MODULATORY EFFECTS OF L-CARNITINE ON TAMOXIFEN TOXICITY AND ONCOLYTIC ACTIVITY: *IN VIVO* STUDY

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ABSTRACT

The aim of this study was to investigate the protective effect of L-carnitine (L-CAR) in tamoxifen (TAM)–induced toxicity and antitumor activity.Adult female rats were randomly divided into 4 groups. Group I was served as control, groups II and III were injected with (10mg/kg, P.O) TAM and L-CAR (300 mg /kg, i.p.) respectively while, group IV was treated with both compounds.The treatment continued daily for 28 days. Administration of TAM resulted in significant increase in serum lipid profiles, liver enzymes and bilirubin level. In addition, TAM produced significant increase in lipid peroxides (LPO) level accompanied with significant decrease in superoxide dismutase (SOD) activity of hepatic and uterus tissues and significant decrease in glutathione (GSH) content of uterus tissue. Administration of L-CAR prior to TAM treatment decreased significantly serum lipids and liver enzymes and significantly increased SOD activity in liver and uterus tissues compared to TAM treated group. Furthermore, it restored LPO and GSH levels in uterus tissue. On the other hand, the apoptotic markers of caspases 9 and 3 were not detected in liver of all the treated groups. Histopathologically, alterations in the liver and uterus structures after TAM treatment which was attenuated by L-CAR administration. The antitumor effect and survival of the combined treatment of Ehrlich Ascites Carcinoma (EAC)-bearing mice was less than each one alone. In addition, L-CAR interestingly increased survival rate of EAC-bearing mice more than TAM treated group. In conclusion, L-CAR has beneficial effects regarding TAM toxicity; however, it interferes with its antitumor effect.

Key words: Tamoxifen, L-carnitine, organ toxicity, antitumor activity, antioxidants

1- INTRODUCTION

Breast cancer is the leading cause of cancer deaths in women worldwide **(Buijs *et al.*, 2008)**. Tamoxifen (TAM), 1-[4-(2-dimethyl-aminoethoxy) phenyl] - 1, 2-diphenyl-1-butene), a non-steroidal antiestrogen drug, is selective estrogen receptor modulator used in prevention and treatment of all stages of hormone-responsive breast cancer **(Matsuoka *et al.*, 2009**). Beside long treatment with TAM and its widespread uses, attention has been focused on its adverse effects, particularly liver and endometrium toxicity **(Stanley *et al.*, 2001)**. According to studies, adjuvant TAM significantly increases women's risk of subsequently developing endometrial carcinoma **(Singh *et al.*, 2007)**. Fatty liver was observed in more than 30% of breast cancer patients who received TAM treatment **(Albukhari *et al.*, 2009)**. Hepatotoxicity has been described with toxic hepatitis, multifocal hepatic fatty infiltration, submassive hepatic necrosis and cirrhosis **(Parvez *et al.*, 2006)**. In

addition, TAM leads to oxidative liver damage and has been elucidated to be a hepatocarcinogen in rodents which is due to overproduction of oxygen radical during TAM metabolism **(Senkus-Konefka *et al.*, 2004)**. It was demonstrated that TAM caused DNA-adducts in rat liver and in the endometrium **(Chao *et al.*, 2011; Wang *et al.*, 2009)**.

L-CAR (L-trimethyl-3-hydroxy-ammoniabutanoate) is quaternary ammonium compound biosynthesized from amino acids lysine and methionine **(Gulcin, 2006)**. It plays anessential role in human intermediary metabolism **(Doberenz *et al.*, 2007)**. The most prominent function lies in its role in the transport of activated long-chain fatty acids from the cytosol to the mitochondrial matrix where β-oxidation takes place and production of cellular energy **(Vamos *et al.*, 2010)**. L-CAR has antioxidant properties and can reduce oxidative stress and acts as a free-radical scavenger. L-CAR has protective effects against acute and chronic doxorubicin toxicity in rat **(Sayed-Ahmed, 2010)**. In addition, it significantly inhibited preneoplastic lesions and hepatocarcinogenesis in animals **(Hoang *et al.*, 2007)**.Therefore, the current study was designed to test the ability of L-CAR to protect liver and endometrial tissues of TAM–treated rats from toxicity without impediments the antitumor effect.

2-MATERIAL AND METHODS

2.1. Material

2.1.1. Animals

Adult female Wistar albino rats weighing 120–170 g were obtained from the Egyptian Organization for Biological Products and Vaccines (VACSERA, Giza, Egypt) and female Swiss albino mice weighing 18-20g were obtained from animal facility, Pharmacology unit, National Cancer Institute (NCI), Cairo University, Egypt. Animals were kept under standard conditions and were allowed free access to a standard requirement diet and water ad.Libitum. Animals were kept under a controlled lighting condition (light: dark, 13 h: 11 h). The animals' treatment protocol has been approved by the animal care committee of the National Cancer Institute, Cairo University, Egypt.

2.1.2. Drug

TAM citrate was a kind gift from Medical Union Pharmaceuticals Company (MUP), Cairo, Egypt. It is obtained as white powder soluble in sterile water and was diluted to the required concentration before use.

2.1.3. Chemicals

L-CAR was a generous donation from the Pharmacology Unit, National Cancer Institute (NCI), Cairo University, Egypt. All other chemicals and solvents used were of the highest purity grade available.

2.2.Methods

2.2.1. Toxicity study

2.2.1.1. Experimental design

Twenty four female rats were divided randomly into four groups (6 animals each). Rats of group I was administeredwith 0.5 ml of normal saline and served as control group. Rats of group II were administered TAM (10mg/kg, P.O) (Perumal et al., 2005).Rats of group III were injected with L-CAR (300mg/kg, i.p.) **(Muthuswamy *et al.*, 2006)**. Rats of

group IV were injected with L-CAR (300mg/kg, i.p.) 1 hour later; rats were treated with TAM (10mg/kg, P.O). The treatment schedule wascontinued once/day for 28 consecutive days.

Twenty-four hours after the last treatment, animals were anesthetized after exposure to ether in desiccators kept in a well-functioning hood. Blood samples were collected by heart puncture and serum samples were separated. Livers and uterus were quickly excised, washed with saline, blotted with a piece of filter paper and homogenized using a Branson sonifier (250, VWR Scientific, Danbury, CT, USA).

