.13%	62.58%	
Albumin mean ± SE (g/dl)	3.83±0.119	2.288**** ±0.15	2.77**** ±0.08	2.92**** ±0.11	3.7**** ±0.108	3.5**** ±0.17	3.028**** ±0.18	3.62**** ±0.127	
%Change	……	-40.26%	-27.67%	-23.75%	-3.93%	-8.61%	-21.14%	-5.48%	
ALP(U/L) mean ± SE	148.9± 2.186	427.82*** *±12.89	140.08*** *±0.702	174.34** **±1.88	174.92± 2.46	193.92** **±1.3	174.24** **±8.03	187.32** **±2.33	
%Change	.....	52.79%	-50%	-37.85%	-37.52%	-54.6%	-37.77%	-33.1%	

Table (13): Collective table of kidney function tests among different studied groups:

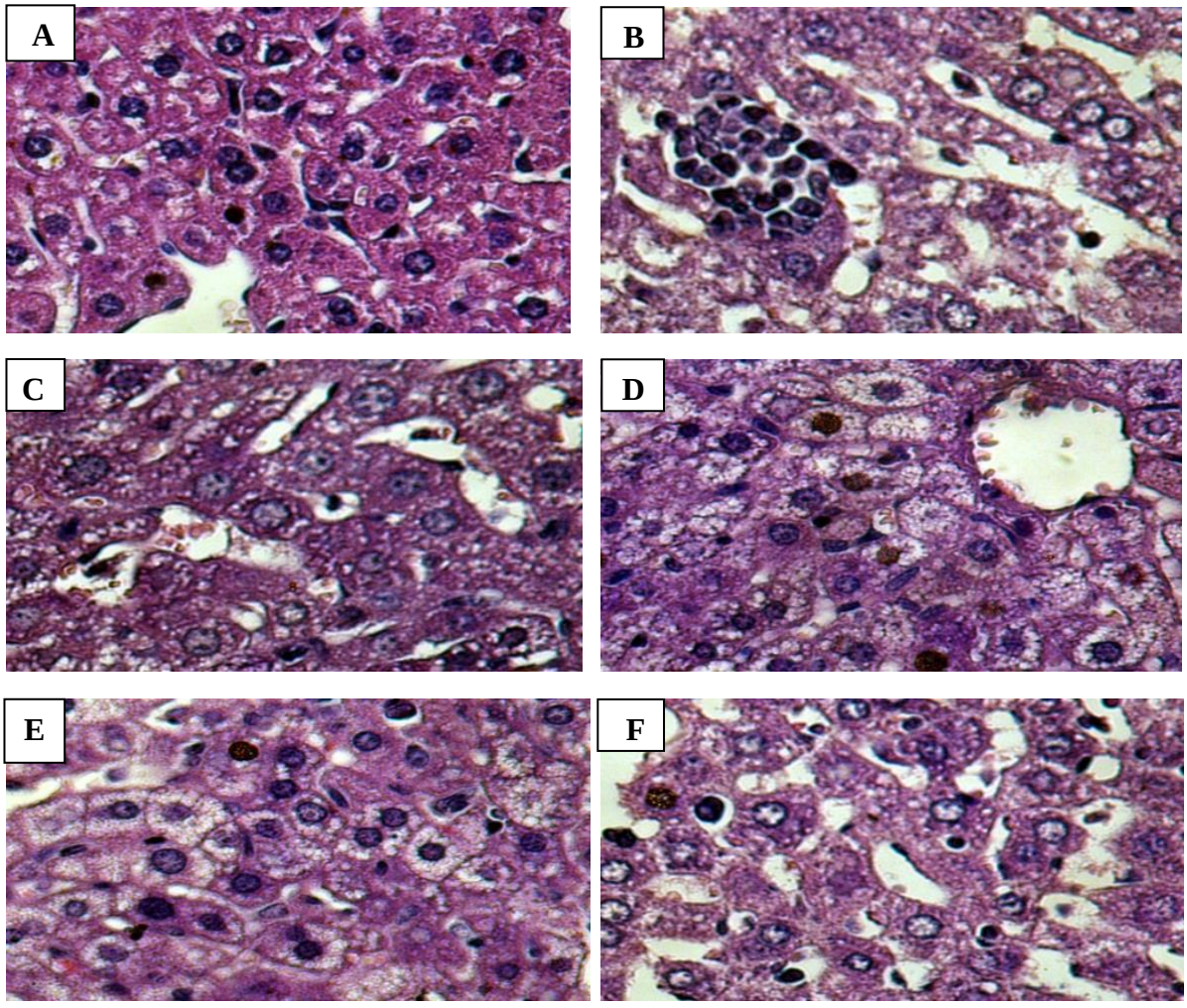
Mice Groups	**Group (1) Negative Control**	**Group(2) Positive Control**	**Group (3) Preventive saffron aqueous extract**	**Group(4) Therapeutic Saffron aqueous extract**	**Group (5) Preventive crocin**	**Group(6) Therapeutic crocin**	**Group (7) Preventive safranal**	**Group(8) Therapeutic Safranal**	**P value**
urea mean ± SE (mg/dL)	35.87± 2.17	98.03**** ±4.74	37.92**** ± 2.46	90.07**** ±2.09	80.4**** ± 3.08	77.24*** *±3.06	97.08*** *±1.79	37**** ±2.65	<0.0001
%Change	……	173.2%	5.7%	151.1%	124.1%	115.3%	170.6%	76.6%	

