



Modulatory Effects of Taurine Combined with Letrozole on Ehrlich Ascites Carcinoma in Mice

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Abstract

Background: Estrogen dependent cancer is a common type of malignancy which is complicated and involve many co-regulators. Aromatase enzyme, integrin $\beta 4$ (ITGB4) and histone deacetylase 4 (HDAC4) are strongly related to this type of carcinogenesis. Additionally, estrogen reliant on oxidative stress is highly involved. Aromatase inhibitors are the first line management for such types of cancer, and third generation letrozole (LET) is the most recent and commonly used one. Lately, new approaches in tumor management include supplements that might have antitumor effects are under investigation. Taurine (TAU) is chosen to be combined with LET on Ehrlich ascites carcinoma model for its antioxidant and potential anticancer effects.

Method: Sixty Swiss-albino female mice were divided into five groups (n = 12): Control group (Normal), EAC group, LET group, TAU group and LET+TAU group. TAU (150 mg/kg, orally) was given day after day for 14 days along with LET (5 mg/kg, orally). After 14 days, mice were sacrificed, ascetic fluid, blood and liver were isolated immediately. HDAC4, ITGB4 and aromatase enzyme genes expression were estimated in liver, vascular endothelial growth factor (VEGF) content was determined by ELISA method in liver, and some oxidative status biomarkers were measured in blood.

Results: Treatment with TAU significantly down regulated hepatic HDAC4, ITGB4 and aromatase genes expression, content of hepatic VEGF and improved the anti-oxidant characteristics in EAC mice.

Conclusion: TAU was observed to improve the anticancer characteristics of LET and might have beneficial role as supplement therapy that has potential anticancer activity in estrogen dependent cancer management protocols.

Keywords: Aromatase; Estrogen; Histone Deacetylase; Integrin $\beta 4$; Letrozole; Taurine

Abbreviations

Ais: Aromatase Inhibitors; Cdna: Complementary DNA; COX-2: Cyclooxygenase-2; EAC: Ehrlich Ascites Arcinoma; ER: Estrogen Receptor; GSH: Glutathione; HDAC4: Histone Deacetylase4; IHC: Immunohistochemical; ITGB4: Integrin Beta 4; LET: Letrozole; MDA: Malondialdehyde; NO: Nitric Oxide; P21waf1/Cip1: Cyclin-Dependent Kinase Inhibitor; PUMA: P53 Up Regulated Modulator Of Apoptosis; Qrt-PCR: Quantitative Real-Time Reverse Transcriptase Polymerase Chain Reaction; SOD: Superoxide Dismutase; TAU: Taurine; VEGF: Vascular Endothelial Growth Factor

Introduction

Estrogen dependent cancer occurrence rates are growing worldwide making it one of the most important causes of cancer-related deaths in women. This type of cancer is associated with increased estrogen blood levels and up-regulation of estrogen receptors (ER) which is involved in tumor initiation, development, and progression [1]. The hormonal effects of estrogen are mediated by its binding to one of the structurally and functionally distinct ERs (ER α and ER β) [2]. ER signaling is many-sided, involving numerous co-regulatory proteins including enzymes and receptors that induce extra-

nuclear and extracellular actions. These co-regulators are strictly controlled under normal conditions and miss-expressed during carcinogenesis to promote initiation, development and metastasis of cancer cells [3].

Aromatase enzyme is the primary co-regulator of estrogen level, act through aromatization of testosterone, and its miss expression leads to increasing the level of estrogen which in turn up-regulates ER and starts the carcinogenic pathway of estrogen [4].

Histone deacetylase4 (HDAC4) is another co-regulator enzyme that might be involved in the malignancy development in breast tissue [5]. HDAC4 overexpression and mutations are chiefly found in breast cancer compared with bladder, colorectal and renal cancer [6]. HDAC4 is found to be overexpressed and participates in tumor cell survival by deacetylating the signal transducer and activator of transcription 1 (STAT1) [7]. Inhibition of HDAC4 activity in clinical and experimental trials indicates potential benefit in treating cancer [8].