2.2.1.2. Determination of lipid profile

Lipid profile was determined using a standard commercial kit (Spectrum diagnostics,Cairo, Egypt). Serum triglycerides (TG), total cholesterol (CH), high-densitylipoprotein-cholesterol (HDL) were determined according to the methods describedby **Tietz *et al.* (1959), Ellefson and Caraway (1976)** and **Warnick and Wood (1995)**, respectively. Low-density lipoprotein-cholesterol (LDL) was calculated bysubtracting the HDL-cholesterol from total cholesterols described by Terpstra et al.(1982).

2.2.1.3. Determination of liver function test

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities weredetermined according to the method of **Zilva and Pannall (1979)** usingspectrophotometric kit (Spectrum diagnostics, Cairo, Egypt). Total and directbilirubin levels were determined according to the method of **Malloy and Evelyn (1937)**.

2.2.1.4. Determination of tissue superoxide dismutase (SOD)

Superoxide dismutase (SOD) activity in liver and uterus homogenates was determined according to the method of **Minami and Yoshikawa (1979)**. This method is based on thegeneration of superoxide anions by pyrogallol autoxidation, detection of generatedsuperoxide anions by nitro blue tetrazolium (NBT) formazan color development andmeasurement of the amount of generated superoxide anions scavenged by SOD (theinhibitory level of formazan color development). The formazan color developed wasdetermined spectrophotometrically (Spectronic 501, Shimadzu, Japan). Enzymaticactivity was expressed inform of μg/g of tissue.

2.2.1.5. Determination of tissue lipid peroxidation

Malondialdehyde (MDA) levels in liver and uterus tissues homogenates were determined spectrophotometrically using the method of **Buege and Aust (1978)**. MDA content was measured at 535 nm. The results were expressed as nmol/g tissue.

2.2.1.6.. Determination of tissue reduced glutathione

Reduced glutathione (GSH) was determined according to the methods of **Ellman (1959)**, it is based on the reduction of Ellman's reagent [5,5'-dithio-bs- (2-nitrobenzoic acid)] by SH groups to form 1mole of 2-nitro-5- mercaptobenzoic acid per mole of SH. The optical density was measured at 412 nm against a reagent blank and the results were expressed as μmol/g tissue.

2.2.1.7. Determination of tissue total nitrate/nitrite (NO(x))

Total nitrate/nitrite (NO(x)) was measured as stable end product, nitrite, according to the method of **Miranda *et al.* (2001)**. The assay is based on the reduction of nitrate by vanadium trichloride combined with detection by the acidic Griess reaction. The diazotization of sulfanilic acid with nitrite at acidic pH and subsequent coupling with N-(10-naphthyl) ethylenediamine produced an intensely colored product that is measured

spectrophotometrically at 540 nm. The absorbance at 540 nm was measured using a plate reader. The levels of NO(x) were expressed as nmol/g wet tissue in homogenate.

2.2.1.8. Determination of DNA fragmentation: agarose gel electrophoresis

According to the method of Katoh et al. (1996)the liver tissue was homogenized and lysed in a cold lysis buffer (10 mMTris– HCl, 5mM disodium EDTA, and 0.5% Triton X-100, pH 8.0) for 10 min at 4 °C. The DNA was sequentially extracted twice using half volumes of phenol/chloroform and incubated at 55 °C for 10 min. After centrifugation at 3000 rpm for 20 min, the upper layer was incubated with proteinase K at 37 °C for 60 min followed by incubation with ribonuclease at 37 °C for 60 min. The DNA was precipitated by adding 10 M ammonium acetate and 100% ethanol and maintained at −20 °C overnight. DNA was collected by centrifugation at 15,000 x g for 20 min, air-dried, and resuspended in TE buffer (10 mMTris–HCl, 5mM EDTA, pH 7.4). Agarose gel electrophoresis **(Yokozawa and Dong, 2001)** was carried out for the analysis of DNA fragmentation. The resulting DNA preparations were electrophoresed through a 1.4% agarose gel containing ethidium bromide using TBE buffer (Tris-boric acid–EDTA buffer, pH 8.3) at 40 V for 5 h. Equal quantities of DNA (based on optical density measurements at 260 nm) were loaded in each lane, and a molecular DNA marker was used as a molecular mass standard. DNA fragmentation was visualized and photographed under ultraviolet illumination.

2.2.1.9. Determination of apoptotic markers: western blotting

The livers were homogenized with phosphate buffer saline (PBS) followed by centrifugation at 11000 rpm 4˚C for 15 min. Protein content in the resulting supernatant was determined using Bradford reagent (Thermo scientific, USA). Equal volume of supernatant was mixed with 1x loading buffer and 5 µl of β-Mercaptoethanoland boiled for 5 min. Proteins were separated by sodium dodecyl sulfatepolyacrylamidegel electrophoresis (SDS–PAGE) and then electrotransferred onto polyvinylidene fluoride (PVDF) membrane using semidry transfer apparatus (Biometra, Germany).The membrane was blocked according to manufacture instruction of chromogenic western max detection kit (Ameresco, USA). After blocking with dilution buffer plus 1% Tween-20 (DBT) plus 1%bovine serum albumin (BSA), the membrane blots was incubated with indicated primary antibody (in DBT) at4˚C over night then washed three times with DBT buffer, and incubated with horseradish peroxidase-conjugated secondary antibody for 4 h at room temperature. After washing three times with DBT buffer, the protein bands were visualized by 3,3'-Diaminobenzidine (DAB) chromogen which gives brown precipitate at the reaction site, specific protein bands on these transferred membranes were detected using the following antibodies:Purfied antiβ-actin antibody was obtained from(Biolegend, USA) and anti-mousecaspase 3 monoclonal antibody were obtained from (Bioscience, USA).Relative expression of proteins was evaluated by normalizing the expression of proteins with quantitative housekeeping protein β-actin **(Salami and Karami-Tehrani, 2003)**.

2.2.2. Histopathological study

The samples were fixed in 10% neutral buffered formalin, dehydrated through alcohols, cleared in xylene and then embedded in paraffin wax. Sections (5 mm thick) were stained with haematoxylin and eosin **(Albukhari *et al.*, 2009)**.

