

Antitumor activity of L-Lysine alpha-oxidase enzyme against Ehrlich Ascites Carcinoma in adult female albino mice

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Abstract

L-lysine alpha-oxidase (LOX) is an interested enzyme due to its potential applications in biotechnology and tumor therapy. The objective of this study was to determine the antitumor activity of L-Lysine alpha-oxidase enzyme against Ehrlich ascites carcinoma in adult female albino mice. Adult female albino mice (20-25g) were divided into four groups (10 animals each) and treated for two weeks as follows: group (1) normal mice orally administration with saline and served as control, group (2) (LOX enzyme group): mice were injected with LOX enzyme doses of 300 U/kg daily for one week, group (3) animals female mice were interprotenally injected with 2.5×10^6 EAC/ml of Ehrlich ascites carcinoma for solid tumor induction, group (4) (LOX enzyme-Tumor group): mice bearing EAC treated with LOX enzyme at the same doses of group (2). Results: Administration of LOX enzyme in combination with EAC ameliorated the EAC-induced liver deterioration. This was monitored from the significant reduction in the level of serum TNF- α , AFP, CEA, ALAT, ASAT, GGT, ALP, cholesterol and triglycerides as well as hepatic MDA and nitric oxide coupled with an improvement in the levels of serum albumin as well as hepatic SOD and GSH. Moreover, the histopathological findings showed a potential protection as the enzyme succeeded in prevention of ascites carcinoma. Inoculation of EAC It could be concluded that LOX enzyme administrated to mice reduce tumor size, inhibit systemic inflammation, improving liver function profile, modulating lipid peroxidation and augmenting antioxidant defense system in EAC bearing mice.

Keywords: Ehrlich ascites carcinoma, lox, mice.

Introduction

Human cancer is as a major communal health problem world-wide. Every year, ten millions of people are diagnosed with cancer disease around the world, and more than half of the patients eventually die from it [1]. Resistance to conventional anticancer therapies in patients with advanced solid tumors [2]. In addition to the side effects that patients experience from conventional chemotherapy such as nausea, vomiting, hair loss and sometimes bone marrow suppression have prompted the need of alternative cancer therapies.

Important mode of cancer treatment is chemotherapy. It is the process of using anticancer drugs to stop cells from multiplying and dividing however there are few curative chemotherapeutic drugs against cancer due to non-selectivity, high toxicity, numerous side effects and low activity [3]. Therefore, new searching and developing effective anticancer agents have a great impact for the patients and could therefore save many lives. The disease such as cancer, chronic lung disease, severe disorders like autism and multiple sclerosis can be treated by therapeutic enzymes. Almost 60% of drug approved for cancer treatment are of natural sources such as fungi. Interestingly, therapeutic enzymes have a particular ability to mediate high affinity interactions with unrelated proteins relevant in cancer conferring on it the property. The chemotherapeutic

agents in miscellaneous include therapeutically enzymes as L-lysine alpha oxidase form an enzyme family with diverse N-termini and conserved C-terminal L-lysine alpha oxidase domains. The first identified member of this enzyme family is L-lysine alpha oxidase [4]. LOX is composed of a four hundred and seventeen residue polypeptide in a four chained dimer structure with every portion of the dimer containing of two distinct chains. LOX is comprised of alpha helices that account for 17.9% of the structure, and beta sheets for 38.3% of the structure [5]. L-lysine alpha-oxidase found in mammals [6], snakes [7], insects [8], marine organisms (mollusks and fish) [9], and microorganisms including bacteria [10], yeasts [11] and fungi [12].

Human LOX is synthesized as a 48 kD prepro-enzyme with a 21 amino-acid signal sequence at the N-terminus [13]. The enzymes treatment of cancer has a long history, first in 1902 with the work of Dr John Beard; a professor at the University of Edinburgh proposed that the pancreatic proteolytic enzyme trypsin represent powerful anti-cancer tool phenotypic revertants [14].