Other co-regulatory proteins involved directly with the estrogen carcinogenic pathway is trans-membrane receptor integrin $\beta 4$ (ITGB4) [9]. Up-regulation of integrin receptor mediates extracellular matrix synthesis, cell adhesion and invasion [10]. ER α overexpression in breast cancer has shown to contribute in overexpression of Δ NP63 gene which is a transcription factor of ITGB4 that in turn promotes cell adhesion and survival [11]. Also, Overexpression of ITGB4 has been strongly related to the violent behavior of ER positive breast cancer [12].

Considering treatment, interfering with estrogen production is the most direct protocol to be effective in estrogen dependent cancer [4]. Letrozole (LET), a third generation aromatase inhibitor, is the first line therapy of estrogen dependent cancer by inhibiting aromatase enzyme activity and interfering with the body's ability to produce estrogen [13]. However, LET side effects may include loss of libido, joint stiffness and loss of bone mineral density with consequent increased risk of fracture [14]. This made it suitable to search for supplements that may improve its anti-cancer effect and even reduce side effects.

Recently the discovery of supplements that might have antitumor effects is a target of many studies to increase treatment efficacy [15]. One of these compounds is taurine (TAU) 2-amino ethane sulfonic acid, endogenously synthesized from amino acids containing sulfur (methionine and cysteine in the presence of vitamin B6)

and a normal constituent of the human diet [16]. TAU was found to have a significant anti-inflammatory and antioxidant effects [17]. Moreover TAU was investigated for its anticancer effect, where it showed significant induction of p53 upregulated modulator of apoptosis (PUMA) [18] and a considerable antiangiogenic effect by down regulation of vascular endothelial growth factor (VEGF) [19].

Moreover, a link between TAU and estrogen was recently established as it is found that estradiol decreases taurine concentration by decreasing cysteine sulfinic acid decarboxylase activity via the ER α in the liver of female mice [20]. This comply with the study that strongly suggested the measurement of TAU level in serum of patients for the primary detection of malignant changes in the breast accompanied with elevated estrogen level [21]. All These observations made it suitable to try combining TAU with LET and assess its potential anticancer activity as supplement for estrogen reliant on cancer in mice inoculated with Ehrlich ascites carcinoma cells.

Materials and Methods

Drugs

LET tablets (2.5 mg, Femara[®], Novartis, Switzerland) were crushed, finely powdered and dispersed in distilled water [22], TAU 99% pure was purchased from Sigma-Aldrich St. Louis, MO (USA).

Animals and experimental protocols

The ethical committee of Faculty of Pharmacy, Mansoura University, Mansoura, Egypt, approved the animal study, in agreement with "Laboratory Animal Care Principles" (NIH publication No. 85-23, revised 1985). The doses of TAU and LET used in this study were in the range of those used in other studies, applied for the same animal species [22,23].

Ehrlich ascites carcinoma (EAC) line as a spontaneous breast cancer of a mammary origin was purchased from the National Cancer Institute (NCI), Cairo University, Cairo, Egypt. EAC line functioned as the original cancer from which an ascites variant was obtained. EAC cells were used for *in vivo* experiments. The tumor cells were maintained in animal bank by serial intraperitoneal (i.p.) transplantations of 10 days-old viable tumor cells (1×10^6) in 1 ml of normal saline. Cell viability was checked by trypan blue exclusion method [24] and was not less than 95%.

Total of sixty Swiss-albino female mice were used as the following:

Forty-eight Swiss-albino female mice were injected i.p. with 0.1 ml cell suspension containing 1×10^6 cells on day zero for tumor

induction. On the next day, mice were randomized and divided into four groups (n = 12 each), and the fifth group of 12 mice with same age and weight was kept under same conditions as normal control without EAC cells. Tested compounds were given orally day after day (seven separate doses).

- **Group 1 (Normal group):** Mice were orally 0.2 ml of normal saline.
- **Group 2 (EAC group):** Mice were given orally 0.2 ml of normal saline.
- **Group 3 (LET group):** Mice were given orally 0.2 mL of letrozole solution (5 mg/kg).
- **Group 4 (TAU group):** Mice were given orally 0.2 mL of taurine solution (150 mg/kg).
- **Group 5 (LET+TAU group):** Mice were given orally letrozole (5 mg/kg) and taurine (150 mg/kg) with final volume up to 0.2mL

Sample preparation

Twenty-four hours after the last treatment, mice of each group were fasted overnight. Ascetic fluid was aspirated to assess tumor volume and percentage of cell viability. Whole blood samples were collected from retro-orbital vein puncture in heparin lined tubes for the study of antioxidant activity.