2.2.3. Antitumor activity

2.2.3.1. Percentage survival of animals

Forty female albino mice inoculated with Ehrlich Ascites Carcinoma (EAC) cells (2.5 X 10^6cells/0.1ml). Each mouse was injected i.p. with EAC cells. Twenty-four hours

after cell inoculation, 40 mice wereclassified into four groups.GroupI; animals were injected with 0.2 ml of saline and servedas control.GroupII; animals were injected once with TAM (10 mg/kg, i.p.). GroupIII; animals were injected once with L-car (300 mg/kg, i.p.). GroupIV; animals were injected once with L-car (300mg/kg, i.p.) followed by TAM in a dose of (10 mg/kg, i.p.) with an in-between interval of 30 min.

The change in the percent survival of animals was recorded daily during a period of 45 days following treatment.

Percent survival= number of living animals/10 X100.

2.2.3.2. Tumor volume:

Forty female mice were used and solid tumor was transplanted subcutaneous in the right thigh of the lower limb of each mouse. Mice with a palpable tumor mass (100mm^3) that developed within 7 days after implantation were divided into 4 groups: Group I**:** ten mice were injected intraperitoneally four times every other day with normal saline and served as control group. Group II**:** ten mice were injected four times every other day with TAM (10mg/kg, i.p.). GroupIII**:** ten mice were injected four times every other day with L-car (300mg/kg, i.p.). Group IV**:** ten mice were injected with L-car (300mg/kg, i.p.), 30 min later, injected with TAM (10mg/kg, i.p.)four times every other day. The change in tumor volume was measured every other day using a Vernir caliper and calculated by following formula according to **Osman *et al.* (1993)**.

TumourVolum (mm^3)= 4$(A/2)^2$X (B/2)/ 3.

Where A and B were denote the minor and major tumor axis,respectively.

2.3. Statistical analysis

Differences between obtained values (mean±S.E.M. n = 6) were carried out by one way analysis of variance (ANOVA) followed by the Tukey–Kramer multiple comparison test. A p value of 0.05 or less was taken as a criterion for a statistically significant difference.

3. RESULTS

3.1. Effect on serum lipid profile

Treatment of rats with TAM(10mg/kg for 28 days) produced significant increase in serum levels of TG, Ch and LDL by (85.36%, 40.78% and 129.07%); respectively as compared to the control group. The combined effect of L-CAR and TAM resulted in significant decrease in levels of TG by (25.88%) as compared to TAM group but it is significantly high in compared to control group. On the other hand, the levels of CH and LDL was significantly decreased and reached to normal level (Table 1).

Table (1): Effect of Tamoxifen (TAM), L-carnitine (L-CAR) and their combination on the level of triglycerides (TG), cholesterol (CH), high density lipoprotein (HDL) and low density lipoprotein (LDL) in serum of female rats.

Treatment	TG (mg/dl)	CH (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
Control	62.16±5.02	87.39±7.47	52.49±3.35	35.60±2.16
TAM	115.22±6.41 [a]	123.03±11.41 [a]	41.48±2.63	81.55±8.08 [a]
L-CAR	75.17±5.69	86.88±7.48	50.93±4.55	35.95±4.51
L-CAR+TAM	85.41±3.79 [ab]	87.79±6.63 [b]	50.79±4.81	36.99±3.66 [b]

The values are expressed as mean ± S.E.M of 6 rats/ group. P value is significant≤ 0.05 using ANOVA followed by Tukey-Kramer as a post ANOVA test. [a,b] Significantly different from the control group and TAM treated group respectively.
TAM (10mg/kg, P.O) and L-CAR (300mg/kg, i.p.) were administered for 28 consecutive days. In the groups treated with two drug regimens, the animals were pretreated with L-CAR 1 hour prior to TAM and the administration was continued as described above.

3.2. Effect on serum liver function

AST, ALT, total bilirubin and direct bilirubin of rats treated with tamoxifen were significantly elevated by (20.85%, 86.57%, 104.65%and 172.09%), respectively compared to the control group. On other hand, treatment with both L-CAR and TAM significantly decreased AST (19.12%), ALT (54.78%), total bilirubin (45.08%) and direct bilirubin (28.21%), as compared to the TAM treated group, which still significantly increased compared to the control group (Table 2).

Table (2): Effect of Tamoxifen (TAM), L-carnitine (L-CAR) and their combination on the level of aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin and direct bilirubin in serum of female rats.

Treatment	AST (U/l)	ALT (U/l)	Total bilirubin (mg/dl)	Direct bilirubin (mg/dl)
Control	65.61±2.08	55.53±9.04	1.29±0.04	0.43±0.03
TAM	79.29±3.02 [a]	103.60±4.31 [a]	2.64±0.09 [a]	1.17±0.02 [a]
L-CAR	66.77±1.54	48.16±5.51	1.34±0.09	0.41±0.04
L-CAR+TAM	64.13±4.44 [b]	46.85±8.31 [b]	1.45±0.08 [b]	0.84±0.08 [ab]

3.3 Oxidative status markers

Figure1A-D shows the effects of TAM, L-CAR and their combination on the activity of SOD, level of LPO, GSH content and level of NO(x), respectively, in liver and uterus tissues. TAM treatmentinduced a significant decrease in the activity of SOD by (30.06, 20.31%)in liver and uterus, respectively. Administration of L-CAR prior to TAM induced significant increase in SOD activity (44.8%) in liver tissue and no significant change in uterus compared to the TAM treated group (Figure1-A).

Treatment with TAM showed significant increase by (102.55, 118.44%) in LPO levels of liver and uterus,respectively. Treatment with both L-CAR and TAM induced no significant decrease in LPO level of liver compared to the TAM treated group while induced significant decrease in the level of LPO (34.63%)in uterus compared to the TAM treated group, however, the value is still high significant when compared with control group (Figure 1-B).

Administration of TAM resulted in a significant decrease by (62.38%) in uterus GSH level and no significant change in liver (Figure 1-C). On other hand, administration of L-CAR prior to TAM induced significant increase(100%) in GSH level of uterus compared to the TAM treated group, however, the value is significantly decrease compared to the control group while the value of GSH still high significant in liver compared to the control group.

