# Potential protective effect of Colostrum or Coenzyme Q10 against experimentally induced carcinogenesis

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**Abstract:** The role of Colostrum (50 mg/kg) or Coenzyme Q10 (100 mg/kg) on oxidative stress induced by Erlich ascites carcinoma [EAC] ($0.2X10^6$ cells) and $CCL_4$ (1.5 ml / kg) in female mice were evaluated. Viability, arginase activity, α L-fucosidase and $Bcl_2$ were significantly exceeded in EAC + $CCL_4$ treated mice. In addition P53 level significantly decreased. These abnormalities are accompanied by increased the lipid peroxidation product (MDA), protein carbonyl PC and decline in antioxidant enzyme activity as well as GSH, CAT, SOD, GST level. It seems that mice pretreated with Colostrum or Coenzyme Q10 then with EAC + $CCL_4$ reveal marked protection as antitumor substance.

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**Key words:** Colostrum, Coenzyme Q10, Ehrlich ascites carcinoma, $CCL_4$, Antioxidant.

## 1. Introduction

Cancer continuous to represent the largest cause of mortality in the world and claims over 6 million lives every year **(Abdullaev *et al.*, 2000)**. An extremely promising strategy for cancer prevention is chemoprevention, which is defined as the use of synthetic or natural agents (alone or combination) to prevent the development of cancer in humans **(Gupta *et al.*, 2004)**.

Colostrum also known colloquially as beestings, bisnings or first milk, Colostrum is a form of milk produced by the mammary glands of mammals (including humans) in late pregnancy. Most species will generate colostrum just prior to giving birth. Colostrum contains antibodies to protect the newborn against disease, as well as being lower in fat, and higher in protein than ordinary milk **(Hibbeln *et al.*, 2006; Sakai *et al.*, 2010).**

Colostrum shows promise in the treatment or prevention of a variety of diseases, **Ateb and ErdoUrul (2003) and Arokiyaraj *et al.* (2008)** CoQ10 is found in the membranes of many organelles. Since its primary function in cells is in generating energy, the highest concentration is found on the inner membrane of the mitochondrion. Some other organelles that contain CoQ10 include endoplasmic reticulum, peroxisomes, lysosomes, and vesicles.

The antioxidant nature of CoQ10 derives from its energy carrier function. As an energy carrier, the CoQ10 molecule continuously goes through oxidation-reduction cycle. As it accepts electrons, it becomes reduced. As it gives up electrons, it becomes oxidized. In its reduced form, the CoQ10 molecule holds electrons rather loosely, so this CoQ molecule will quite easily give up one or both electrons and, thus, act as an antioxidant. CoQ10 inhibits lipid peroxidation by preventing the production of lipid peroxyl radicals (LOO). Moreover, $CoQH_2$ reduces the initial perferryl radical and singlet oxygen, with concomitant formation of ubisemiquinone and $H_2O_2$. This quenching of the initiating perferryl radicals, which prevent propagation of lipid peroxidation, protects not only lipids, but also proteins from oxidation. In addition, the reduced form of CoQ effectively regenerates vitamin E from the a-tocopheroxyl radical, thereby interfering with the propagation step **(McConnell *et al.*, 2001)**.

The goal of this study is to test whether Colostrum or Coenzyme Q10 inhibit the proliferation of tumour cells without damaging the normal cells, in experimentally liquid tumour induced by Erlich ascites and $CCL_4$ in female mice.

## 2. Materials and Methods

**Materials:**

Corn oil is oil extracted from the germ of corn (maize), 100% pure donated from supermarket. Cows Bovine Colostrum is a milky fluid that comes from the breasts of the first few days after giving birth, before true milk appears. Coenzyme Q10 is donated from Sigma Aldrich. Ehrlish ascites carcinoma cells were purchased from National Institute of Cancer, Cairo, Egypt. Carbon tetra chloride ($CCL_4$) is donated from Sigma Aldrich.