L-lysine alpha-oxidase was also recognized as a target for the anti-oncogenic transcription factor, interferon regulatory element-1 (IRE-1), which manifests tumor suppressor activity and contributes to the development of human hematopoietic malignancies [15].

[16] reported that malignant human breast carcinomas and broncho-pulmonary carcinomas LOX was highly expressed in hypertrophic scar-like stroma, surrounding the *in situ* tumor which forms a peritumor barrier, type I, III, and IV collagens and elastin were found to be co-distributed. The aim of research in the cancer field is selectively targeting and destroying malignant cells and preventing their spread by use natural microbial enzyme.

Materials and Methods

Animals

The present study used female mice as experimental subjects. Three weeks old female Swiss albino mice weighing 20-25 g were obtained from animal house of faculty of medicine (Assiut branch, Egypt).

Induction of Ehrlich Ascites Carcinoma

Ehrlich Ascites Carcinoma (EAC) cells were reported to show greater initial growth and total cell count in female mice [17]. A line of EAC cells was obtained from the Cancer Biology Department of the National Cancer Institute (Cairo, Egypt) and maintained by weekly intra-peritoneal transplantation of 2.5×10^6 cells / mouse. EAC cells were obtained from donor mice (Swiss albino) of 20-25 g body weight and suspended in sterile isotonic saline. A fixed number of viable cells (usually 2×10^6 cells/20 g body weight) were injected intraperitoneally in the thigh of each recipient mouse [18]. The viability of the cells was 99% as judged by trypan blue exclusion assay.

L-lysine alpha-oxidase enzyme Preparation

L-lysine alpha-oxidase was produced with *Tichoderma harzianum* Rifai AUMC No.848 collected from Assiut University Mycology Center under submerged fermentation using malt extract media, at 25-30 °C and purified to homogeneity through ammonium precipitation, anion exchange (Q FF column) and gel filtration chromatography (sephacryl-200). The enzyme has a molecular weight of approximately 118 kDa using native gel, and found to be about 58 kDa that indicated the enzyme consists of two subunits identical in molecular weight [19].

Experimental design

Adult female mice weighting 20- 25 g were obtained from Animal Colony, animal house of faculty of medicine (Assiut branch, Egypt). Before start the experiment the animals were housed in suitable plastic cages one week for acclimation. Fresh tap water and standard rodent food pellets [20.3% protein (20% casein and 0.3% DL-Methionine), 5% fat (corn oil), 5% fibers, 3.7% salt mixture and 1% vitamin mixture, obtained from Meladco Company, El-Obour City, Cairo, Egypt] were always available. All animals received human care in compliance with the standard institutional criteria for the care and use of experimental animals as cited by animal ethical committee of National Research Center, Egypt number FWA00014747. After animals being acclimatized with the experimental conditions, they were randomly divided into four groups (10 animals each); group 1) healthy mice orally administered with saline (0.4 ml/kg/day) and act as control, group 2): mice were IP injected with LOX enzyme 300U/kg daily for two week according to [20], group 3): animals were injection with EAC cells, each mice was injected, once; inter with 2.5×10^6 EAC/ml to induce the formation solid tumors [21] and group

4): mice bearing EAC treated with LOX enzyme at the same dose of group 2.

Blood and Tissue Sampling

At the end of the study period after two weeks, animals were fasted overnight. Following diethyl ether anesthesia, and using heparinized capillary tubes, blood specimens were collected from the retro-orbital plexus into vacutainer collecting tubes and left 20 minutes to clot, then centrifuged at 3000 rpm for 10 minutes using cooling centrifuge (IEC centra-4R, International Equipment Co., USA). The sera were separated, divided into aliquots and stored at -80°C. After blood collection, the animals were rapidly sacrificed and the right liver lobe of each animal was dissected out, washed with saline, dried, rolled in a piece of aluminum foil and stored at -80°C for homogenization biochemical determinations. The other liver lobe was soaked in a formalin-saline (10%) buffer; immediately sectioned, stained and prepared for microscopic examination.

