Efficacy of Colostrum or Coenzyme Q10 against some organ dysfunction induced by CCL_4 and EAC in mice

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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] ($0.02X10^7$ cells) and CCL_4 (1.5 ml / kg) in female mice were evaluated. Casepase 9 and Casepase 3 as well as Bax-1 were significantly decreased in EAC treated mice. In addition CD4, CD8, CD95 and Sub G1 significantly increased. These abnormalities are accompanied by increased the serum ALT, AST, T. bilirubin, Creatinine, Urea and uric acid and decline in Total protein and Albumin as well as disturbe mineral levels. Mice pretreated with Colostrum or Coenzyme Q10 then with EAC + CCL_4 showed marked protection against induced tumor substance.

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1. Introduction

Hepatorenal Toxicity (HRT) is a particular and common type of kidney failure that affects with liver cirrhosis or, less commonly, with fulminant liver failure, **(Arroyo *et al.*, 1996)**. HRT involves constriction of the blood vessels of the kidneys and dilation of blood vessels in the splanchnic circulation, which supplies the intestines, **Ginès and Arroyo (1999).**

Colostrum expressed on day 4 of lactation, and breast milk expressed on day 8. Colostrum often has a yellow hue compared to breast milk. Colostrum has antioxidant components, such as lactoferrin, and hemopexin, which binds free heme in the body, **Gutteridge and Smith (1988)**.

Hagiwara *et al.* (2000) reported that, IgG levels in natural colostrums is active towards 19 specific human pathogens were just as high as in hyperimmune colostrum, and natural colostrum nearly always had higher antibody titers than did the hyperimmune version.

Coenzyme Q10 is an oil-soluble, vitamin-like substance which is present in most eukaryotic cells, primarily in the mitochondria. It is a component of the electron transport chain and participates in aerobic cellular respiration, generating energy in the form of ATP, Therefore, those organs with the highest energy requirements such as the heart, liver and kidney have the highest CoQ10 concentrations, There are three redox states of coenzyme Q10: fully oxidized (ubiquinone), semiquinone (ubisemiquinone), and fully reduced (ubiquinol). The capacity of this molecule to exist in a completely oxidized form and a completely reduced form enables it to perform its functions in the electron transport chain and as an antioxidant respectively **Montero *et al.* (2008)**.

CoQ10 is also being investigated as a treatment for cancer, and as relief from cancer treatment side-effects **(Mortensen *et al.*, 1998).**

The main purpose of the current study is to explore the hepatorenal toxicity complications occurred due to Ehrlich ascites carcinoma (EAC) cells and CCL_4 and to determine whatever Colostrum or Co enzyme Q10 may protect these organs from EAC and CCL_4 adverse effect in female mice.

2. Materials and Methods:

Materials:

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

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 mice per cage, in a controlled environment (25 ºC ±2, 50-60% relative humidity and 12- hour light-dark cycle).

Animal groups:

The animals were fed standard rodent diet and allowed water *ad libitum* for a week to acclimatized,

and they were randomly assigned to 7 groups: control untreated group, corn oil treated group (corn oil orally administered daily at a dose of 1.5 ml / kg), colostrum treated group (colostrum orally administered daily at a dose of 50 mg / kg), coenzyme Q10 treated group (coenzyme Q10 orally administered at a dose of 100 mg / kg from the first day to the last day), colostrum+EAC+CCL_4 group (colostrum orally administered daily at a dose of 50 mg / kg and CCL_4 administered daily orally at a dose of 1.5 ml / kg, 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 administered orally at a dose of 100 mg / kg and CCL_4 administred daily orally at a dose of 1.5 ml / kg, while EAC were injected intrapretonial in the 21th day only by dose ($0.02x10^7$ cells)), EAC+CCL_4 group (Erlich ascites carcinoma cells where injected intrapretonial in the 21th day only by dose ($0.02x10^7$ cells) while CCL_4 administred daily orally at a dose of 1.5 ml / kg).