Creatinine mean ± SE (mg/dL)	0.349± 0.021	0.848**** ±0.0382	0.458****± 0.02175	0.46****± 0.0457	0.351****± 0.01438	0.48****± 0.02607	0.533**** ±0.0265	0.725****± 0.02088	
%Change	…… …….	50%	11%	12%	2%	14%	55%	111.7%	
Uric acid mean ± SE (u/L)	3.5±0. 19	5.57****± 0.16	4.32****±0 .12	4.07****± 0.11	3.58****± 0.15	4.13**** ±0.09	5.02**** ±0.28	5.33****± 0.37	
%Change	…… …..	59%	16%	23%	18%	10%	52%	43%	

Table (14): Collective table of heart function tests among different studied groups:

Mice Groups	**Group (1) Negative Control**	**Group(2) Positive Control**	**Group (3) Preventive saffron aqueous extract**	**Group(4) Therapeutic Saffron aqueous extract**	**Group (5) Preventive crocin**	**Group(6) Therapeutic crocin**	**Group (7) Preventive safranal**	**Group(8) Therapeutic Safranal**	**P value**
CK – MB mean ± SE (U/L)	4.58±0 .50	21.38**** ±0.265	13.90**** ±0.257	17.44**** ±0.29	5.57****± 0.257	8.62****±0 .259	14.7****± 0.11	7.69****±0 .17	0.0 001
% Change	……..	373.3%	208.8%	286.6%	23.7%	91.5%	226.6%	68.8%	
LDH mean ±	2158.4 ±9.61	4748****± 254.98	2181.4*** *±12.22	2286.4*** *±12.64	2178.6** **±7.40	2348.1*** *±21.74	2902.2** **±84.5	2538.1*** *±34.09	