Biochemical measurements

The activity of serum aminotransferases (ALAT and ASAT) was determined as described by [22] using reagent kits purchased from Human Gesell Schaft fur Biochemical und Diagnostic mbH, Germany. Serum GGT activity was measured according to the kinetic method described by [23] using reagent kits purchased from Bio Systems S.A. Costa Brava 30, Barcelona, Spain. Serum alkaline phosphatase (ALP) activity was assayed according [24] method using DiaSys reagent kits purchased from Dia Sys Diagnostic systems GmbH Germany. Serum total proteins, albumin, urea, creatinine and uric acid concentrations were evaluated according to Johnson *et al.*, [25] and Young, [26] using reagent kits purchased manual from Dia Sys Diagnostic systems GmbH Germany. Serum total cholesterol, triglycerides, HDL and LDL levels were determined according to Artiss & Zak [27], Cole *et al.*, [28], Wieland & Seidel [29] and Lopes-Virella *et al.*, [30] respectively, using Dia Sys reagent kits purchased from DiaSys Diagnostic System GmbH, Germany. Tumor necrosis factor-alpha, alpha fetoprotein and carcinoembryonic antigen were using ELISA (Dynatech Microplate Reader Model MR 5000, 478 Bay Street, Suite A213 Midland, ON, Canada) reagent kits purchased from Sino Gene Clon Biotech Co., Ltd, No.9 Bo Yuan Road, Yu Hang District 311112, Hang Zhou, China.

Tissue Homogenization and biochemistry

A specimen from liver was homogenized in ice-cold phosphate buffer (50 mM, pH 7.4) to give 10% homogenate (w/v). The homogenate was centrifuged at 5000 rpm for 20 minutes to remove the nuclear and mitochondrial fractions; the supernatant was divided into aliquots and stored at -80°C till the biochemical measurements.

Liver lipid peroxidation end product malondialdehyde (MDA), level was determined chemically according to the method described by Ruiz-Larrea, [31]. In brief 0.5 ml of supernatant homogenate was added to 4.5 ml working reagent [0.8 g thiobarbituric acid (TBA) dissolved in 100 ml perchloric acid (10%), and mixed with trichloroacetic acid (20%) in a ratio 1 to 3 v/v,]. In a boiling- shaking water bath, the sample-reagent mixture was left for 20 minutes, then carried to cool to room temperature and centrifuged for 5 minutes at 3000 rpm. Immediately, the absorbance of the

clear pink supernatant was measured photometrically at 535nm against reagent blank (0.5 ml distilled water + 4.5 ml working reagent). MDA level was calculated according to the following formula: $MDA (nmol/g \text{ tissue}) = \left[\frac{A_{535} \times 10^9}{(1.56 \times 10^5) \times 10^3} \right] \times AD \times 10$; where, $1.56 \times 10^5 M^{-1} L^{-1} cm^{-1}$ = extinction coefficient of MDA, AD is assay dilution (10). Reduced glutathione (GSH) was determined according to the method of Beutler *et al.*[32]; Superoxide dismutase (SOD) and nitric oxide (NO) were determined according to the method of Sinha [33], Masayasu and Hiroshi [34] and Gross *et al.* [35], respectively. Using reagent kits obtained from Biodiagnostic Co., Dokki, and Giza, Egypt.

Histopathological investigation

Parts of the excised liver tissues were fixed in formaldehyde-Saline (10%) buffer for 24 h, and then embedded in paraffin blocks. Sections of 4 to 5 μm thickness were stained with hematoxylin and eosin (H&E) for histopathological examination using light microscope.

Statistical analysis

The obtained data were subjected to one way analysis of variance (ANOVA) followed by post- hoc (Tukey) test at $p \leq 0.05$ using statistical analysis system (SAS) program software; copyright (c) 1998 by SAS Institute Inc., Cary, NC, USA [36].

Results

The obtained data showed that intraperitoneal injection of female mice with the LOX enzyme didn't significantly change either tumor cell count, AFP, TNF-alpha or CEA, while EAC-induced animals revealed a significant increases in these measurements when both groups were compared to healthy group. In addition, treatment of EAC-induced animals with LOX enzyme resulted in a significant reduction in tumor cell count, AFP, TNF-alpha or CEA in compared to the untreated EAC-induced animals group (table 1).