After 21 days from CCL_4 administration, EAC was injected, then after other 12 day mice were sacrificed by cervical decapitation after slight other anesthesia. Blood samples were collected in heparinized tubes and tissue samples (liver & kidney) were quickly separated and weighed, stored at -20° C for future analysis.

Estimated parameters:

Fresh tissue specimens were transported to the laboratory in isotonic saline and prepared according to the method described by **(Tribukait *et al.*, 1975)** as follow:

The tissues were washed with isotone tris EDTA buffer, 3.029 g. of 0.1 M tris (hydroxymethyl aminomethane (CAT. No T-1378, sigma chemical company), 1.022 g. of 0.07 M sodium chloride (ADWIC) and 0.47 g. of 0.005 M EDTA (CAT. No E-6758, sigma). They were dissolved in 250 ml of distilled water and then the PH was adjusted at 7.5 by using 1N HCL.

The cell suspension was centrifuged at 3875882 Xg for 10 min. Where upon the supernatent was aspirated. If they were macroscopically contaminated with blood, it was then subjected to haemolysis with filtered tap water for 10 mins. After centrifugation and aspiration of the supernatent the cell is fucosidase in ice-cold 96-100% ethanol (BDH) in approximately 1 ml for each sample. These fucosidase cells stored indefinitely in a refrigerator until running the sample.

BAX was determined in different cell suspension samples using the method of **Desagher et *al.* (1999)** Caspase 9 was estimated in cell suspension of different sample using the method of **Cho *et al.* (2004)**. CD4 was estimated according to the method of **Kwong *et al.* (1998)**. CD8 were determined in different cell suspension samples by **Gao and Jakobsen (2000)**. CD95 was estimated using the method described by **Lichter *et al.* (1992)**

Serum sodium and potassium were assessed as described by **Trinder (1951)** and **Sunderman and Sunderman (1958)** respectively by using colorimetric and turbidimteric kits. (ALT) and (AST) were assessed as described by **Reitman and Frankel (1957)** using Colorimetric kit. T. bilirubin content was assessed as described by **Walter and Gerade (1970)** using Colorimetric kit. Serum Albumin content and T.protien content were assessed as described by **Doumas *et al.* (1971)** and **Gornal *et al.* (1949)** respectively using Colorimetric kit. Creatinine content was assessed as described by **Schirmeister *et al.* (1964)** using Colorimetric kit. Urea content and uric acid were assessed as described by **Fawcett and Soctt (1960) and Barham and Trinder (1972)** respectively using urease – berthelot method and enzymatic Colorimetric kit. All kits were purchased from (Biodiagnostic company, 29 Tahreer St., Dokki, Giza, Egypt). 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:

See Table(1)- Table(3).

4. Discussion

Despite advances in the cellular and molecular knowledge, hepatorenal toxicity remains one of the major public health problems throughout the world. It encompasses various pathological entities and a wide range of clinical behaviors. Hepatorenal toxicity is a multistep process in which an accumulation of genetic events leads to a progressively dysplastic cellular appearance, deregulated cell growth, and finally, carcinoma **(Anne *et al.*, 2004)**. The search for new sources of biologically active compounds is important for the discovery of new drugs for the treatment of hepatorenal toxicity.

The present data showed that, EACc inoculation and CCL_4 induces significant decrease in caspase 9 and caspase 3%, this result agree with the previous study of **Chen *et al.* (2005)** who reported that, the increased cytotoxicity in response to SRC inhibition was associated with a large increase in processing and activation of caspase 3, activation of caspase 3 seems to be independent of cytochrome C release and caspase 9 activation, the SRC tyrosine Kinase may provide an important target for small molecule inhibition in cancer. Also, **Tenev *et al.* (2001)** demonstrated that, the pro-caspase-3 can sensitize cells to proteasome inhibitor-induced apoptosis. The

administration of colostrum and coenzyme Q10 showed increase in caspase 3 and caspase 9 %, maybe due to an immunomodulatory effect of bovine colostrum lactoferrin **(Kelloff *et al.* 1994)** and coenzyme Q10 (**Folkers *et al.*, 1982)**.