**Animals and Diets:**

Female Swiss albino mice weighing 23 ±3 g were purchased from animal house of National institute of cancer for experimental animals, Cairo, Egypt. They were raired in the animal house of

Zoology Department, Faculty of science, Mansoura University, Egypt.

The animals were housed in cages in groups, 12 mouse per cage, in a controlled environment (25±2 ºC, 50-60% relative humidity and 12- hour light-dark cycle).

The animals were fed standard rodent diet and allowed water ad libitum for a week, and were then randomly assigned to 7 groups: control untreated group, corn oil group (corn oil orally administred at adose of 1.5 ml / kg from the first day to the last day), colostrum group (colostrum orally administred at a dose of 50 mg / kg from the first day to the last day), coenzyme Q10 group (coenzyme Q10 orally administred at adose of 100 mg / kg from the first day to the last day), colostrum+EAC+$CCL_4$ group (colostrum administred orally at adose of 50 mg / kg and $CCL_4$ administred orally at a dose of 1.5 ml / kg from the first day to the last day, while EAC were injected intradermal in the 21th day only at a dose ($0.02x10^7$ cells)), coenzyme Q10 +EAC+$CCL_4$ group (coenzyme Q10 administred orally at a dose of 100 mg / kg and $CCL_4$ administred orally at a dose of 1.5 ml / kg from the first day to the last day, while EAC were injected pretonial in the 21 th day only by dose ($0.02x10^7$ cells)), EAC+$CCL_4$ group (Erlich ascites carcinoma cells where injected pretonial in the 21 th day only by dose ($0.02x10^7$ cells) while $CCL_4$ administrated orally at a dose of 1.5 ml / kg from the first day to the last day ).

After 21 th day from $CCL_4$ adminstration, EAC was injected, then after 12 day mice were scarificed by cervical decapitation and viliability was assessed. Blood samples were collected in heparinized tubes and tissue samples (liver & kidney) were quickly separeted and weighed, stored at -20° C for future analysis.

Viability of EAC:

The fresh cells were stained with trypan blue dye according to the method of, **(Boyse -Wetzel *et al.*, 1964)** the cells that did not stained were viable and non viable cells were counted. The percentage of residual cell viability was calculated using the following formula:

$$\% \text{ Residual Cells} = \frac{\text{Negative Trypan Blue Cells}}{\text{Positive Trypan Blue Cells + Negative Trypan Blue Cells}} \times 100$$

**Tumour markers**

Arginase activity was determined by colorimetric kit described by Marsch et al., 1965 (Biodiagnostic company, 29 Tahreer St., Dokki, Giza, Egypt). α L Fucosidase activity was determined using the method of El-Houseini et al., 2005 ( Biodiagnostic company, 29 Tahreer St., Dokki, Giza, Egypt ). P53 was assessed in cell suspension using the method Descripied by Sironi et al., (1999) Bcl2 was estimated in cell suspension using the method of pezzella et al., (1990).

**Oxidative stress biomarkers**

Malondialdehyde was assessed as described by Ohkawa, et al. (1979) by using Colorimetric kit purchased from (Biodiagnostic company, 29 Tahreer St., Dokki, Giza, Egypt). Protein carbonyl content was measured by first forming labelled protein hydrazone derivatives using 2, 4- dinitrophenylhydrazide Smith et al., (1991). GSH was assessed as described by Beutler et.al, (1963) using Colorimetric kit purchased from (Biodiagnostic company, 29 Tahreer St., Dokki, Giza, Egypt). Super oxide was assessed as described by Nishikimi et al., (1986) using Colorimetric kit purchased from (Biodiagnostic company, 29 Tahreer St., Dokki, Giza, Egypt). Catalase was assessed as described by Aebi, (1984) using Colorimetric kit purchased from (Biodiagnostic company, 29 Tahreer St., Dokki, Giza, Egypt). Glutathione-S-transefrase was assessed as described by Habig and Pabst (1974) using Colorimetric kit purchased from (Biodiagnostic company, 29 Tahreer St., Dokki, Giza, Egypt).