After the decapitation of mice, livers were dissected out, dried on filter paper and divided into three parts. The first part was fixed in buffered formalin (El-Nasr Chemicals Co, Cairo, Egypt) for histopathological and immunohistochemical examination. The second part was weighed and homogenized in ice-cold buffer of sodium potassium phosphate (0.01 M, pH 7.4) containing 1.15% KCl with a glass homogenizer. Centrifugation of the homogenates for 5 min (5000×g) at 4°C and the resulted clear solution was collected to be stored at -80°C for further biochemical measurements. The last part was immersed in liquid nitrogen and stored at -80°C for assessment of gene expression by Quantitative Real-time reverse transcriptase Polymerase Chain Reaction (qRT-PCR).

Biochemical examination

Antitumor characteristics represented in ascetic tumor volume (ml), and percentage of tumor cell viability were detected by Trypan blue exclusion method [24].

Oxidative status

Superoxide dismutase (SOD), glutathione (GSH), malondialdehyde (MDA), and nitric oxide (NO) were determined in blood spectrophotometrically using Eppendorf Biophotometer plus (Eppendorf®, Hamburg, Germany) according to the methods of Nishikimi, *et al.* (1972), Ellman, *et al.* (1959), Montgomery and Dymock, (1961), and Satoh, (1978), respectively, using assay kits provided by Bio Diagnostic, Giza, Egypt.

Enzyme immunosorbent assay

ELISA kit supplied from Cloud Clone Corp (USA) was used for the determination of vascular endothelial growth factor (VEGF) content in liver homogenate following the company protocol. The absorbances of ELISA test results were read by the standard microtiter plate reader at 450 nm.

Immunohistochemical (IHC) determination of estrogen receptor in liver sections

The BioModule™ IHC Staining Kit (purchased from ThermoFisher scientific, UK) utilizes indirect staining method to detect antigens. Slides were photographed using Olympus® digital camera installed on Olympus® microscope with 0.5 X photo adaptor, using 40 X objective and saved as TIFF. The resulted images were analyzed on Intel® Core I3® based computer using VideoTest Morphology® software (Russia) with a specific built-in routine for object counting and analysis.

Expression level of histone deacetylase 4 (HDAC4), integrin beta4 (ITGB4), and aromatase enzyme genes were determined in liver homogenate using q RT-PCR

RNA was extracted from the harvested liver tissue using Qia-gen RNeasy Plus Mini kit. The pure RNA samples were reverse-transcribed cDNA. Amplification and online monitoring were performed using The Thermo Scientific Maxima® SYBR Green/ROX qPCR Master Mix (2X) by Rotor-Gene Q (Qiagen, USA). Meanwhile, mouse Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as a housekeeping gene and an internal reference control. Primer Express 3.0 was used for gene specific PCR primers design (Applied Biosystems, USA) (Table 1). Each primer was blasted on NCBI/Blast to ensure its specificity to the required gene. Melting curve analysis of the PCR products were performed to verify their identity and specificity. Mouse HDAC4, ITGB4, aromatase enzyme and GAPDH mRNA relative expression were determined by $2^{-\Delta\Delta Ct}$ method [27].

Name	Sequence	Accession number	Amplicon size
GAPDH F	5'- ATGGTGAAGGTCGGTGTGAAC-3'	NM_008084.2	250
GAPDH R	5'-TTGATGTTAGTGGGGTCTCGC-3'		
HDAC4 F	5'-TCCCCATCCATTCACAAGCT-3'	NM_207225.2	150
HDAC4 R	5'-CTGCTGGAAGCTGTTGCTTGT-3'		
ITGB4 F	5'-GAAGGAGTTGCAGGTGAAGC-3'	NM_001005608.2	91
ITGB4 R	5'-GCTGAGTTGGACTTGAAGC-3'		
aromatase F	5'-TCCCCATCCATTCACAAGCT-3'	NM_007810.4	150
aromatase R	5'-CTGCTGGAAGCTGTTGCTTGT-3'		

Table 1: The primer set used for the amplification of mice GAPDH, HDAC4, ITGB4 and aromatase genes.