SE (U/L)									
%Change	……	119.98%	1.06%	5.93%	0.93%	8.79%	34.46%	17.59%	



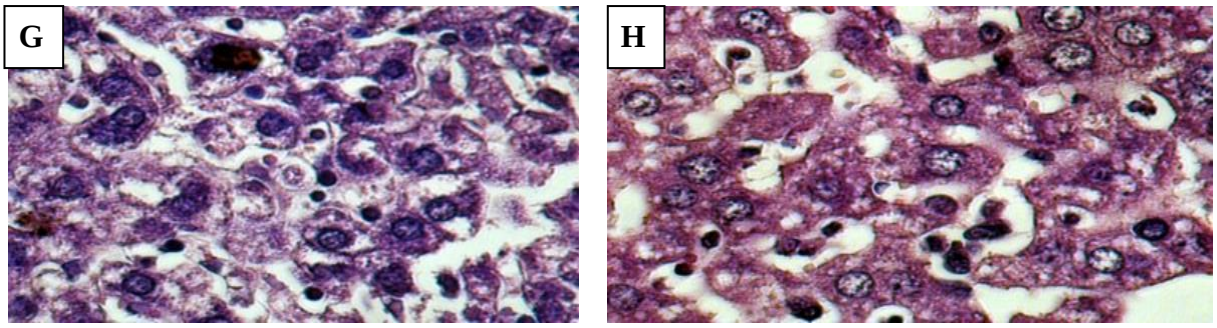
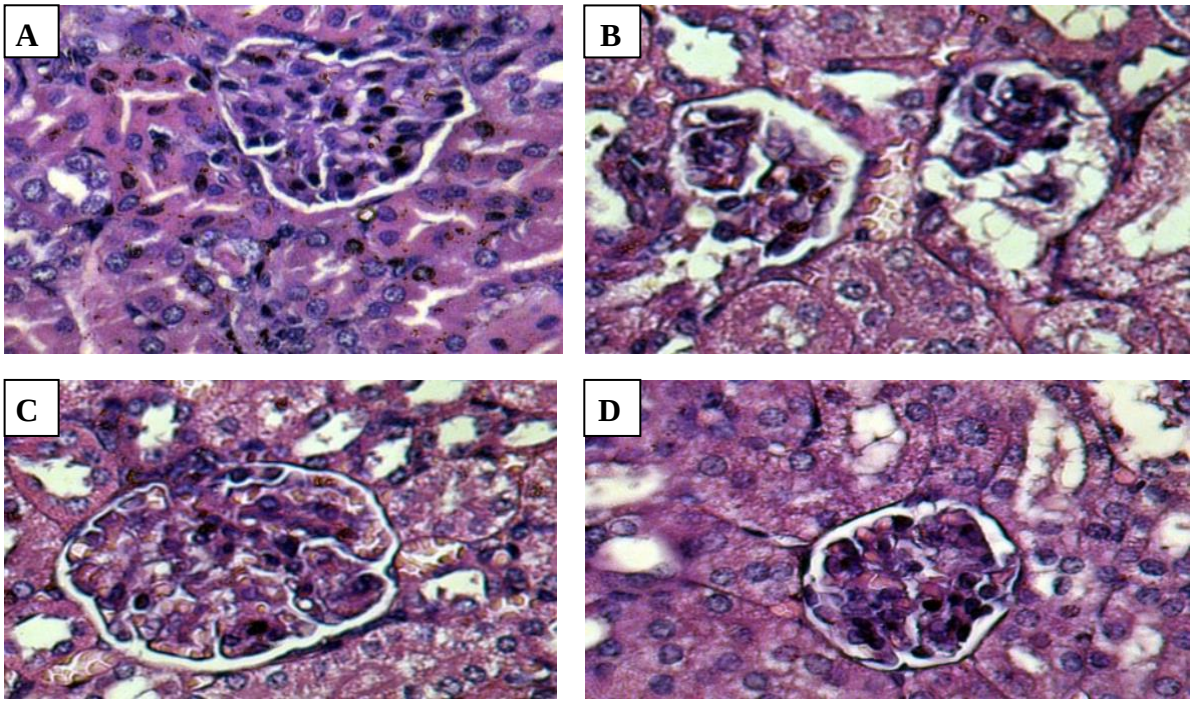


Figure (7)(1liver): High power micrographs of mouse liver in groups respectively stained with H&E A) negative contro; group revealed normal histological pattern with normal central vein is surrounded by radiating cords of hepatocytes . B)positive control groupshowed marked degeneration and necrosis, as well revealed that infiltrated, marked degree of cellular anaplasia, pleomorphism, and anisocytosis, with nuclear vascularity and some tumor cells were differentiated into gland-like structures surrounding a lumen containing eosinophilic material C) preventive group of saffronexhibited moderate degree of improvement in hepatocytes that exhibits moderate vacuolated hepatocytes . D) therapeutic group of saffron exhibited a moderate degree of hepatocytes damage, where moderate atrophy. E) preventive group of crocin exhibited a good degree of hepatocytes damage with mild atrophy. F) therapeutic group of crocin revealed moderate improvement in liver structure where mild cellular infiltrations, moderate atrophied vacuolar degeneration hepatocytes. G) preventive group of safranal exhibited mild improvement with marked necrosis; with marked vacuolations and degeneration in hepatocytes H) therapeutic group of safranal exhibited moderate improvement in liver structure