**Histological technique**

Mice were sacrificed by decapitation under light isoflurane anesthesia. Kidneys and liver were rapidly dissected out, tiny pieces from both organs were immediately fixed in 10% formol saline for paraffin sections (5 µ thickness) stained with H & E and Masson'strichrome stains **(Bancroft and Layton, 2013)**.

**Statistical Analysis:**

The results were analysed by One Way ANOVA (analysis of variance) test and compared using Tukey test. The results were expressed as mean ± standard error (SE). The values of $p \leq 0.05$ were considered statistically significant **(Snedecor and Cochran, 1982)**.

## 3. Results

Administration Colostrum or Coenzyme Q10, prior to EAC+ $CCl_4$ inoculation resulted a significant decrease ($P<0.05$) in the viability as compared to EAC+ $CCl_4$ group (Table1). EAC implantation and $CCl_4$ administration causes significant increase ($P<0.05$) in the serum arginase and α L-fucosidase activities as compared to their activates in control group. Administration Colostrum or Coenzyme Q10, prior to EAC+ $CCl_4$ inoculation produced significant amelioration ($P<0.05$) in the serum arginase, L-fucosidase and in Bcl2 and proliferation in P53 activities as compared to that of EAC control group.

Table (2) showed that EAC+ $CCl_4$ induces significant increase ($P<0.05$) in MDA and PC and significant decrease ($P<0.05$) in GSH, CAT, SOD and GST as compared to that of the normal control group. Administration of Colostrum or Coenzyme Q10 prior

to EAC + $CCl_4$ inoculation produced significant decrease ($P<0.05$) in MDA and PC and significant increase in GSH, CAT, SOD and GST as compared to that of EAC+ $CCl_4$ group.

**Table (1) Protective effect of Colostrum or Cooenzyme Q10 on vailability, serum arginase and alpha L fucosidase activities as a tumour marker as well as Bcl2 % as antiapoptotic marker, P53 as apoptoic and oncogenic markers in control and different treated mice groups**

| Animal groups | Control | Corn Oil | Colostrum | Coenzyme Q10 | Erlich+ccl4 | Colostrum+ Erlich+ccl4 | Coenzyme Q10+ Erlich+ccl4 |
|---|---|---|---|---|---|---|---|
| Vailability % | | | | | 25.6±0.4 | 10.5±0.1[b] | 20.2±0.3[b] |
| Arginase (U/L) | 145.5±9.3 | 145.1±6.5 | 143.2±6.9 | 142.2±5.9 | 318.2±7.4[ab] | 203.7±7.4[ab] | 249.7±8.8[ab] |
| alpha L fucosidase (U/L) | 2.76±0.04 | 2.81±0.04 | 2.83±0.05 | 2.85±0.03 | 8.41±0.2[ab] | 5.14±0.1[ab] | 5.99±0.3[ab] |
| BCL-2 (liver) % | 46.7±0.5 | 46.4±0.9 | 49.8±0.7 | 46.2±1.4 | 63.6±0.9[a] | 54.03±1.3[ab] | 60.7±2.4[a] |
| BCL-2 (kidney)% | 46.6±0.5 | 47.7±0.6 | 47.0±0.6 | 43.7±1.2 | 66.7±0.5[a] | 54.7±1.2[ab] | 57.6±0.8[ab] |
| P53 (liver) % | 42.8±0.7 | 47.4±0.7[a] | 44.9±1.2 | 45.5±0.5 | 25.7±0.8[a] | 38.3±1.2[ab] | 33.1±0.9[ab] |
| P53 (kidney) % | 43.5±0.7 | 44.4±0.9 | 46.5±0.5 | 47.9±0.5[a] | 26.5±0.9[a] | 36.9±1.1[ab] | 34.2±1.2[ab] |

[a] significant against control group $p\leq 0.05$; [b] significant against Ehrlich + CCl4 group group $p\leq 0.05$.