Table 1: Tumor cell count, AFP (ng/ml), TNF-α (pg/ml) and CEA (ng/ml) of treated and control animals groups.

	Tumor cell	AFP	TNF-α	CEA
	Count × 10 ⁶	(ng/ml)	(pg/ml)	(ng/ml)
Control	0±0 ^c	24.3±4.98 ^c	79.3±5.64 ^c	1.55±0.23 ^c
LOX	0±0 ^c	22.43±3.12 ^c	70.4±4.32 ^c	1.46±1.64 ^c
EAC	4739 ±12.84 ^a	97.4±5.21 ^a	197.5±4.65 ^a	59.37±2.47 ^a
EAC+LOX	74.7±32.8 ^b	56.44±4.34 ^b	112.43±6.4 ^b	39.39±1.23 ^b

Data are presented as mean ±S.E. Within each column, means with different superscript letters are significantly different ($P \leq 0.05$) using one way (Tukey tests) ANOVA test. -LOX (L-Lysine Alpha-Oxidase), EAC (Ehrlich Ascites Carcinoma)

The obtained results showed that intraperitoneal injection of female mice with the LOX enzyme didn't significantly change of serum ALAT, ASAT, GGT and ALP activities, while EAC-induced animals revealed a significant increases in these measurements when both groups were compared to healthy group. In addition, treatment of EAC-induced animals with LOX enzyme resulted in a significant reduction in of serum ALAT, ASAT, GGT and ALP activities in compared to the untreated EAC-induced animals group (table 2).

Table 2: Serum ALAT, ASAT, GGT and ALP activities of treated and control animals groups.

	ALAT (U/L)	ASAT (U/L)	GGT (U/L)	ALP (U/L)
Control	49.33±2.01 ^c	55.67±1.65 ^c	118.33±1.64 ^c	13±0.12 ^c
LOX	44±1.63 ^c	53.33±2.32 ^c	121.12±2.44 ^c	12±0.4 ^c
EAC	106.67±2.71 ^b	140.7±4.19 ^b	162.33±3.47 ^a	25±0.81 ^a
EAC+LOX	69.33±1.54 ^a	72.33±2.43 ^a	92.66±2.65 ^d	16.67±1.49 ^b

Data are presented as mean ±S.E. Within each column, means with different superscript letters are significantly different ($P \leq 0.05$) using one way (Tukey tests) ANOVA test. -LOX (L-Lysine Alpha-Oxidase), EAC (Ehrlich Ascites Carcinoma)

The obtained data showed that intraperitoneal injection of female mice with the LOX enzyme didn't significantly change of serum total protein, albumin, globulin and A/G ratio levels, while EAC-induced animals revealed a significant decreases in these measurements when both groups were compared to control group. In addition, treatment of EAC-induced animals with LOX enzyme resulted in a significant reduction in of serum total protein, albumin, globulin and A/G ratio levels in compared to the untreated EAC-induced animals group (table 3).

Table 3: serum total protein, albumin, Globulin and A/G ratio levels of treated and control animals groups.

	Total protein	Albumin	Globulin	A/G ratio
	g/dl	g/dl	g/dl	
Control	6.8±0.21 ^d	3.85±0.02 ^a	3.1±0.04 ^a	1.19±0.12 ^d
LOX	6.8±0.6 ^d	3.9±0.02 ^a	2.66±0.04 ^a	1.23±0.02 ^d
EAC	5.37±0.05 ^c	2.85±0.02 ^c	2.44±0.02 ^a	0.82±0.028 ^c
EAC+LOX	6.6±0.12 ^a	3.2±0.03 ^d	2.7±0.02 ^a	1.5±0.04 ^a

Data are presented as mean ±S.E. Within each column, means with different superscript letters are significantly different ($P \leq 0.05$) using one way (Tukey tests) ANOVA test. -LOX (L-Lysine Alpha-Oxidase), EAC (Ehrlich Ascites Carcinoma)

The obtained data showed that intraperitoneal injection of female mice with the LOX enzyme didn't significantly change of serum cholesterol, triglycerides, HDL and LDL levels, while EAC-induced animals revealed a significant increase in serum total cholesterol, triglycerides and LDL coupled with decreased in HDL levels were compared to control group. In addition, treatment of EAC-induced animals with LOX enzyme resulted in a significant reduction in serum total cholesterol, triglycerides and LDL coupled with increased in HDL compared to the untreated EAC-induced animals group (table 4).