Table (1) Protective effect of Colostrum or Coenzyme Q10 on Casepase 9,3 as an apoptotic marker, Bax-1 as a primary apoptotic marker, CD4 as T helper cells marker, CD8 as T cytotoxic marker, CD95 as fast ligand apoptotic marker as well as Sub G1 as apoptosis phase in control and different treated mice groups ($\bar{X}$±S.E)

Animal groups / Parameters	Control	Corn Oil	Colostrum	Coenzyme Q10	Erlich+ CCL_4	Colostrum+ Erlich+ CCL_4	Coenzyme Q10+ Erlich+ CCL_4
(liver) Caspase 9 %	46.7±0.5	51.9±0.6[a]	53.1±0.6[a]	57.4±0.8[a]	29.8±0.7[a]	42.4±0.6[ab]	33.6±0.9[ab]
(kidney) Caspase 9 %	48.1±0.5	52.1±0.8[a]	57.7±0.4[a]	56.9±0.5[a]	26.8±0.7[a]	45.3±0.5[b]	33.9±1.1[ab]
(liver) Caspase 3 %	58.9±0.5	56.5±0.3	63.3±0.5[a]	63.9±0.9[a]	26.2±1.2[a]	41.5±0.9[ab]	33.9±0.6[ab]
(kidney) Caspase 3%	58.4±0.3	58.3±0.4	66.5±0.4[a]	65.9±1.1[a]	28.7±0.6[a]	42.7±0.8[ab]	36.2±0.9[ab]
(liver) Bax-1 %	47.08±0.8	48.02±0.9	54.4±0.4[a]	48.4±0.4	30.8±0.5[a]	40.7±0.7[ab]	33.8±0.8[ab]
(kidney) Bax-1 %	46.7±0.6	47.5±0.6	52.3±0.6[a]	48.3±0.4	31.5±1.1[a]	38.5±0.5[ab]	33.9±0.9[a]
(liver) CD4 %	24.1±0.4	26.4±0.5[a]	24.9±0.4	25.8±0.6	43.4±0.4[a]	32.4±0.3[ab]	36.2±0.3[ab]
(kidney) CD4 %	25.36±0.5	26.8±0.5	25.48±0.4	26.3±0.5	41.6±0.5[a]	32.8±0.6[ab]	36.4±0.7[ab]
(liver) CD8 %	31.9±0.7	34.9±0.6[a]	31.5±0.4	35.3±0.5[a]	53.4±0.7[a]	40.3±0.7[ab]	46.6±0.6[ab]
(kidney) CD8 %	32.1±0.7	35.5±0.4[a]	32.7±0.5	37.9±0.4[a]	53.9±0.5[a]	40.6±0.6[ab]	46.7±0.5[ab]
(liver) CD95 %	41.83±1.2	46.5±0.5[a]	41.80±0.7	42.7±0.8	75.6±0.5[a]	50.8±0.8[ab]	53.8±0.9[ab]
(kidney) CD95 %	39.1±0.4	45.5±0.5[a]	39.7±0.6	42.2±0.7[a]	74.03±1.2[a]	48.8±0.4[ab]	55.6±0.5[ab]
(liver) Sub G1 %	41.9±0.7	44.02±1.1	42.7±0.7	45.2±0.5	62.6±0.9[a]	48.8±0.7[ab]	55.9±0.9[ab]
(kidney) Sub G1 %	43.1±0.3	43.5±0.7	43.3±0.6	47.4±0.5[a]	63.1±0.9[a]	49.9±1.1[ab]	55.1±0.5[ab]

[a] significant relative to control group p≤ 0.05; [b] significant relative to Ehrlich + CCl_4 group group p≤ 0.05.