**Table (2): The antioxidant effect of Colostrum or Coenzyme Q10 in MDA, GSH, CAT, SOD, GST and PC in control and different treated mice groups.**

| Animal groups | Control | Corn Oil | Colostrum | Coenzyme Q10 | Erlich+ccl4 | Colostrum+ Erlich+ccl4 | Coenzyme Q10+ Erlich+ccl4 |
|---|---|---|---|---|---|---|---|
| MDA (liver) (nmol / g.tissue) | 70.7±0.5 | 71.1±0.5 | 31.5±0.4[a] | 42.1±0.4[a] | 158.5±0.6[a] | 132.9±0.5[ab] | 140.1±0.4[ab] |
| MDA (kidney) (nmol / g.tissue) | 67.6±0.5 | 67.2±0.5 | 27.6±0.5[a] | 38.1±0.4[a] | 154.6±0.4[a] | 134.7±0.5[ab] | 139.9±0.5[ab] |
| PC (liver) (µmol DNPH / mg. wet tissue) | 8.9±0.7 | 8.9±0.6 | 6.4±0.1[a] | 7.4±0.1 | 16.9±0.2[a] | 12.9±0.1[ab] | 13.9±0.2[ab] |
| PC (kidney) (µmol DNPH / mg. wet tissue) | 7.5±0.1 | 7.4±0.2 | 6.5±0.2[a] | 7.3±0.2 | 15.7±0.3[a] | 12.2±0.2[ab] | 13.2±0.1[ab] |
| GSH (liver) (mg / g. tissue) | 49.5±0.8 | 51.7±0.6 | 57.1±1.8[a] | 56.9±0.3[a] | 25.1±0.5[a] | 35.7±0.6[ab] | 30.8±0.8[ab] |
| GSH (kidney) (mg / g. tissue) | 39.6±0.3 | 41.4±0.4 | 49.5±0.4[a] | 46.8±0.4[a] | 14.9±0.3[a] | 25.4±0.3[ab] | 20.2±0.4[ab] |
| SOD (liver) (U / g. tissue) | 27.8±0.2 | 28.8±0.1 | 25.6±0.3 | 24.6±0.6 | 20.5±0.4[a] | 22.8±0.7[a] | 21.4±0.9[a] |
| SOD (kidney) (U / g. tissue) | 25.6±0.2 | 28.2±0.2 | 23.6±0.9 | 22.1±0.9 | 17.2±2.8[a] | 20.6±1.5 | 18.4±2.2[a] |
| CAT (liver) (U / g. tissue) | 116.9±0.3 | 118.1±0.3[a] | 115.3±0.2[a] | 114.4±0.4[a] | 32.7.±0.2[a] | 81.7±0.2[ab] | 57.1±0.2[ab] |
| CAT (kidney) (U / g. tissue) | 114.9±0.2 | 115.8±0.2 | 113.2±0.2[a] | 111.8±0.3[a] | 30.7±0.2[a] | 79.5±0.2[ab] | 55.1±0.2[ab] |
| GST (liver) (U / g. tissue) | 7.1±0.1 | 7.6±0.1[a] | 8.8±0.1[a] | 8.2±0.1[a] | 3.7±0.1[a] | 4.8±0.1[ab] | 4.3±0.1[ab] |
| GST (kidney) (U / g. tissue) | 6.4±0.1 | 7.1±0.1[a] | 8.2±0.1[a] | 7.6±0.1[a] | 3.1±0.1[a] | 4.3±0.1[ab] | 3.6±0.1[ab] |

[a] significant against control group $p\leq 0.05$; [b] significant against Ehrlich + CCl4 group group $p\leq 0.05$.

## 4. Discussion

In this study, Administration of both Colostrum and Coenzyme Q10 to the normal animals for 33 day did not produce any significant modification in the animal's body weight gain by the end of the experimental period, compared to the normal control, this result indicate that both Colostrum and Coenzyme Q10, have produced no toxic biocompatible, biodegradable effect or afavorable effect on the growth rate of the animal used, a view which in agreements with **Vinsova and Vavrikova (2008)**.

