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# Beneficial Roles of Quercetin Nanoparticles Extracted from *Euphorbia Helioscopia* Against Lung Damage in Mice

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**Abstract**

This study aimed to evaluate the beneficial roles of quercetin nanoparticles against lung damage induced by Ehrlich ascetic's carcinoma (EAC) model in mice. Quercetin was purified from the ethanolic extract of *Euphorbia helioscopia*. All the confirmation chemical analysis as TLC and HPLC were performed. The conversion of quercetin to nanoparticles was carried out using poly vinyl alcohol (PVA) method. The *in vivo* study was carried on 90 albino mice weighed 25-30 g which were divided randomly into 6 groups; where G1 was the control group, G2 was administered with Quercetin, G3 was injected with quercetin nanoparticles, G4 is the EAC groups, G5 was injected with both EAC and quercetin, and G6 included mice injected with both EAC and quercetin nanoparticles. The lung damage and improvement prognosis were evaluated by the estimation of different oxidative stress biomarkers as reduced glutathione, malondialdehyde, superoxide dismutase, and catalase. In addition, some anti-inflammatory parameters as tumor necrosis factor-α and interleukin-6 were measured. The obtained results indicated the excellent therapeutic role of quercetin in general and the greater effect of quercetin nanoparticles in the lung tissue's improvement and recovery against the damage induced by Ehrlich model. The histological and immunohistopatholgical examinations confirmed these results.

**Key words:** Quercetin; Lung damage; Malondialdehyde; *Euphorbia helioscopia*, interleukin-6; Quercetin nanoparticles.

## 1. Introduction

Empirical tumors have major significance in modeling. Ehrlich ascites carcinoma (EAC) is one of the widespread tumors **[1, 2]**. Spontaneous murine mammary adenocarcinoma that strained breast cancer is renowned as Ehrlich tumors **[3-6]**. EAC is an undifferentiated tumor owning high capability for transplantation, non-regression, short lived, proven malignancy, and high proliferation capacity **[7, 8]**. However, it can encourage lung toxicity and oxidative stress leading to lung damage **[9].**

*Euphorbia helioscopia L* extract has potential effects as anticancer folk medicine protecting against different types such as liver cancer, esophagus cancer, bronchitis, nasopharyngeal carcinoma and glomerulonephrities. *Euphorbia helioscopia L* contains several of secondary metabolites according to phytochemical studies. Terpenoids and flavonoid are the prime biologically active substance.

Quercetin (3, 4', 5, 5', 7-pentahydroxy-Flavone) is the mainly flavonoid component isolated from *E. helioscopia*. It plays an important role in the medicinal impact in different biological activities of *E. helioscopia* **[10].**

Quercetin has strong antioxidant, anti-inflammatory, and anticancer properties. It is presented as pro-apoptotic flavonoid on tumor **[11].** It plays an important role in inhibition of proliferation of cancer cell. It impulses cell cycle arrest such as G2/M arrest or G1 arrest in many types of cells. Furthermore it intercedes apoptosis due to stress protein inducing, disturbance of microtubules and mitochondria, releasing of cytochrome c, and caspases activation **[12].**

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None the less, the curative efficacy of Quercetin is disserving by its low water solubility. In the concerned of unraveling; the solubility problem of Quercetin and provision take longer cycle times and acceptable metabolic processes **[11].**

This study aimed to solve the less solubility issue of quercetin extracted form *E. helioscopia* by its conversion into nanoparticles by using the emulsion technique of poly vinyl alcohol (PVA) and evaluate the beneficial effects of quercetin nanoparticles against lung damage induced by EAC in mice.

## 2. Material and methods

### 2.1 Plant Material

The whole flowering plant of *Euphorbia helioscopia Linn.* was collected from its natural habitats from wheat farms in El-Gharbia governorate in Egypt in March 2020. The plant was kindly authenticated by staff members of Department of Botany, Faculty of Science, Tanta University, Tanta, Egypt. A voucher specimen (EH 202) was deposited in Pharmacognosy department, Faculty of Pharmacy, Damanhour University, Egypt.