Treatment of animals with TAM treatment induced non- significant change in level of NO(x) in the liver and uterus compared to the control group. Treatment with both L-CAR

and TAM induced significant increase (29.26%) in the level of NO(x)in uterus tissue compared to TAM treated group (Figure 1-D).

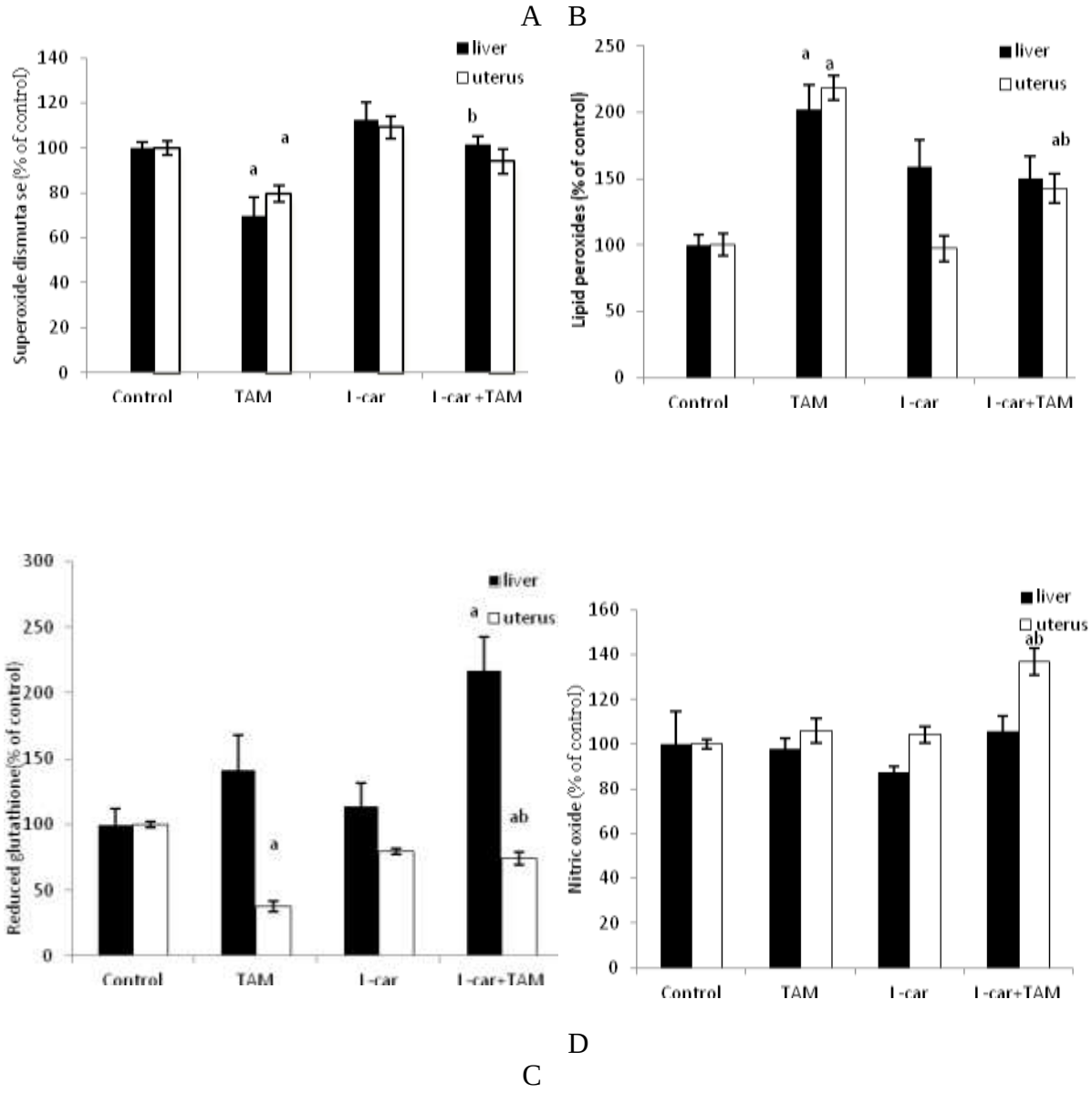


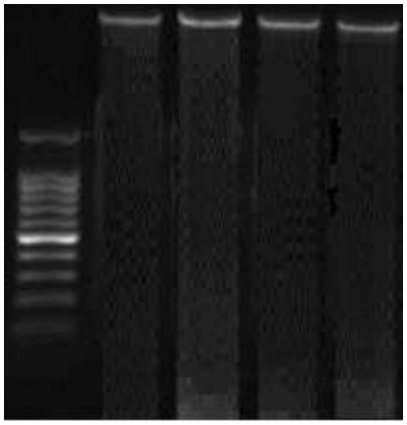
Figure (1): Effect of Tamoxifen (TAM), L-carnitine (L-CAR) and their combination on (A)superoxide dismutase expressed as µg/g tissue (SOD) activity, (B) lipid peroxides expressed as nmol/g tissue (LPO) level, (C) reduced glutathione expressed as µmol/g tissue (GSH) level, (D) total nitrate/nitrite expressed as nmol/g tissue (NO(x)) level in female rats for 28 consecutive days.

Data are presented as % of control ± S.E.M of 6 rats/ group. [a,b] Significantly different from the control group and TAM treated group at $p \leq 0.05$ using ANOVA followed by Tukey-Kramer as a post ANOVA test.

TAM (10mg/kg, P.O) and L-CAR (300mg/kg, i.p.) were administered for 28consecutive days. In the groups treated with two drug regimens, the animals were pretreated with L-CAR 1 hour prior to TAM and the administration was continued as described above.

3.4. DNA fragmentation

Figure (2) shows the gel electrophoresis of DNA in liver tissue of female rat treated with TAM (10 mg/kg, P.O) and/or L-CAR (300mg/kg, i.p.) for 28 consecutive days did not show any change indicating no DNA fragmentation.



M 1 2 3 4

Figure (2): Gel electrophoresis of DNA in hepatic tissue, M: molecular weight marker, lane 1: liver DNA of normal control female rat, lane 2: liver DNA of female rat treated with TAM (10mg/kg,P.O), lane 3: liver DNA of female rat treated with L-CAR (300mg/kg, i.p.), lane 4: liver DNA of female rat treated with L-CAR 1 hour prior to tamoxifen for 28 consecutive days. In the groups treated with two drug regimens, the animals were pretreated with L-CAR 1 hour prior to TAM and the administration was continued as described above.