In the present results, EACc inoculation and $CCL_4$ induces significant increase in Availability of EAC cells %; this result agree with **Bergamini-santos *et al.* (2004)** that may lead to the neutrophilic inflammatory response which is essential to Ehrlich tumor controlling. The administration of Colostrum or Coenzyme Q10 to Ehrlich ascites and $CCl_4$ mice treated group showed significant decrease in Availability when compared with the mice loaded with EAC-$CCL_4$, this result may be attributed to the anti-inflammatory action of bovine colostrum lactoferrin **(Nishino *et al.*, 2002)** and antioxidant acivity of coenzyme Q10 **(Folkers *et al.*, 1993)**.

The present data showed that, EACc inoculation and $CCL_4$ induces significant increase in serum arginase activity compared to that of the normal control, this result agree with **Erbas *et al.* (2007)** who observed that, in the tumor induced by Ehrlich ascites in female mice there was a significant increase in serum arginase activity. The administration of colostrum or coenzyme Q10 showed significant decrease serum arginase activity when compared with the mice loaded with EAC-$CCL_4$, this result may be attributed to the anti-inflammatory action of bovine colostrum lactoferrin **(Wakabayashi, 2000)** and antioxidant activity of coenzyme Q10 **(Littarru and Tiano, 2007).**

EACc inoculation and $CCL_4$ induces significant increase in Serum α L fucosidase (AFU) activity as compared to that of the normal control, this result agree with **Grizzi *et al.* (2007)** who noted that, in the tumor induced by Ehrlich ascites in female mice there was a significant increase in AFU activity which indicate the appearance of cancer. The administration of colostrum or coenzyme Q10 showed decrease in AFU activity when compared with the mice loaded with liquid tumor, this result agree with **Kawamori *et al.* (1999)** and may be due to the anti-inflammatory action of bovine colostrum lactoferrin, while Coenzyme Q10 exhibited significant antitumor and might be show oxidative metabolism, an explantion which in accordance with **Overvad *et al.* (1999)**.

The present data showed that in the liquid tumor induced by Ehrlich ascites and $CCL_4$ in female mice, there was a significant increase in $Bcl_2$ % when compared with normal one, this result agree with the previous study of **Tsujimoto *et al.* (1985)** who noted

that, $BcL_2$, is as a proto-oncogene exceed in a follicular B-cell lymphoma as a proto-oncogene. The administration of colostrum or coenzyme $Q_{10}$ pre EACc and $CCL_4$ showed decrease in $Bcl_2$ % when compared with the mice loaded with liquid tumor only, These results agree with **Tsuda *et al.* (2002)** who noted that, the mechanism of bovine colostrum lactoferrin cellular defense and coenzyme Q10 against ROS generation as reported by **Beyer, (1996).**