### 2.2 Extraction of Quercetin from *Euphorbia helioscopia Linn*

#### 2.2.1Chemical and instruments used in the extraction

All solvents and Chemicals were purchased from Sigma Chemical Co. (St., Louis, USA). Quercetin was purchased from Sigma-Aldrich. Solvent evaporation and concentration were performed on Rotavapor Heidolphvv 2000l and determination of flavonoidal content using Spectrophotometer (Optima SP-300, Japan). TLC analysis was done on precoated silica gel 60 F254 plates (Germany). Sephadex LH20 (Pharmazie) was used for purification. Melting points were determined using Fisher- Johns melting point apparatus. UV detection of the TLC plates was done using Camag, Switzerland UV Lamp. HPLC (Preklin-Elmer) C18 column (250 × 4.6 mm; 5 μm) with UV-detector system.IR ( Perkin- Elmer) apparatus was used.The 1H and 13 C -NMR spectra were recorded in DMSO-d6 using TMS asinternal standard, on Varian-Mercury BB 400 NMR. . Poly vinyl alcohol (PVA) was purchased from Fisher Scientific, UK.

#### 2.2.2 Preparation of the total ethanolic extract

The air-dried powder (2.5 Kg) was defatted with light petroleum till exhaustion. Then the air- dried defatted powder was macerated with 70% alcohol for 24 hr then filtered. Extraction was repeated three times till exhaustion. The collected ethanolic extracts were concentrated under reduced pressure in Rotavapor to produce waxy green residue (150g). The residue was extracted with ethyl acetate several times. Ethyl acetate fractions were subjected to silica gel column chromatography. Elution was started with chloroform then polarity was increased with chloroform: ethyl acetate (50:50), ethyl acetate, ethyl acetate: methanol (90:10) and methanol. Similar fractions were collected, dried. To give a compound which shown a single spot on the Column was monitored using TLC plate developed in Toluene: ethyl acetate: formic acid (6:2:0.8) and sprayed with Aluminum chloride reagent. Another solvent system used was n- butanol: acetic acid: water (2:2:6) BAW. Similar fractions were mixed and separate on developed on preparative TLC plates. Purification of the isolated compound Quercetin was done using Sephadix LH20 column. This isolated compound was further confirmed by using FTIR, 400 MHz 1H – NMR and FAB- MS spectroscopy. Isolated compound was performed. 1H NMR (400 MHz) spectra were recorded on Bruker Advance 400 in CDCl3. The FAB mass spectra were recorded on a Jeol SX102/Da-600 mass spectrophotometer.

#### 2.2.3 TLC analysis

10 mg of extract was dissolved in 1 ml ethanol. Ten microliters were deposited on silica gel F 254 plate then developed in using hexane: ethyl acetate: acetic acid (2: 2: 1) as the solvent system. The plate is visualized under UV then sprayed with anisaldehyde/ $H_2SO_4$ and then dried and heated to 120°C . Ethanolic extract showed 3 spots with Rf values: 0.26, 0.42 and 0.88.

#### 2.2.4 HPLC analysis:

HPLC analysis of the total ethanolic extract was done using a C18 column as the stationary phase. Several trials confirmed better separation using acetonitrile: methanol (50:50) as mobile phase at a flow rate of 0.5 ml/min. Quercetin was used as a standard. The isolated compound was injected and spiked with the standard quercetin in another HPLC run under the same previous conditions.

### 2.3 Preparation of nanoparticles of Quercetin

Quercetin nanoparticles were prepared by using emulsion diffusion technique. One gram of quercetin was dissolved in 90% ethyl alcohol, and an equal weight of PVA (One g) was solved in 10 ml of distilled water; the two solutions were holed on a magnetic stirrer for 30 minutes. Then the Quercetin solution was inserted drop by drop on aqueous PVA with stirring for another 30 minutes [13]. Quercetin nanoparticles was analyzed using transmission electron microscope (TEM) (Jeol, GEM-100CX), Faculty of Science, Alexandria University, Egypt.

### 2.4. 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 **[8,9].**

**2.5 In vivo study**

This study was carried out on 90 albino mice weighed average (25-30 g). They were obtained from Zoology Department, Faculty of Science, Tanta University, Egypt. All animals were adapted for a week prior the experiment proceeding under standard laboratory conditions (Temperature 23 ± 2 °C, humidity of 55 ± 5%. Light was on a12:12 hr light/dark cycle).