3.5. Effect on the level of caspase 3 proteins

The level of caspase 3 proteins were determined by western immunoblotting analysis. As it is observed there was no marked alteration in the level of caspase 3 proteins by TAM (Figure 3). While, L-CAR alone and with TAM induced activation in level of caspase 3.

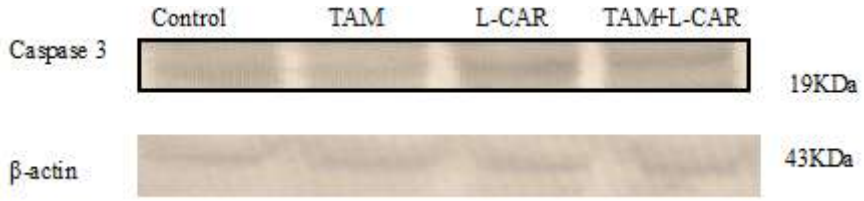


Figure (3): Effect of TAM, L-CAR and their combination on caspase 3 in hepatic tissue using western blot technique. TAM (10mg/kg, P.O) and L-CAR (300mg/kg, i.p.) were administered for 28consecutive days. In the groups treated with two drug regimens, the animals were pretreated with L-CAR 1 hour prior to TAM and the administration was continued as described above.

3.6. Histopathological findings

The protection effect of L-CAR against TAM-induced hepatotoxicity was further confirmed by conventional histopathological examination. Liver sections in the control group showed branching cords of hepatocytes are radiating from the central vein (v). The hepatocytes (H) are having vesicular nuclei and some binucleated cells are separated by sinusoids (S) lined by flat endothelial cells and kupffer cells Figure 4 (a). In Figure 4 (d) Liver section of rat liver treated with L-CAR group showing similar histological structure as control. Figure 4(b, c) photomicrograph of rat liver of TAM treated group showing most of hepatocytes (H) around dilated and congested portal vein (P) with empty cytoplasm and dark nuclei, thickening and congested central vein (v), and some hepatocytes (H) appear with cytoplasmic vacuolation. In combined group L-CAR and TAM showed relatively normal of hepatocytes around the portal vein (p), bile duct (b) and hepatic artery (A). However, the remaining cells are moderately swollen and vacuolated. Figure 4 (e, f).