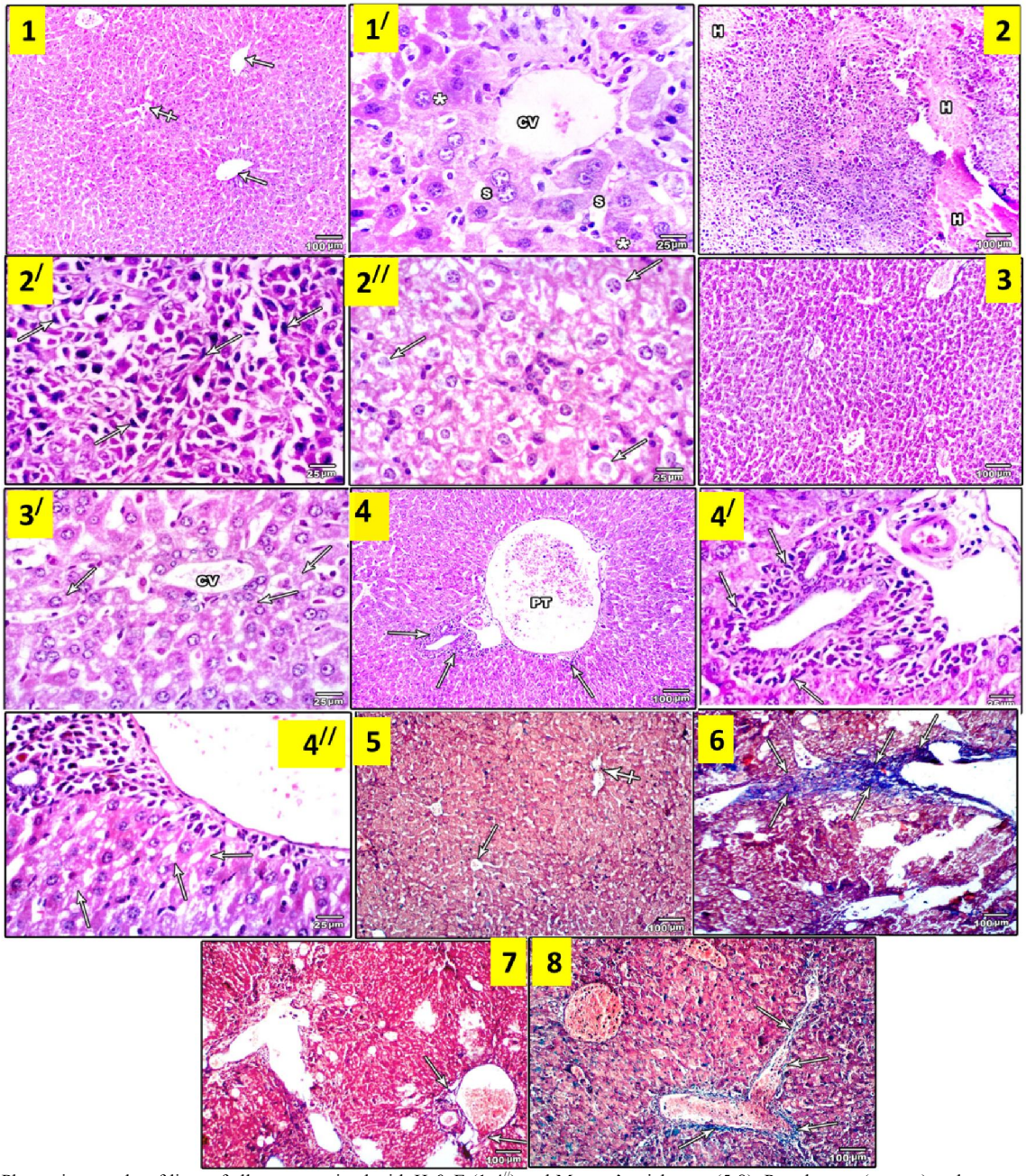


Fig. a. Photomicrographs of liver of all groups stained with H & E (1-4$^{//}$) and Masson's trichrome (5-8). Portal tracts (arrows) and a central vein (crossed arrow) are seen in the control group (1). A higher magnification (1$^{/}$) showing the central vein (CV) and hepatocytes (*) separated by blood sinusoids (S). EAC + $CCL_4$ group (2) demonstrates massive infiltration with Erlichs cells and wide areas of haemorrhage inbetween (H). A higher magnification showing Erlichs cells (arrows) (2$^{/}$) and marked vacuolation of the majority of hepatocytes (arrows) (2$^{//}$). Note the absence of infiltrating Erlichs cells in Colostrum + EAC + $CCL_4$ group (3). The higher magnification (3$^{/}$) shows hepatocytes with minimal cytoplasmic vacuolation (arrows). Coenzyme Q10 + EAC + $CCL_4$ group demonstrates small areas of focal infiltration (arrows) around portal tract (PT) (4 & 4$^{/}$) and minimal cytoplasmic vacuolation (arrows) in the hepatocytes (4$^{//}$). Masson's trichrome of control group (5) demonstrates thin connective tissue at the areas of portal tracts (crossed arrow) and in the wall of central vein (arrow). EAC + $CCL_4$ group liver shows extensive fibrosis (arrow) in liver parenchyma (6). Colostrum + EAC + $CCL_4$ group (7) displays slightly larger amount of connective tissue (arrows) as compared to the control group. Coenzyme Q10 + EAC + $CCL_4$ group (8) illustrates moderate amount of fibrous tissue (arrows). (1,2,3, 4,5,6,7,8): ×100, scale bar = 100 µm). (1$^{/}$,2$^{/}$,2$^{//}$,3$^{/}$,4$^{/}$,4$^{//}$: ×400, scale bar = 25 µm).