Animal were lied on a standard diet, Egyptian Company of Oils and Soap, Kafr- El-Zayat; Egypt) and water ad libitum. The protocol of experiment was confirmed by Local Ethics Committee and Animals Research of the Tanta University, Egypt ( IACUC-SCI-TU-00179) .

The mice were randomly divided into six groups (fifteen for each). The first group (G1) included mice were injected with only saline and served as the control group, the second group (G2) were administered with only quercetin (50 mg/kg) 3 time aweek for one month **[14];** the mice in the group (G3) were injected with only quercetin nanoparticles (50 mg /kg) 3 time aweek for one month **[15]** , the fourth group (G4) was injected with Maurine Ehrlich was generated by i.p injection of 2×106 viable tumor cells, (G5) included EAC mice treated after 14 days of the injection with quercetin 3 time aweek for a month and the sixth group (G6) included EAC mice treated after 14 days of the injection with quercetin nanoparticles by the recommended doses.

**2.6 Homogenate preparation**

Lung tissues were used for detection of antioxidant parameters. To unhand any red blood cells and clots from tissue, it was irrigation with phosphate buffered saline (PBS) pH 7.4 containing 0.16 mg/ml heparin. At that time the tissue was homogenized in 5-10 ml cold buffer (around 50 mM potassium phosphate pH 7.5, 1mM EDTA) per gram tissue utilizing tissue homogenizer. At that time the sample was centrifuged at 4000 rpm for 15 minutes at 4°c. The supernatant was lifted for examine and side on ice.

**2.7 Determination of antioxidant parameters**

Determination of reduced glutathione was done by the colorimetric technique by using the kit of BioDiagnositic, Egypt, CAT. No GR2511 according to the oxidation of GSH by 5,5'-dithiobis-2-nitrobenzoic acid [DTNB], and the end product yellow colored complex is measured at 412 nm **[16].**

Lipid peroxide (Malondialdehyde) was detected by colorimetric methods. Thiobarbituricacid reactive product was resulted by reaction of Thiobarbituric acid (TBA) with Malondialdehyde (MDA) in acidic medium at temperature of 95oc for 30 min. The end product pink output can be estimated at 534nm **[17].**

Super oxides dismutase (SOD) (EC 1.15.1.1) estimation was determined by the colorimetric technique by utilizing the BioDiagnositickit, Egypt, CAT. No # SD2521. This experiment bulid on the capacity of enzyme to inhibit the phenazinemethosulphate mediated reduction of nitro-blue tetrazolin dye. Absorbance determined at 560 nm more than 5 minutes for both control and sample at 25ºC **[18].**

Catalase enzyme (EC 1.11.1.6) was estimated by colorimetric method according to BioDiagnositic kit, Egypt (CAT. No # SD2517). The reaction was based on the response between the catalase and the recognized amount of $H_2O_2$ to produce a Quinoneimine dye, which measured at 510 nm [19].

**2.8 Determination of interleukin-6 (IL-6) and Tumor necrosis factor-alpha (TNFα)**

Detection of the IL-6 and TNFα were carried out using mouse ELISA Kit purchased from abcam, USA (CAT. No. ab222503 and CAT. No ab208348, respectively).

**2.9. Histopathological Examination**

Fixed lung from various gatherings were prepared for paraffin sections, stained with haematoxylin and eosin for histological examination as indicated by Beltagy *et al.,* **[20,21]**

**2.10. Immunohistochemical investigation**

Distribution of protein expression of apoptotic p53 stained in lung tissue was examined in deparaffinized sections (5 μm) using avidin–biotin-peroxidase immunohistochemical method (Elite-ABC, Vector Laboratories, CA, USA) method against P53 antibody (dilution 1:200 DAKO Japan Co., Ltd., Tokyo, Japan) according to Tousson *et al.,* **[22, 23]**

**2.11. Statistical Analysis**

All the data were expressed as means ± S.D. The statistical analysis of data (one-way ANOVA) was done using GraphPad Prism 5 (GraphPad Software Inc., San-Diego, California USA). p-Values <0.05 we were be considered as statistically significant.