Accession number: The accession number of the sequence in the Entrez Nucleotide Database. GAPDH: Glyceraldehyde 3-phosphate dehydrogenase. HDAC4: Histone deacetylase 4. ITGB4: Integrin beta 4. F: Forward R: Reverse.

Data management and statistical analysis

All values were expressed as mean ± S.E. Data was analyzed by ANOVA using the statistics package for Social Science Software (SPSS version 15.0 Chicago, IL, USA). Differences were considered statistically significant when p < 0.05.

Results

Effect of taurine, letrozole and their combination on EAC model in mice were detected by evaluation of the following Antitumor characteristics (tumor volume and tumor cell viability)

Figure 1A illustrates that treatment with TAU significantly reduced tumor volume by 48% and 24.3% compared to EAC group and LET group, respectively (p < 0.05). Also, the combined therapy shows a significant reduction in tumor volume by 74.6%, 24.3% and 51.2% compared to EAC group, LET group and TAU group, respectively, (p < 0.05).

Figure 1B demonstrates that percentage of tumor cell viability, in TAU group, is significantly decreased by 26.5% compared to EAC group (p < 0.05). While the LET+TAU group shows a significant decrease in tumor cell viability (p < 0.05) by 37.4%, 5.21% and 14.8% compared to EAC group, LET group and TAU group, respectively.

Oxidative Status

Figure 2A shows a significant increase in the activity of SOD in TAU group by 1.4 fold compared to EAC group (p < 0.05), and a significant increase in LET+TAU group by 1.58 fold compared to EAC group (p < 0.05).

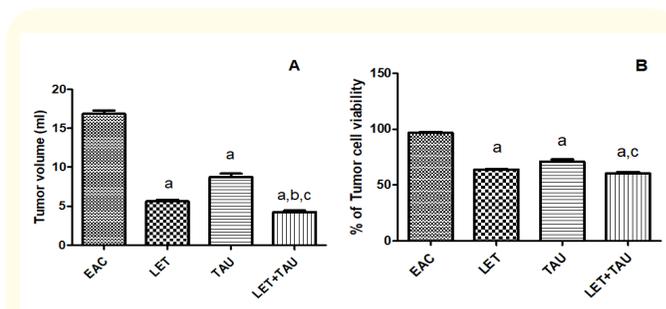


Figure 1: Effect of Taurine (TAU), Letrozole (LET) and their combination (LET+TAU) on Ehrlich ascites carcinoma (EAC) model in mice. (A) Ascetic tumor volume (ml). (B) Percentage of tumor cell viability. Values were represented as mean ± SE. Number of mice in each group (n=12). Significance at (p<0.05). a: Significant compared to EAC group. b: significant compared to LET group. c: Significant compared to TAU group.

Figure 2B demonstrates that TAU group exhibit a significant increase in blood GSH level by 1.89 fold compared to EAC group (p < 0.05). While LET+TAU group significantly increased GSH level by 2.58 fold, 1.4 fold and 1.36 fold compared to EAC group, LET group and TAU group, respectively (p < 0.05).

Figure 2C illustrates a significant decrease in NO level by 48.9% in TAU group compared to EAC group (p < 0.05), and a significant decrease by 53.4%, 3.5% and 8.6% in LET+TAU group compared to EAC group, LET group and TAU group, respectively (p < 0.05).

Figure 2D shows that treatment with TAU significantly reduced MDA level by 20.6% and 5.7% (p < 0.05) compared to EAC group,

while the combined therapy significantly reduced MDA level by 23.5% compared to EAC group ($p < 0.05$).