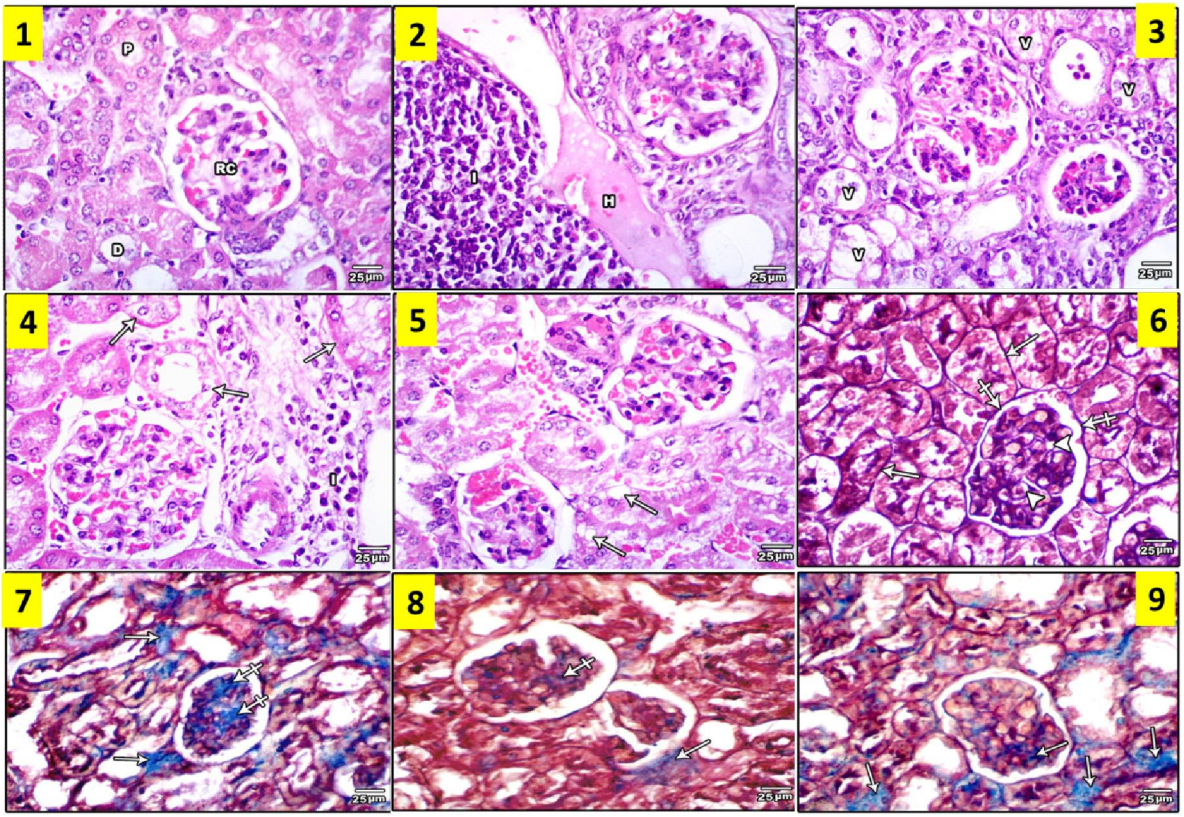


Fig. b. Photomicrographs of the renal cortex of all groups stained with H & E (1-5) and Masson's trichrome (6-9). Renal corpuscles (RC) proximal (P) and distal (D) convoluted tubules are seen in the control group (1). Erlich+$CCl_4$ group displays severe interstitial nephritis (I) haemorrhage (H) and marked vacuolation of renal tubules lining cells (2 and 3). Coenzyme Q10+ Erlich+$CCL_4$ group shows minimal vacuolation (arrows) and interstitial nephritis (I) (4). Colostrum + EAC + $CCL_4$ group displays minimal cytoplasmic vacuolation (arrows) (5). Masson's trichrome of control group (6) demonstrates thin connective tissue around renal tubules (arrows), around renal corpuscles (crossed arrows) and in the renal glomerulus (head arrows). EAC + $CCL_4$ group (7) kidney shows massive interstitial (arrows) and glomerular fibrosis (crossed arrows). Colostrum + EAC + $CCL_4$ group (8) illustrates minimal amount of interstitial (arrow) and glomerular (crossed arrow). Coenzyme Q10 + EAC + $CCL_4$ group (9) shows slightly less amount of fibrosis (arrows) as compared to EAC + $CCL_4$ group (7). (×400, scale bar = 25 µm).

The reduced P53 in tumor loaded group is not unexpected, and agree with **Wilson *et al.* (1997)** and may be as a result of the tumor suppressor function of P53 as mentioned by **Irani *et al.* (2011)**. The tumor-suppressor protein p53 accumulates when DNA is damaged due to a chain of biochemical factors. Part of this pathway includes alpha-interferon and beta-interferon, which induce transcription of the p53 gene, resulting in the increase of P53 protein level and enhancement of cancer cell-apoptosis. P53 prevents the cell from replicating by stopping the cell cycle at G1, or interphase, to give the cell time to repair, however it will induce apoptosis if damage is extensive and repair efforts fail. Any disruption to the regulation of the p53 or interferon genes will result in impaired apoptosis and the possible formation of tumors (**Takaoka *et al.*, 2003)**. This apoptosis may be attributed due to the activation of P53 leading to DNA damage as reported by **Wilson *et al.* (1997)**, the administration of colostrum or coenzyme Q10 showed significant increase in P53 % when compared with that in the mice loaded with liquid tumor only, these results may be attributed to the potant antioxidant acivity of bovine colostrum lactoferrin **(Owour and Kong, 2002)**; **Folkers *et al.*, 1993)** respectively.