Table (2): The effect of Colostrum or Coenzyme Q10 on liver and kidney function tests in control and different treated mice groups ($\bar{X}$±S.E)

Animal groups / Parameters	Control	Corn Oil	Colostrum	Coenzyme Q10	Erlich+ CCL_4	Colostrum+ Erlich+ CCL_4	Coenzyme Q10+ Erlich+ CCL_4
ALT (U/L)	40.4±1.01	53.1±0.8[a]	40.8±1.1	33.4±0.8[a]	85.3±1.3[a]	64.9±1.12[ab]	75.8±1.1[ab]
AST (U/L)	36.9±0.7	43±0.8[a]	22.9±0.8[a]	33.8±0.6	75.2±1.3[a]	54.9±0.8[ab]	65.5±1.2[ab]
T. Bilirubin (gm /dL)	0.02±0.005	0.07±0.02	0.03±0.004	0.1±0.001	1.4±0.1[a]	0.2±0.03[ab]	0.5±0.01[ab]
Albumin (gm /dL)	4.3±0.2	3.4±0.1[a]	4.1±0.1	4.5±0.2	1.3±0.1[a]	3.5±0.2[ab]	2.4±0.2[ab]
Total protein (gm /dL)	7.4±0.2	7.1±0.2	7.5±0.3	8.4±0.3	3.8±0.2[a]	6±0.2[a]	4.8±0.3[ab]
Creatinine (mg /dL)	0.5±0.2	0.7±0.1	0.6±0.1	0.5±0.1	7.8±0.2[a]	3.1±0.3[ab]	4.2±0.2[ab]
Urea (mg /dL)	26.7±3.2	28.5±2.9	26.8±2.8	26.2±2.7	90.5±2.2[a]	40.5±2.8[ab]	53.2±1.5[ab]
Uric acid (mg /dL)	5±0.2	3.4±0.3[a]	6.5±0.1[a]	7.5±0.1[a]	12.8±0.6[a]	8.5±0.1[ab]	9.6±0.1[ab]

[a] significant against control group p≤ 0.05; [b] significant against Ehrlich + CCl_4 group group p≤ 0.05.

Table (3) Effect of Colostrum or Coenzyme Q10 on serum sodium and potassium concentrations in control and different treated mice groups ($\bar{X}$±S.E)

Animal groups / Parameters	Control	Corn Oil	Colostrum	Coenzyme Q10	Erlich+ CCL_4	Colostrum+ Erlich+ CCL_4	Coenzyme Q10+ Erlich+ CCL_4
Sodium (mEq / L)	144.5±2.9	151.2±0.9	141.8±2.7	137.5±1.5	85.2±1.8[a]	116±1.6[ab]	94.5±1.7[ab]
Potassium (mEq / L)	4.5±0.3	5.2±0.2	5.8±0.2[a]	6.2±0.4[a]	14.2±0.4[a]	9.9±0.2[ab]	11.7±0.2[ab]

[a] significant against control group p≤ 0.05; [b] significant against Ehrlich + CCl_4 group group p≤ 0.05.

Administration of colostrum or coenzyme Q10 can ameliorate EAC-CCL_4 mediated hepatic apoptosis in mice, such as endogenous antioxidant compounds. EAC-CCL_4 has an anti-apoptotic effect through the inhibition of hepatic apoptosis decreased bax-1. It significantly inhibited hepatocyte apoptosis by down-regulating the expression of caspase-3 and inhibiting the release of cytochrome C from mitochondria into the cytoplasm **(Tang *et al.*, 2007)**. Here we demonstrated that intervention with EAC-CCL_4 or the early stage of chronic liver disease effectively attenuated bax-1 -dependent hepatocyte apoptosis and liver fibrosis, thus retarding disease progression in animals **(Shah *et al.*, 2016)**. **Yasuda *et al.* (2000)** showed that EAC-CCL_4 can induce acute hepatocellular damage which is characterized by necrotic cell death, while **Shi *et al.* (1998)** indicated that, a substantial number of hepatocytes undergo apoptosis in the acute stage after EAC-CCL_4 administration. In the present study, both apoptosis and necrosis occurred in the CC L_4-induced chronic liver injury model, but the colostrum, coenzyme Q10 upregulate by induce apoptosis increasing bax-1. **(Tsuda *et al.*, 2006; Crane *et al.*, 1994)**.