**3. Result**

**3.1 HPLC analysis of *E. helioscopia* extract**

HPLC profile showed 3 peaks using UV-Vis detection at 360 nm as shown in Figure1. Rt of peaks were 2.82, 3.65 and 4.84. The former two peaks were large and broad. The third peak showed the same retention time Rt= 4.8 with that of the authentic the flavonoid; Quercetin. Also, the isolated compound was detected by HPLC as Quercetin.

### 3.2 Isolation of the compound Quercetin from the aerial part of the plant *Euphorbia helioscopia*

The isolated compound Quercetin resulted from different silica gel columns, preparative TLC and Sephadex LH 20 was collected and weighed.

It was pale yellow needles obtained has m.p. 191-193°C, Rf: 0.79 (BAW), Rf= 0.94 in Ethyl acetate: methanol: formic acid: water (5:3:1:1) and Rf= 0.97 Ethyl acetate: pyridine: methanol: water (16:4:2:1). The total isolated compound weighed 88mg. Thus the yield was 0.09mg/ g powder. IR spectrum of the isolated compound in KBr pellet exhibits peaks at 3624, 3473, 3286 cm-1 (O-H), 1707 cm-1 (C=O), 1649 cm-1, 1408 cm-1 (C-C=C), 1275, 1245, 769.8 $cm^{-1}$ (C-H). The spectrum was superimposed with that of the authentic quercetin in the fingerprint region.

The results of 1H- NMR (DMSO-$d_6$, 400 MHz) of the compound Qurcetin is tabulated in Table (1) 6.18 (1H, d, J = 1.9 Hz, H-6), 6.41 (1H, d, J = 1.9 Hz, H-8), 7.07 (1H, d, J = 2.0 Hz, H-2′), 6.88 (1H, d, J = 2.0 Hz, H-5′), 7.54 (1H, d, J = 8.5, 2.0 Hz, H-6′). 12.84 (1H,S. OH at C5. All data were identical with that of Quercetin.

ESI-MS of the isolated compound Quercetin showed *m/z*: 302 [$M^+$] corresponding to molecular formula (C15H10O7). As supported by1H -NMR, on the basis of their analysis.

molecular formula $C_{15}H_{10}O_7$ suggesting that it is Quercetin. Spectral data were confirmed by comparing with the previously reported data **[24].**

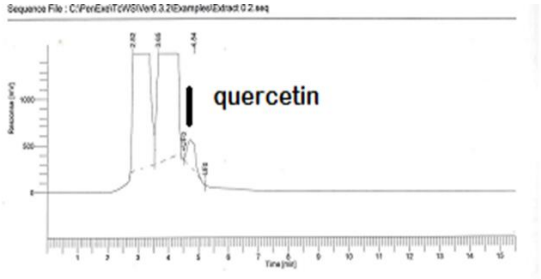


**Fig1:** HPLC of the total ethanolic extract of E. helioscopia

### 3.3 Quercetin Nanoparticles

The morphology of the investigated Quercetin nanoparticles showed a rode shape nanostructure as shown in Figure 2. The obtained particle sizes of such nanoparticles ranged from 30 to 40 nm. The obtained size recommended to be in the safe range for use in therapeutics **[25].**

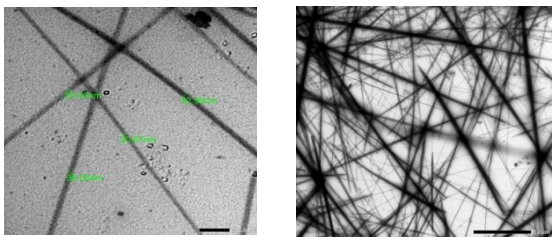

**Fig 2*:*** TEM Quercetin nanoparticles

### 3.4. Detection of antioxidant in lung tissue:

The results show there is no significant changes in GSH, SOD, MDA and catalase between, G2 and G3 in lung tissue if compared with the control (G1). On the other hand, significant increases in MDA and significant decreases in GSH, SOD and catalase were recorded in G4 compared with GPI in lung tissue. MDA was significantly decreased in lung after treatment with both quercetin extract (G5) and quercetin nanoparticles (G6) if compared with mice did not receive any treatment (G4). While, GSH, SOD and catalase were significantly increased in both G5 and G6 in comparison with G4 (Table 1).