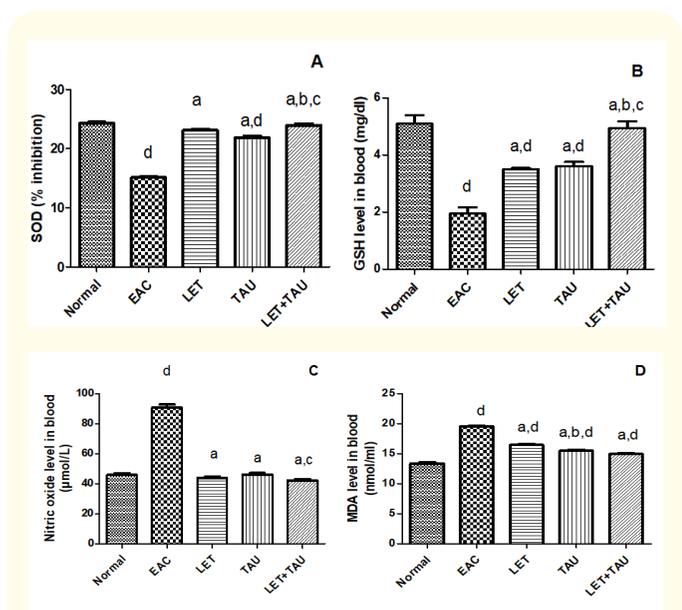


Figure 2: Effect of Taurine (TAU), Letrozole (LET) and their combination (LET+TAU) on Ehrlich ascites carcinoma (EAC) model in mice. (A) Blood super oxide dismutase (SOD) (% inhibition). (B) Blood glutathione (GSH) level (mg/dl). (C) Blood nitric oxide (NO) level ($\mu\text{mol/L}$). (D) Blood malondialdehyde (MDA) level (nmol/ml). Values were represented as mean \pm SE. Number of mice in each group ($n=12$). Significance at ($p < 0.05$). a: Significant compared to EAC group. b: significant compared to LET group. c: Significant compared to TAU group. d: Significant compared to normal group.

Vascular Endothelial Growth Factor (VEGF) in hepatic homogenate

Figure 3 demonstrates a significant reduction in VEGF content in TAU group by 67.7% and 21% ($p < 0.05$) compared to EAC group and LET group, respectively, and a significant reduction in LET+TAU group by 73.5%, 27.8% and 18% compared to EAC group, LET group and TAU group, respectively ($p < 0.05$).

Estrogen receptor expression in hepatic homogenate

Figure 4 illustrates a significant reduction in the percentage of ER positive cells in TAU group by 57.9% and 25.6% ($p < 0.05$) compared to EAC group and LET group, respectively, and a significant reduction in LET+TAU group by 66.7%, 41% and 20.6% compared to EAC group, LET group and TAU group, respectively ($p < 0.05$).

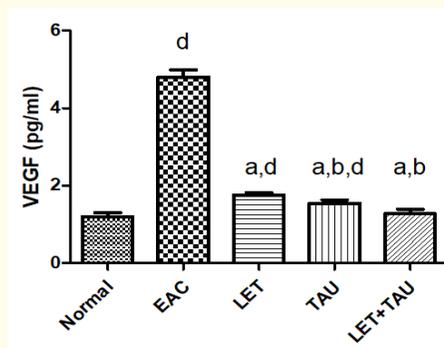


Figure 3: Effect of Taurine (TAU), Letrozole (LET) and their combination (LET+TAU) on hepatic vascular endothelial growth factor (VEGF) content (pg/ml) in Ehrlich ascites carcinoma (EAC) model in mice. Values were represented as mean \pm SE. Number of mice in each group ($n=12$). Significance at ($p < 0.05$). a: Significant compared to EAC group. b: significant compared to LET group. d: Significant compared to normal group.

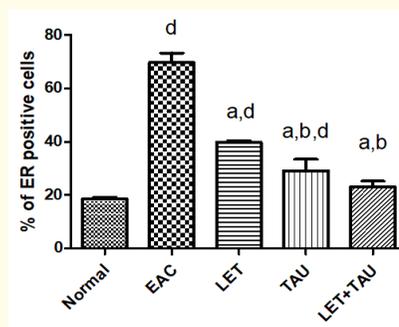


Figure 4: Effect of Taurine (TAU), Letrozole (LET) and their combination (LET+TAU) on percentage of estrogen receptor (ER) positive cells in liver of Ehrlich ascites carcinoma (EAC) model in mice. Values were represented as mean \pm SE. Number of mice in each group ($n=12$). Significance at ($p < 0.05$). a: Significant compared to EAC group. b: significant compared to LET group. d: Significant compared to normal group.

Hepatic expression level of aromatase enzyme, integrin beta4, and histone deacetylase 4 using qRT-PCR.