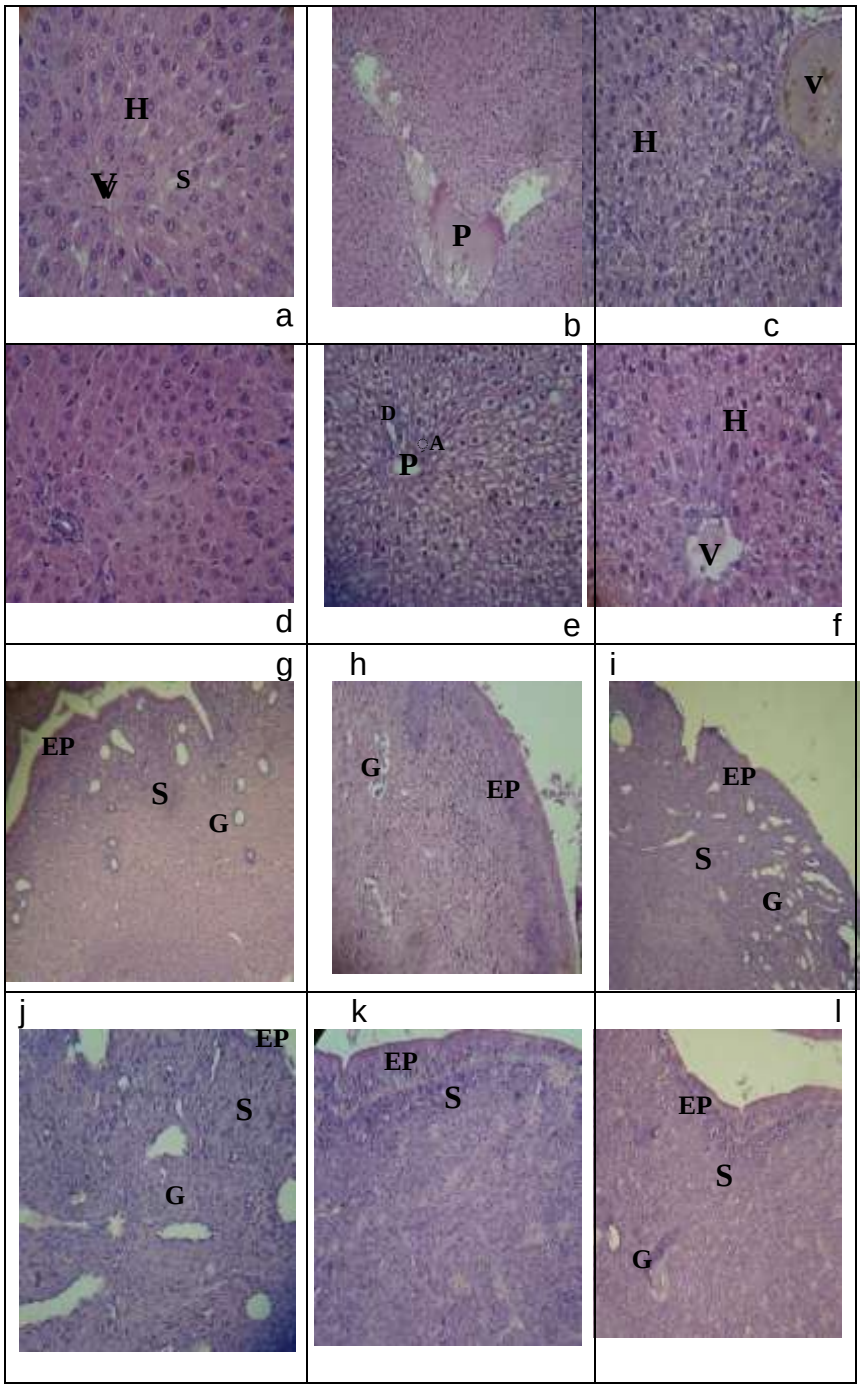


Figure (4): Effect l-carnitine pretreatment on TAM-induced hepatic and endometrium toxicities in female rats. (a): liver section of the normal control group did not show histopathological changes. (d): photomicrograph of rat liver of treated L-carnitine group showing similar histological structure as control. (b, c): photomicrograph of rat liver of TAM treated group showing most of hepatocytes (H) around dilated and congested portal vein (p) with empty cytoplasma and dark nuclei, thickening and congested central vein (v) .and some hepatocytes (H) appear with cytoplasmic vacuolation. (e,f) : photomicrograph of rat liver of L-carnitine+TAM treated group showing relatively normal of hepatocytes around the portal vein (p) ,bile duct (b) and hepatic artery (A) and remarkably healthy hepatocytes. (g)**:** photomicrograph of rat endometrium of control group did not show histopathological changes. (h)**:** photomicrograph of rat endometrium of L-carnitine treated group showing no histological changes were seen as compared to the control group. (I,j)**:** photomicrograph of rat endometrium of TAM treated group showing degeneration surface epithelium (EP), partially ulcerated, edematous stroma (S) contains tortuous glands (G), hyperplasia stroma (S) contain cells with deeply stained

nuclei and endometrial glands (G) within its wide lumen. (k, l)**:** photomicrograph of rat endometrium of L-carnitine+TAM treated group showing recovery of the surface epithelium (EP) nearly normal cellular stroma (S). **(**1-6, 10) X400; (7-9, 11, 12) X100. H & E.

Photomicrograph of rat endometrium of control group showing surface columnar epithelium (EP) and highly cellular stroma (S) contains numerous corkscrew endometrial glands (G) Figure 4 (g). Section of rat endometrium of L-CAR treated group showing no histological changes were seen as compared to the control group Figure 4 (h). photomicrograph of rat endometrium of TAM treated group showing degeneration surface epithelium (EP), partially ulcerated and edematous stroma (S) contains tortuous glands (G), hyperplasia stroma (S) contain cells with deeply stained nuclei and endometrial glands (G) within its wide lumen Figure 4 (i, j). photomicrograph of rat endometrium of L-CAR with TAM treated group showing recovery of the surface epithelium (EP) nearly normal cellular stroma (S) and most of the endometrium histological architure Figure 4 (k, l).

3.7. Antitumor activity

3.7.1.Percent survival of animals

Figure (5) showed the percent survival of Ehrlich Ascites Carcinoma EAC-bearing mice treated with TAM(10mg/kg single i.p.) and/or L-CAR (300mg/kg single i.p.).At day 23 of the experiment, the percentage of survival was 0% in control untreated tumor-bearing mice, while at day 24 of experiment, the percentage of survival was 0% in TAM treated group. On the other hand, at day 41, the percentage of survival was 0% in mice treated with L-CAR, whereas at day 19, the percentage of survival was 0% in mice treated with L-CAR and TAM.

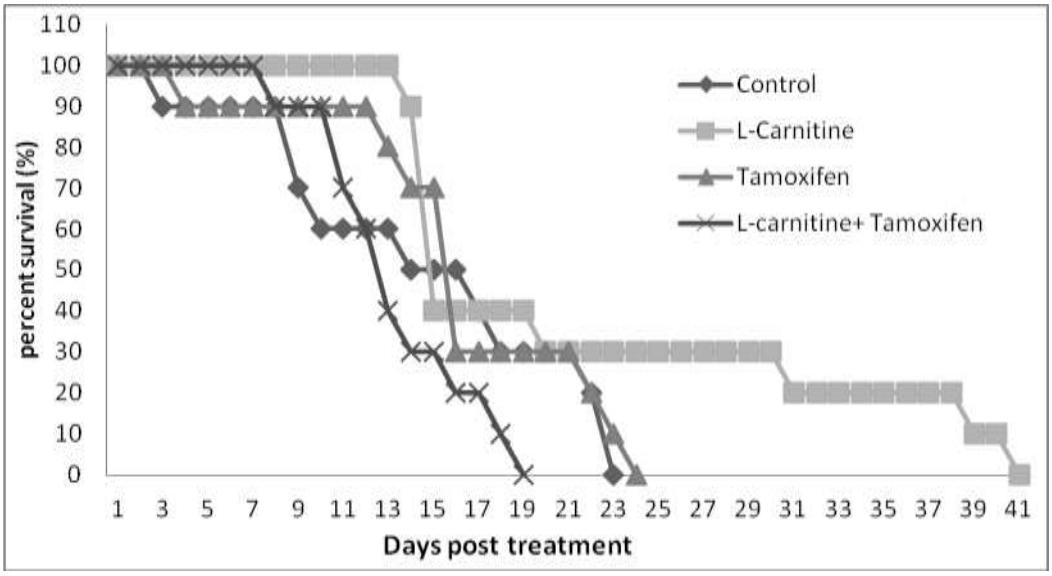


Figure (5): Effect of Tamoxifen (10mg/kg), L-carnitine (300mg/kg) and their combination on the percentage survival rate of EAC-bearing mice.

3.7.2.Tumor volume

Figure (6) showed the effect of TAM, L-CAR and their combination on the growth of solid Ehrlich carcinoma. The tumor volume of control group showed progressive increase, whereas treatment of mice four times every other day with TAM (10mg/kg, i.p.) resulted in significant decrease in tumor volume as compared to control group. L-CAR alone induced non-significant decrease in tumor volume compared to control group. Pretreatment with L-

CAR in the combined group resulted in significant decrease in tumor volume compared to control group.