The present data showed that, EACc inoculation and CCL_4 induces significant increase in CD4, CD8

and CD95 %, these results agree with the study of **Zhao *et al*. (2008)** who demonstrated that, Cellular and humoral immune responses are essential for the prevention and defense against inflammation and toxicity. The administration of colostrum or coenzyme Q10 showed decrease CD4, CD8 and CD95 %, which reveals an immunomodulatory effect of bovine colostrum lactoferrin **(Kelloff *et al.*, 1994)** and coenzyme Q10 **(Folkers *et al*., 1982).**

Liver and Kidney tissues of the EAC-CCL_4 animal group and treated with colostrum and coenzyme Q10 showed an accumulation of cells in the Sub G1 of the cell cycle. In this study the population of cells in the Sub G1increased profoundly in EAC-CCL_4 animal group, while the decrease in the Sub G1 after the administration of colostrum and coenzyme Q10 was observed which indicated that bovine colostrum and coenzyme Q10 could delay or inhibit cell cycle progression through Sub G1 (**Owuor and Kong, 2002; Beyer 1992**). This delay may be attributed to the ability of both bovine colostrum and coenzyme Q10 as antioxidant activity to bind with DNA arresting cell cycle and causes apoptosis (**Tsuda *et al*., 2006; Sakata *et al*., 2008**). The role played by ROS in cellular processes including DNA damage, mitochondrial dysfunction, activation of signaling pathways and activation of transcription factors also leading to upregulate genes as mentioned by **Schumacker (2006)**.

Alterations of the entire liver functions, with degenerative changes, were observed after EAC-CCL_4 administration. CCl4 causes an increase in the ALT, AST, T. bilirubin and decrease in Albumin, Total protein since liver damage releases these enzymes and proteins in the blood circulation after the administration of hepatotoxin; such as EAC-CCL_4 **(Kew, 2000)**. The toxicity of CCl_4 is initiated by formation of a reactive metabolite trichlormethyl radical ($CCl3^{\cdot}$) and trichloromethylperoxy radical ($CCl3OO^{\cdot}$) by liver microsomal CYP450 which attacks and destroys polyunsaturated fatty acids **(Vajdovich *et al*., 1995)**, the result indicate the improvement of liver function markers after adminstration of bovine colostrum **(Oh *et al*., 2010)** and coenzyme Q10 **(Folkers *et al*., 1982).**

Nephrotoxicity induced in mice by EAC-CCL_4 was manifested by a marked increase in serum urea, uric acid and creatinine levels and distruction in secretion of hormones, gluconeogenesis and extracellular homeostasis of pH and blood components. These results were previously reported (**Ozturk *et al*., 2003; Makni *et al*., 2013; Rahmat *et al*., 2014)**. Uric acid level was the most affected renal marker, followed by creatinine level and then urea concentration. Mean while, **Bashandy and Al-Wasel (2011)**, demonstrated that urea and creatinine levels were elevated by EAC-CCL_4 intoxication. Colostrum and Coenzyme Q10 used in these experiments improve the damaging loaded on liver and kidney these results may be attributed to scavenging of oxidative stresss that's result in EAC-CCL_4 exposed (**Owour and Kong, 2002**; **Folkers *et al*., 1993)** respectively with improvement in reabsorption, synthesis and enezymatic action.

EAC-CCL_4 results in increases potassium, and decreased in sodium concentrartions, but when administred with colostrum or coenzyme Q10, the result showed increased in sodium and decreased in potassium, that's mean improvement of Na-K levels, this results may be attributed to scavenging action as well as antioxidative role of colostrum **(Oh *et al*., 2010)** as well as coenzyme Q10 (**Beyer et *al*., 1992)**

In conclusion, the present study provided obvious evidence on the beneficial effects of colostrum and Coenzyme Q10 in reducing hepatorenal toxicity and counteracting the metabolic disorders associated with female mice inoculated with ehrlish assicites carcinoma cells and CCl_4 model and may offer novel approaches to cancer therapy.

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