Figure 5A illustrates a significant decrease in hepatic aromatase enzyme gene expression by 45.2% and 43% in TAU group compared to EAC group and LET group, respectively ($p < 0.05$) and a significant reduction of the hepatic aromatase enzyme gene expression in LET+TAU group by 45% and 43% compared to EAC group and LET group, respectively ($p < 0.05$).

Figure 5B shows that treatment with TAU and LET separately exhibited significant reduction in hepatic ITGB4 gene expression by 34.6% and 42.3%, respectively, compared to EAC group ($p < 0.05$) and the combined therapy shows significant reduction in hepatic ITGB4 gene expression by 65.4%, 40% and 47% compared to EAC group, LET group and TAU group, respectively ($p < 0.05$).

Figure 5C represents a significant decrease in hepatic HDAC4 gene expression by 43.8% and 40% in TAU group compared to EAC group and LET group, respectively ($p < 0.05$) and a significant reduction of the hepatic HDAC4 gene expression in LET+TAU group by 44% and 41% compared to EAC group and LET group, respectively ($p < 0.05$).

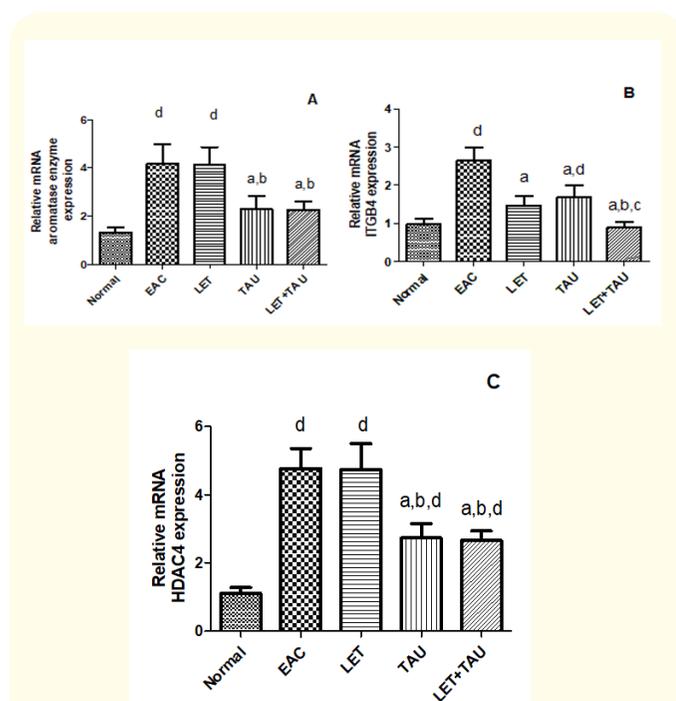


Figure 5: Effect of Taurine (TAU), Letrozole (LET) and their combination (LET+TAU) on liver of Ehrlich ascites carcinoma (EAC) model in mice. (A) Relative mRNA aromatase enzyme (CY19A1) expression. (B) Relative mRNA integrin $\beta 4$ (ITGB4) expression. (C) Relative mRNA histone deacetylase 4 (HDAC4) expression. Values were represented as mean \pm SE. Number of mice in each group ($n=12$). Significance at ($p < 0.05$). a: Significant compared to EAC group. b: significant compared to LET group. c: Significant compared to TAU group. d: Significant compared to normal group.

Discussion

Supplement aided strategies for cancer management are gaining great concern in research field recently. Also, many antioxi-

dants show promising results in malignancy prognosis with potential anticancer effects. This study was performed to evaluate the antitumor activity of TAU either alone or combined with LET as a possible modulatory supplement. This was assessed by measuring some biochemical parameters including oxidative stress biomarkers, VEGF, ER and the expression of three genes involved in the carcinogenesis process induced by estrogen related pathway, which are aromatase enzyme, ITGB4 and HDAC4 using EAC model in mice.