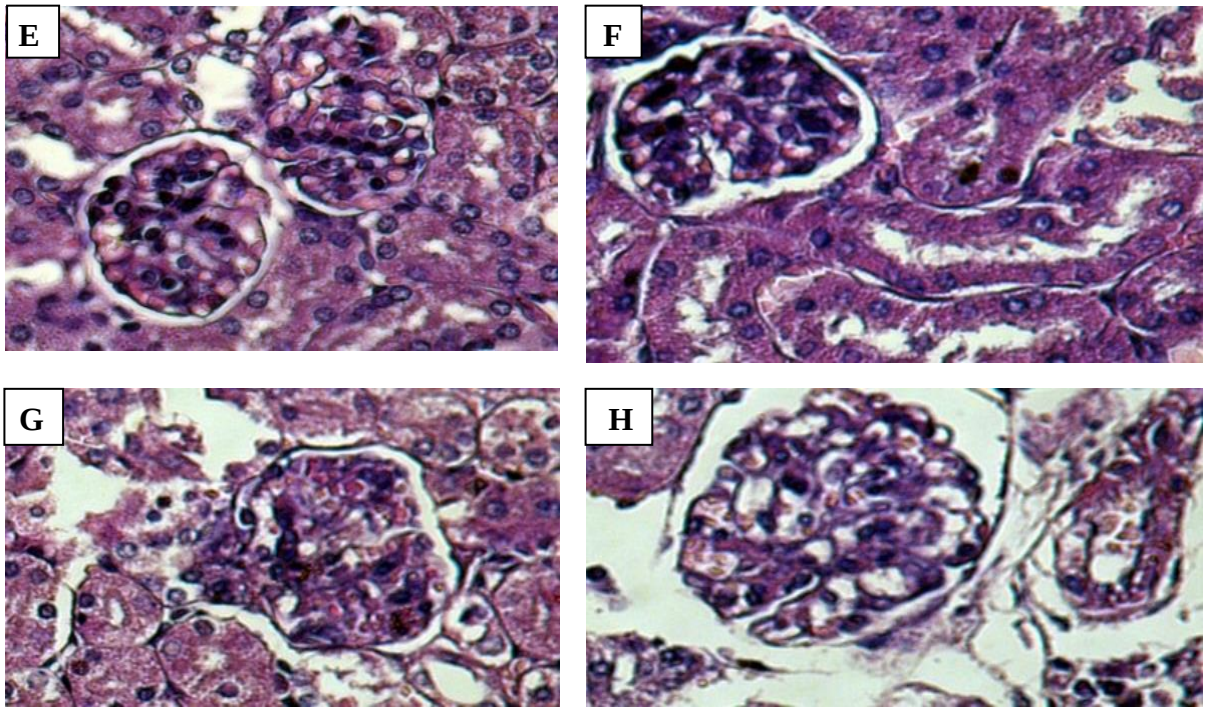


Figure (8)(kidney): High power micrographs of mouse kidney in groups respectively stained with H&E A) negative control group revealed normal histological structure .B) positive control group showed variable pathological changes in glomeruli and some parts of the urinary tubules .C)preventive group of saffron showed a good improvement in kidney structure were observed in kidney sections . D) therapeutic group of saffron revealed moderate improvement in Malpighian corpuscles and renal tubules .E))preventive group of crocin revealed marked improvement of the kidney structure F)therapeutic group of crocin revealed moderate degree of improvements with moderate degenerated to the renal tissues, moderate to marked necrosis with some of apoptotic cells were observed G) preventive group of safranal revealed mild degree of marked improvement of the kidney structure where marked degenerated to the renal tissues. H) therapeutic group of safranal revealed mild degree of marked improvement of the kidney structure where marked degenerated to the renal tissues.