### 3.5 Determination of IL-6 and TNF-α:

There were no significant changes in the both measured pro-inflammatory parameters (TNF-α and IL-6) between mice in groups G1, G2 and G3. The concentration of TNF-α and IL6 were significantly elevated in the in the lung tissues of mice in G4 if compared with the control group.

After treatment with quercetin and its nanoparticles (G5 and G6), the concentrations of both TNF-α and IL-6 showed significant decrease in comparison with the obtained values of mice in G1 (Table 2).

### 3.6 Lung histopathology

Lung sections in control (G1), quercetin (Q; G2) and quercetin NPs (Q-NPs; G3) groups revealed a normal lung architecture, normal spongy structure with typical alveoli, alveolar sacs, thin interalveolar septa, bronchioles, and normal pulmonary vessels (Figure 3A-3C). Lung sections in EAC (G4) showed thickened wall of bronchiole with moderately congested blood capillaries, marked macrophages infiltration in the alveolar spaces, degenerated and severe dense and and ongested pulmonary blood vessels (Figure 3D).

Lung sections in the treated EAC mice with quercetin (EAC+Q; G5) revealed mild improvement with decrease in the thickened interalveolar septa and moderate macrophages infiltration in the alveolar spaces (Figure 3E). The treated EAC with quercetin nanoparticles (EAC+Q-NPs; G6) was found to markedly attenuate these signs of EAC-induced lung injury (Figure 3F).

### 3.7 Impact of EAC, Quercetin and Quercetin nanoparticles on P53

Faint to mild positive reactions for P53 expression in interalveolar septa in both lung sections of the control group (G1), quercetin (Q; G2) and quercetin NPs (Q-NPs; G3) groups (Figure 4A-4C). While; lung sections in EAC (G4) showed strong positive reactions for P53 (fig. 4D). In contrast; the treatment of EAC with quercetin (natural or nano forms) showed significant decrease in P53.

The treated EAC with quercetin (EAC+Q; G5) revealed moderate positive reactions for P53 expression while the treated EAC with quercetin nano forms (EAC+Q NPs; G6) showed mild positive reactions for P53 expression (Figure 4E&4F).

**Table 2:** Assessment of the pro-inflammatory markers in lung tissues of different groups

| Biomarker / Group | IL-6 (Pg/g.tissue) | TNF-α (Pg/g.tissue) |
|---|---|---|
| **G1** | 67.13±1.61 | 4.97±0.09 |
| **G2** | 66.47±1.42 | 5.02±0.13 |
| **G3** | 68.30±0.89 | 5.05±0.16 |
| **G4** | 92.59±1.67[a] | 14.76±0.41[a] |
| **G5** | 84.31±2.03[a,b] | 9.22±0.25[a,b] |
| **G6** | 79.22±0.58[a,b] | 7.82±0.23[a,b] |

Data are presented as mean ± S.D,. [a] Significant vs. control group (G1), [b] significant vs. EAC group (G4).

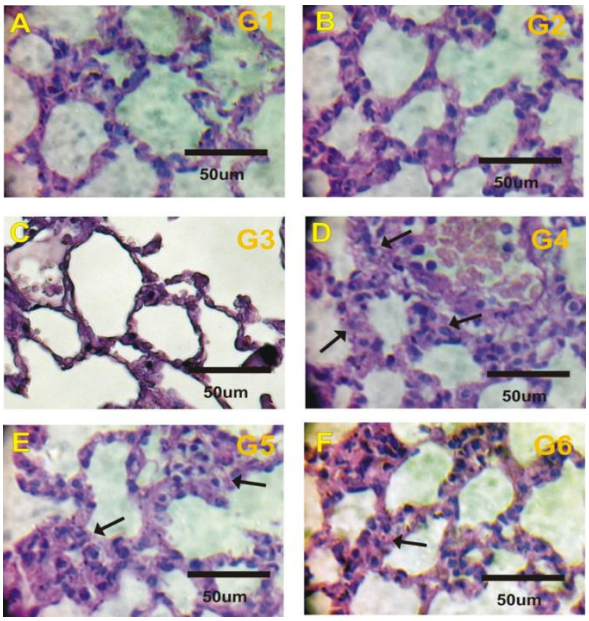


**Fig 3:** Photomicrographs of the mice lung transverse sections stained with H&E in different groups. **A-C:** Normal lung architecture in control (G1), quercetin (Q; G2) and quercetin NPs (Q-NPs; G3). **D:** Thickened wall of bronchiole (arrows) with moderately congested blood capillaries, in EAC (G4). **E:** Mild improvement with decrease in the thickened interalveolar septa and moderate macrophages infiltration in the alveolar spaces in lung sections in the treated EAC mice with quercetin (EAC+Q; G5). **F:** Normal lung architecture except only mild increased in thickened interalveolar septa in treated EAC with quercetin nanoparticles (EAC+Q-NPs; G5).