In the present study, the tumor volume of the EAC group showed an increase due to tumor cell multiplication. There was statistically significant difference in the tumor volumes and percentage of tumor cell viability between the EAC group and TAU, LET and their combination groups. TAU showed antitumor activity compared to EAC group through significantly decreasing both tumor volume and percentage of tumor cell viability. Previously, TAU had been investigated for its antitumor activity and showed promising results in attenuating the growing cancer cells [30]. Therefore, a possible mechanism underlying the anticancer activity of TAU is by induction of PUMA expression which further up/and down-regulate the expression of pro apoptotic and anti apoptotic proteins leading to induction of tumor cell apoptosis [31]. Also, the combined administration of LET and TAU showed significant reduction in tumor volume and percentage of tumor cell viability compared to LET group that may be considered as additive effect, which comply with former recommendations to incorporate TAU in cancer management protocols for its anticancer effect [19,32].

Additionally, a possible mechanism of antitumor activity of TAU is its antioxidant and membrane-stabilizing properties by normalizing the oxidative stress markers causing associated changes in organ pathophysiology [33]. On the other hand, abnormal elevated level of estrogen was concerned to be carcinogenic through oxidative stress mediated pathways in the kidney, liver and breast tissues of various rodent models of cancers causing change in serum markers for oxidative damage [34,35]. All these observations are in agreement with the current results, where TAU and LET+TAU groups showed promising antioxidant effect by significantly affecting the inhibition percentage of SOD and the level of GSH as antioxidant machinery in cells, and significantly reducing NO and MDA levels compared to EAC group and LET treated group, respectively.

Another clue evidence that augment the claim of the anticancer activity of TAU is reducing VEGF content. The existing results clearly demonstrate that treatment with TAU significantly reduced hepatic VEGF content compared to EAC group. This is in agreement with other previous studies proved that TAU significantly reduced the VEGF gene expression [19,38]. Also combining TAU with LET significantly reduced hepatic VEGF content compared to LET group

which suggests an additive mechanism accomplished by the combination of LET and TAU.

In the present study, hepatic HDAC4 gene expression showed a significant reduction upon treatment with TAU compared to EAC group. This also might be an acceptable mechanism of the anticancer effect of TAU, where other studies found that HDAC4 is over expressed during ER dependent carcinogenesis [39], and that the silencing of HDAC4 gene or inhibition of the enzyme activity by HDAC inhibitor induced transcriptional activation of the cyclin-dependent kinase inhibitor (p21^{WAF1/Cip1}) gene, increasing its expression and causing arrested cancer cell growth through subverting the ordinary repair process of DNA-damaging [40,41]. LET group did not show any significant change on hepatic HDAC4 gene expression compared to EAC group, also the effect of the combined therapy did not show any significant effect on hepatic HDAC4 gene expression compared to TAU group.

The existing results clearly demonstrate that LET group does not show any effect on hepatic aromatase enzyme mRNA relative expression which comply with previous study indicated that LET act only as enzyme inhibitor and has no effect on the gene expression [42], while its significant action on the percentage of ER positive cells in liver is related to its inhibitory action on the enzyme activity, reducing the level of estrogen and causing subsequent feedback and down-regulating ER positive cells [43]. On the other hand, the current results clearly showed for the first time that TAU significantly reduced hepatic mRNA relative expression of aromatase enzyme, which in turn down-regulated the percentage of hepatic ER positive cells compared to EAC group (Figure 6).

This might be attributed to the anti-inflammatory effect of TAU which act by decreasing expression of cyclooxygenase-2 [44], and subsequent reduction of the produced levels of prostaglandin E2 [37], which is known to be an important mediator in aromatase gene expression [45]. These findings explain the additive decreasing action of the combined treatment on percentage of ER positive cells compared to either LET group or TAU group individually.

Also, a direct relationship between estrogen and ITGB4 expression through a Δ Np63 mediated pathway was found, where estrogen activated ER α transcription, inducing Δ Np63 gene expression that functions as a transcription factor of ITGB4. The over expressed ITGB4 binds directly to laminin, activating phosphoinositide-3-kinase-protein kinase B/Akt (PI3K-PKB/Akt) pathway signaling which is well-recognized as one of the most commonly miss-regulated pathways in cancer, and increased activation of PI3K-PKB/Akt cascade results in violent behavior of cancer cells through promoting survival and invasion [11].