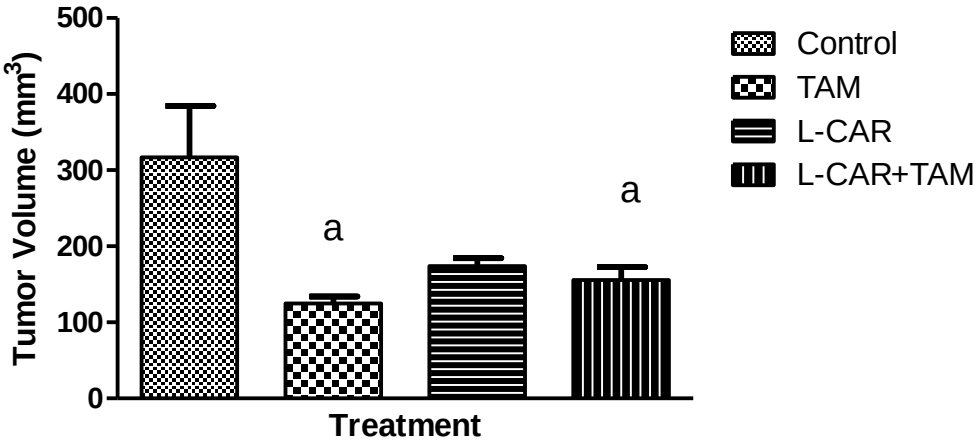


Figure (6): Effect of TAM, L-CAR and their combination on the tumor volume of EAC-bearing mice. Data are expressed as mean ± S.E.M of 6 mice/ group. P value is significant $\leq$ 0.05 using ANOVA followed by Tukey-Kramer as a post ANOVA test. [a] Significantly different from the control group. TAM (10mg/kg, i.p.) and L-CAR (300mg/kg, i.p.) were administered four times. In the groups treated with two drug regimens, the animals were pretreated with L-CAR 1 hour prior to TAM and the administration was continued as described above.

4. DISCUSSION

TAM is an anti-estrogenic drug widely used for the treatment of all stages of breast cancerin spite of its hepaticand endometrium toxicities in rats **(Karki *et al.*, 2000; Stanley *et al.*, 2001)** and human **(Akcay *et al.*, 2000)**. In the present study TAM induced a significant increase in TG, CH and LDL. Our results are in agreement with previous studies **(Akhondi-Meybodi *et al.*, 2011; Lee *et al.*, 2010)**. They reported that,the increased lipid profile after TAM administration was probably due to reduction in hepatic ability of lipid β-oxidation that ultimately enhanced hepatic fat content and hypertriglyceridaemia and increase in the rate of CH biosynthesis in liver by TAM.Increase in TG level within normal range in most of patient while, severe hypertriglyceridemia developed in small number of them was reported in postmenopausal patients receiving adjuvant TAM **(Liu and Yang, 2003)**. However, lower CH and LDL levels were detected by TAM in ovariectomized rats **(Lundeen *et al.*, 1997)** and in postmenopausal women **(Love *et al.*, 1994)**.

Our study demonstrated a significant increase in serum activity of AST, ALT and level of bilirubin in TAM treated rats. Our results are in agreement with the previous findings of **Jain *et al.* (2011)** and **Kumarappan *et al.* (2011)** who reported similar elevation in liver function. TAM has much higher affinity for hepatic tissue, its active metabolite, 4-hydroxytamoxifen induces conformational changes in the lipid bilayers of cell membranes an effect which can lead to an increase in the membrane permeability and facilitates the passage of cytoplasmic enzymes outside the cells leading to the increase in the

aminotransferase activities in blood (Albukhari et al., 2009)**.** L-CAR in this study significantly decreased the levels of TG, CH, LDL, and significantly inhibited the rise in liver function compared to TAM treated group. L-CAR is an essential cofactor in the transport of long-chain fatty acids from the cytosol to mitochondria for subsequent β-oxidation and production of cellular energy. In addition, it exhibits antioxidant and antiapoptotic activity **(Muthuswamy *et al.,* 2006)**. It is very effective in minimizing age-associated disorders, which free radicals are the major cause **(Gulcin, 2006; Gomez-Amores *et al.,* 2006)**. Previous study reported restoration of lipid profiles by L-CAR in rats **(Sidoriak and Volgin, 1996)** and in premenopausal women **(Lofgren *et al.,* 2005)**.

In consistent with previous studies **(Albukhari *et al.,* 2009; Jain *et al.,* 2011; Tabassum et al., 2006)**, our study showed a significant increase in LPO with significant decrease in antioxidant levels in hepatic and endometrium tissue after TAM administration. TAM impaired beta oxidation of fatty acids **(Parvez *et al.,* 2006)** and mitochondrial functions as it acts as an uncoupling agent and a powerful inhibitor of mitochondrial electrontransport chain eventually results in mitochondrial oxidativedamage **(Parvez *et al.,* 2008)**. TAM decreased the activities of catalase, glutathione peroxidase and SOD and content of GSH in liver,the decreased glutathione peroxidase activity leads to H_2O_2 accumulation in the liver, which in turns inactivates SOD **(Kakkar *et al.,* 1997)**. The decreased antioxidant power of TAM-intoxicated rats could be attributed toimpairment of hexose monophosphate (HMP) shunt and thereby reduced NADPHavailability and the ability to recycle substances as GSSG to GSH is decreased **(Stanley *et al.,* 2001)**. However, **Perumal *et al.* (2005)** have shown that TAM inhibited LPO triggered by freeradicals in 7, 12 dimethyl Benz (a) anthracene (DMBA) induced peroxidative damagein rat mammary carcinoma.

Reduction in levels of LPO and elevation in antioxidants in L-CAR treated rats suggested that L CAR scavenges free radicals generated during oxidative stress. L-CAR had an effective superoxide anion radical scavenging, hydrogen peroxide scavenging, total reducing power and metal chelating on ferrous ions activities **(Gulcin, 2006; Kalaiselvi and Panneerselvam, 1998)**.

Nitric oxide (NO(x)) has been implicated in various aspects of cancer biology, including both pro and anti- tumor functions **(Ostad *et al.,* 2009)**. In our study neither TAM nor L-CAR alone had an effect on NO(x) level; however, significant increase in NO(x) in uterus tissue was observed when we use two drugs in combinations. We assume that this dual modulatory effects were not notable until both drugs were used resulting in significant increase in NO(x). On the other hand, enhanced NO(x) production was found by TAM **(Loo *et al.,* 1998)** and L-CAR **(Bueno *et al.,* 2005)** in serum of rats and in breast cancer tissue **(Erbas *et al.,* 2007)**.