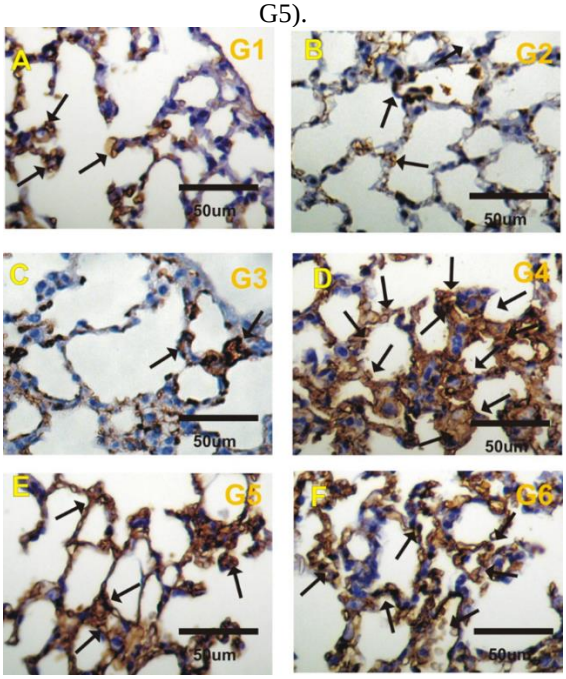


**Fig 4:** Lung sections photomicrographs in the different groups stained with P53. **A-C:** Faint to mild positive reactions for P53 expression in control (G1), quercetin (Q; G2) and quercetin NPs (Q-NPs; G3) groups**. D:** Strong positive reactions for P53 in lung sections in EAC (G4). **E:** Moderate positive reactions for P53 expression in EAC+Q (G5). **F:** Mild positive reactions for P53 expression in EAC+Q NPs (G6).

## 4. Discussion

Euphorbia helioscopia is considered as a cheap source of quercetin as it can be collected from the natural habitats of wheat. Previous study proved the high flavonoidal content of the plant (215.68 mg of RU/g extract [20]. TLC and HPLC of the total extract confirmed quercetin is the major flavonoidal content. Thus, chromatographic separation from the plant extract is recommended. Spectroscopical analysis of the isolated compound by different methods confirmed its identity as quercetin.

**Table 1:** Assessment of antioxidant markers in lung tissues of different groups:

| Biomarker / Groups | GSH (mg/g tissue) | SOD ( U/g tissue) | MDA (nmol/gtissue) | Catalase (U/g tissue) |
|---|---|---|---|---|
| **G1** | 88.3±0.61 | 398.1±0.7 | 612.8±1.56 | 321.8±0.89 |
| **G2** | 87.4±0.52 | 396.5±0.9 | 610.1±2.03 | 324.4±1.46 |
| **G3** | 87.7±0.85 | 399.1±1.8 | 614.8±1.34 | 323.2±0.84 |
| **G4** | 42.3±1.56[a] | 280.7±2.4[a] | 1080.1±3.8[a] | 245.3±2.11[a] |
| **G5** | 48.8±0.67[a,b] | 321.4±3.0[a,b] | 930.5±4.37[a,b] | 265.0±1.70[a,b] |
| **G6** | 84.52±1.5[a,b] | 392.86±3.6[a,b] | 751.58±3.92[ab] | 313.95±2.1[a,b] |

Data are presented as mean ± S.D,. [a] Significant vs. control group (G1), [b]significant vs. EAC group (G4).