The present data showed that EAC inoculation induces significant increase in liver and kidney tissues malondealdehyde (MDA) and protein carbonyl (PC) levels as compared to that of the normal control. The increased MDA level in tumor loaded group may be as a result of the excessive free radical production as reported by **Gupta *et al.* (2004)**. The administration of

colostrum or coenzyme Q10 showed significant reduction in hepatic and tumor tissues malondialdehyde (MDA) and protein carbonyl (PC) levels when compared with the mice loaded with EAC-$CCL_4$, a result which may be attributed to the antioxidant activity of colostrum or may be attributed to the bovine colostrum lactoferrin enhancement of the cell killing activity of cytotoxic T and natural killer cells as reported by **Tsuda *et al.* (2006)** and antioxidant activity of coenzyme $Q_{10}$, a view which in accordance with **Beyer *et al.* (1992)**.

In the present study, the significant decreases of GSH, SOD, CAT and GST, upon EAC-$CCL_4$ administration, indicated that EAC-$CCL_4$ destroyed antioxidant defense system in mice, these free radicals also attack antioxidant defense system, leading to the loss of antioxidant components such as GSH, SOD, CAT and GST (**Cheshchevik *et al.*, 2012)**. Administration of colostrum and Coenzyme $Q_{10}$ improve the antioxidant defense system and protect the Liver and kidney tissue cells from damaging induced by EAC-$CCL_4$ through decreasing the oxidative stress and support the antioxidant defense system **(Black, 2002; Conklin; 2005).**

**In conclusion**, the present study provided obvious evidence on the beneficial effects of colostrum and coenzyme Q10 in reducing tumor growth and counteracting the metabolic disorders associated with female mice inoculated with liquid tumour model and may offer novel approaches to cancer therapy.

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