Our study showed no change in the expression of caspases 3 and intact genomic DNA was observed in normal hepatic tissue treated with TAM. The concept that TAM has favorable antineoplastic effect without any adverse effect on human normal breast tissue was indicated in benign biopsies of patients receiving TAM daily **(de Lima *et al.,* 2003; Walker *et al.,* 1991)**. Moreover, no change in the expression of caspase-3 in MCF-7 cell by TAM however, it exerts a significant caspase-3 dependent apoptosis in ER-MDA-MB468cells and co-incubation with caspase-3 inhibitor abolished the apoptosis was found **(Salami and Karami-Tehrani, 2003)**. L-CAR was reported as an important anti-apoptotic mediator **(Moretti *et al.,* 2002)**. L-CAR was reported to induce apoptosis in hepa1c1c7 cells by regulating Fas ligands and inhibiting the expression of Bcl-2 and inducing the up-regulation of caspase-9 and caspase-3 **(Fan *et al.,* 2009)**. Savitha and **Panneerselvam (2007)** have also observed that the L-CAR protected DNA repairing enzymes, thereby preventing DNA strand

break by maintaining thiol-containing compound and improving the glutathione redox status, in addition, L-CAR decreased the levels of oxidative stress mediated DNA damage during aging in rats. However, our results indicated histopathological changes in architecture of the liver and uterus in TAM treated group which might be attributed to impaired β-oxidation of fatty acids and generation of ROS **(Albukhari *et al.*, 2009)**. It was found that TAM induced DNA adduct formation in rat liver and uterus through metabolic activation to α-hydroxytamoxifen which is sulfonated, forming an electrophilic carbocation that is capable of reacting with DNA yields a DNA adduct **(Costa *et al.*, 2007)**.

L-CAR clearly ameliorated the histopathological changes induced by TAM and showed relatively normal and recovery of liver and uterus tissues. These findings support previous results indicating the antioxidant activity exhibited by L-CAR. In the present study, we have tested the effect of administration of TAM against induction of mammary carcinoma in female mice. Our study demonstrated that TAM decreased tumor volume and increased survival rate compared with the untreated control. **Gabri *et al.* (2004)** reported a significant reduction in tumor volume and a significant increase in the mean survival time of EAC-bearing animals in TAM pretreated group. L-CAR as amonotherapy had an inhibitory effect on tumor volume **(Sayed-Ahmed *et al.*, 1999). Niang and Melka (2000)** reported that Acetyl-L-carnitine prolonged the survival of micewith the ascitic form of leukaemia L1210 and L-CAR treatment prolonged survival ofrats with adriamycin-induced heart failure by improving the myocardial metabolism of fatty acids **(Kawasaki *et al.*, 1996)**.

The combined effect of TAM and L-CAR on the survival in our study was less than the individual drug. It seems that L-CAR antagonize both the antitumor effect and survival of TAM upon administration of both drugs together. The antitumor effect and survival of the combined drug was less than each one alone. Interestingly L-CAR increased survival rate of EAC-bearing mice more than TAM treated group. The antagonistic effect of L-CAR with TAM may be due to interference with TAM metabolism, increase its excretion (Olszowy et al., 2006) or may be due to drug interaction which affects the cytotoxic effect of TAM. Also, this effect might be due to the antioxidant activity of L-CAR **(Gulcin, 2006)**.

5. CONCLUSION:

This study illustrates that although L-CAR ameliorates TAM-induced hepatic and endometrium toxicities by preventing oxidative stress and enhancingantioxidant enzymes, it interferes with its antineoplastic activity.

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تأثيرات ل-كارنتين المعدلة لسمية تاموكسيفين و كذلك لنشاطه المضاد للأورام: دراسة داخل جسم الحى

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أن الهدف من هذه الدراسة الاستدلال و التعرف على التأثير الوقائى ل-كارنتين على السمية المحدثة بدواء التاموكسيفين و فى علاج السرطان. و لتحقيق الأهداف السابقة فقد تم تقسيم الجرذان البيضاء الى اربع مجموعات تتكون كل مجموعة من ستة جرذان تم حقنها على النحو التالى:- المجموعة الاولى تم اعطاء محلول ملحى كمجموعة ضابطة و فى المجموعة التانية التاموكسيفين (١٠مجم / كجم فمويا) و فى المجموعة الثالثة ل- كارنتين (٣٠٠مجم / كجم فى التجويف البريتونى) و الرابعة بال-كارنتين ثم التاموكسيفين بعد ساعة واحدة) واستمرت هذه الدراسة لمدة ٢٨ يوما على التوالى . وقد اظهرت نتائج هذه الدراسة ان التاموكسيفين قد سبب زيادة في معدل الدهون، نشاط انزيمات الكبد و مستويات البيليروبين. هذا بالاضافة الي انه سبب زيادة فى الليبيد بروكسيداشن و انخفاض في نشاط انزيم السوبر اكسيد ديسميوتيز في انسجة الكبـــد والرحم وانخفاض ملحوظ فى الجلوتاثيون المختزل فى انسجة الرحم. من ناحية اخرى اثبتت نتائج هذه الدراسة ان استخدام ل-كارنتين ساعة قبل التاموكسيفين قد قلل من السمية المحدثة بواسطة التاموكسيفين وذالك بتقليل كل من معدل الدهون، نشاط انزيمات الكبد، و زيادة ملحوظة فى نشاط انزيم السوبر اكسيد ديسميوتيز في انسجة الكبـــد والرحم مقارنة باستخدام دواء التاموكسيفين فقط. كما ادى الى تحسن مستويات الليبيد بروكسيداشن و الجلوتاثيون المختزل في انسجة الرحم و من ناحية اخرى لم يحدث اى تنشيط فى مستوى تكوين كسبيس ٩ و٣ فى كل المجموعات. و قد أكدت الدراسة المجهرية للأنسجة الكبد والرحم هذه النتائج البيوكيميائية. و قد أظهرت الدراسة أن معدل البقاء و فاعلية علاج السرطان للتاموكسيفين و ل كارنتين معا فى الفئران البيضاء الحاملة لخلايا سرطان أرليخ الأستسقائى و أرليخ الصلب اقل من كل منهما على حدة. بينما ادى ل- كارنتين الى زيادة فى معدل البقاء مقارنة باستخدام دواء التاموكسيفين فقط . من هذه الدراسة نستخلص ان دواء ل كارنتين قلل من تاثير التاموكسيفين السمي ولكن يجب ان يكون استخدامه محدودا نظرا لتقليله من فاعلية التاموكسيفين في علاج السرطان