Quercetin is a flavonoidal compound has shown important beneficial advantages on many diseases. Quercetin has a very specific character that continuously paying attention to its therapeutic impact on tumors; it is summarized as it has no any toxic effects on normal cells even with high doses. Several studies have improved that quercetin can extend anti-tumor functions in a different mechanisms as preventing cell cycle, enhances apoptosis, and prevents angiogenesis and metastasis **[26].** Our study aimed to solve the less solubility issue of quercetin extracted form *E. helioscopia* by its conversion into nanoparticles by using the emulsion technique with PVA and evaluate the its beneficial effects against lung damage induced by EAC in mice. Ehrlich ascites tumor cells give rise to several morphological and metabolic alteration including changes in oxidant and antioxidant status in the animals **[27].** The oxidative stress involves series of events giving rise to inflammation. Both reactive oxygen species (ROS) and oxidative stress are performed the main causes of cell imperfection and tissue injury [**28-30**] . They also alter amount of cell signaling pathway leading to release the inflammatory mediator like tumor necrosis factor-α (TGFα) and interleukin-6 (IL-6) **[31]**. The cells hold out this condition by using of various defense mechanisms ambit of the scavengers of free radical like glutathione (GSH) and antioxidant enzymes such as catalase, superoxide dismutase **[32].** Our results showed that oxidative stress markers significantly changed in the lung tissue in EAC model (G4). Malondialdehyde (MDA) concentrations significantly increased in EAC model by about 1.8 fold where, reduced glutathione (GSH) concentrations significantly decreased by about 0.5 fold if compared with the normal values obtained in G1. There was also significant decrease in SOD and catalase activities by about 0.7 and 0.8 folds, respectively if compared with G1. Oxidative stress has been related to the hyper-activation of signaling pathways for cell survival, proliferation and migration, in addition of metabolic modification of tumor microenvironment **[33; 28].** The obtained results indicated that both quercetin and quercetin nanoparticles treatment (G5 and G6) have powerful effects on oxidative stress by revealing MDA, GSH, SOD and catalase to their nearly normal values. Our result is compatible with the characterization of Quercetin which has a highly antioxidant activity. It act on various pathway, thorough its can be reacting with reactive species and formation the less reactive phenoxy radicals. Furthermore, the flavonoid disturb the lipid peroxidation by reactive oxygen species neutralization, as well as it is ameliorating the antioxidant enzymes activity **[34].** In the pulmonary damage prognosis stimulated by oxidative stress, many changes in alveolar macrophages role with alteration in production of cytokine may appear to help in the repair processes **[35].** The screening of the model of lung-injury proved by determination of several cytokine especially IL-6, TNFα mediators of inflammation involved the disorders respiratory pathogenesis **[36].** The result obtained in the present study documented significant increase in both TNF-α and IL-6 in EAC model (G4) in comparison with normal values in G1. After treatment with quercetin and quercetin nanoparticles (G5 and G6), the results showed regression in both TNF-α and IL-6 values. That is proving the role of Quercetin on oxidative inhibitors effect and antioxidant fibrous modulating agents, which ameliorate pulmonary fibrosis **[36].** These results were confirmed by the histological sections that indicated marked macrophages infiltration in the alveolar spaces in G4 and after treatment with quercetion (normal and nanoform; G5 and G6) there were degradation in macrophages infiltration. Quercetin have been documented to be the new potentially effective oxidative inhibitors and In addition, several studies confirmed that the anticancer effects of quercetin are related to its ability to induce apoptosis in cancer cells **[33].** The oxidative stress was related to cell survival, proliferation and migration likewise the oxidative damage is appreciate to be the main source of involuntary DNA damage **[32].** The results showed significant increase in P53 expression due to intracellular oxidative stress and DNA damage in lung sections of mice in EAC model (G4). After treatment with both quercetin and quercetin nanoparticles the result confirm that the gradual decrease in P53 expression as moderate to mild, respectively.

## 5. Conclusions

In conclusion, this study confirmed that quercetin nanoparticles (Q-NPs) have excellent therapeutic role in the lung tissues improvement and recovery against the damage induced by EAC model in mice via its antioxidant, anti-inflammatory properties. These results were declared by assessment of MDA, GSH, SOD, catalase, TNF-α and IL-6 in lung tissues. Furthermore, it has anti-apoptotic properties by modulating DNA damage. The histological and immunohistopatholgical examinations confirmed these results.

## 6. Conflicts of interest

There are no conflicts of interest

## 7. References

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