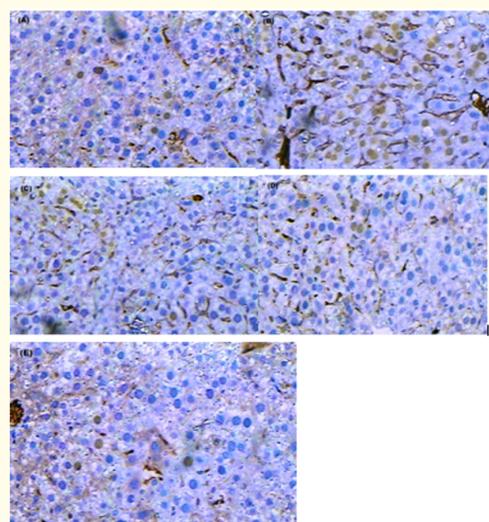


Figure 6: Immunohistochemical analysis of hepatic estrogen receptor (ER) of Ehrlich ascites carcinoma (EAC) model in mice treated with Taurine (TAU), Letrozole (LET) and their combination (LET+TAU) showing: (A) Low expression of ER in normal group. (B) Marked increase of ER expression in EAC group. (C) Marked decrease of ER expression in LET group. (D) Marked decrease of ER expression in TAU group. (E) Marked decrease of ER expression in their combination group.

Both the inhibitory action of LET on aromatase and the activity of TAU by reducing its expression are expected to reduce estrogen level which in turn hinder the ER α - Δ Np63-ITGB4 pathway resulting in reducing ITGB4 gene expression, and this is in agreement with our results which show that both LET and TAU are capable to significantly reduce the mRNA relative expression of hepatic ITGB4 compared to EAC group, and the effect of their combination on ITGB4 expression is significantly lower than the treatment with either LET or TAU each alone.

Moreover, it is stated that occurrence of tumors affects many vital organs especially the liver, even if the site of the tumor does not interfere directly with its functions [46]. Our findings were reinforced by the results of histopathological examination of liver tissues of different groups since liver sections of EAC bearing mice showed severe necrosis with deeply pyknotic nuclei, where liver tissues of EAC bearing mice treated with LET or TAU or their combination recorded fewer lesions and decreased number of necrotic hepatocytes compared to EAC group (Figure 7).

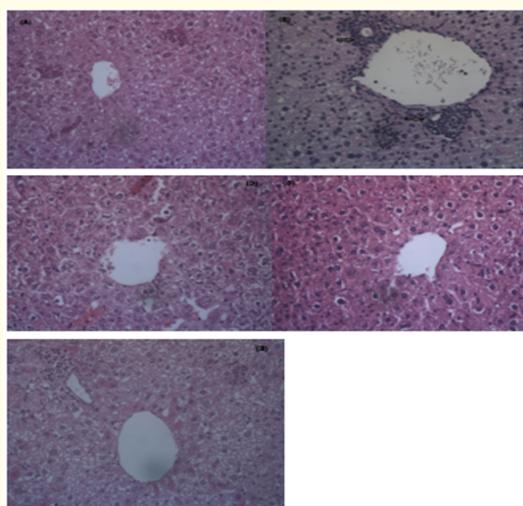


Figure 7: Photomicrographs of hepatic sections of Ehrlich ascites carcinoma (EAC) model in mice treated with Taurine (TAU), Letrozole (LET) and their combination (LET+TAU). H&E stain, X200 showing: (A) normal group. (B) Severe necrosis and deeply pyknotic nuclei of hepatocytes of EAC group (arrow). (C) Decreased number of necrotic hepatocytes and fewer lesions in LET group. (D) Decreased number of necrotic hepatocytes and fewer lesions in TAU group. (E) Decreased number of necrotic hepatocytes and fewer lesions in their combination group.

Nonetheless, these results must be taken with caution and a number of limitations should be borne in mind. First, the study is performed on an animal model not humans where the effects of TAU combined with LET might be affected by this. Second, the age of the tumor in the mice model is too short and this makes the study protocol very compressed.

Conclusion

Collectively, our results suggest that TAU might have a considerable anticancer activity through reversing the oxidative damage produced by estrogen, antiangiogenic effect by reducing hepatic VEGF content and decreasing the gene expression of aromatase enzyme, ITGB4 and HDAC4 which are important genes involved in estrogen dependent carcinogenesis. Thus, these results encourage more studies to assess the efficacy of taurine as a supplementary agent with LET in management of estrogen reliant cancers.

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