Table 4: serum total cholesterol, triglycerides, HDL-cholesterol and LDL-cholesterol levels of treated and control animals groups.

	Cholesterol	Triglycerides	HDL	LDL
	mg/dl	mg/dl	mg/dl	mg/dl
Control	126±1.4 ^c	138.7±3.6 ^a	33.6±1.2 ^d	57.6±3.2 ^e
LOX	117.3±2.49 ^e	144.3±5.0 ^a	29.8±1.65 ^d	56.3±2.2 ^e
EAC	226.6±3.0 ^b	206.3±10.1 ^e	19.5±2.1 ^c	95.4±4.23 ^b
EAC+LOX	122.3±1.24 ^a	158.3±14.9 ^b	35.7±1.14 ^a	53.4±2.7 ^a

Data are presented as mean ±S.E. Within each column, means with different superscript letters are significantly different ($P \leq 0.05$) using one way (Tukey tests) ANOVA test. -LOX (L-Lysine Alpha-Oxidase), EAC (Ehrlich Ascites Carcinoma)

The obtained data showed that intraperitoneal injection of female mice with the LOX enzyme didn't significantly change of serum blood urea, creatinine and uric acid, while EAC-induced animals revealed a significant increase in these measurements when both groups were compared to control group. In addition, treatment of EAC-induced animals with LOX enzyme resulted in a significant reduction in serum blood urea, creatinine and uric acid in compared to the untreated EAC-induced animals group (table 5).

Table 5: serum blood urea, creatinine and uric acid of treated and control animals groups

	Blood urea mg/dl	Creatinine mg/dl	Uric acid mg/dl
Control	58.32±6.61 ^e	1.76±6.21 ^e	4.32±5.3 ^c
LOX	49.23±5.63 ^e	1.69±3.43 ^e	5.34±3.69 ^e
EAC	78.3±7.32 ^b	2.1±7.19 ^b	7.23±5.47 ^b
EAC+LOX	51.33±7.54 ^a	1.54±6.65 ^c	4.46±4.65 ^a

Data are presented as mean ±S.E. Within each column, means with different superscript letters are significantly different ($P \leq 0.05$) using one way (Tukey tests) ANOVA test. -LOX (L-Lysine Alpha-Oxidase), EAC (Ehrlich Ascites Carcinoma) The obtained data showed that intraperitoneal injection of female mice with the LOX enzyme didn't significantly change of hepatic MDA, NO, GSH and SOD levels, while EAC-induced animals revealed a significant increase in liver MDA and NO coupled with decreased in hepatic GSH and SOD levels were compared to control group. In addition, treatment of EAC-induced animals with LOX enzyme resulted in a significant

reduction in hepatic MDA and NO coupled with increased in hepatic GSH and SOD compared to the untreated EAC-induced animals group (table 6).

Table 6: Hepatic malodialdehyde (MDA), nitric oxide (NO) and glutathione reduced (GSH) levels and superoxide dismutase (SOD) activity of treated and control animals groups.

	NO ($\mu\text{mol/g tissue}$)	MDA (nmol/g tissue)	GSH (mg/g tissue)	SOD (U/g tissue)
Control	33.42±3.76 ^e	75.34±5.34 ^e	95.5±6.65 ^d	18.4±6.2 ^e
LOX	35.42±2.49 ^e	73.33±9.1 ^e	91.8±4.2 ^d	22.3±5.2 ^e
EAC	19.21±1.45 ^b	45.32±6.9 ^b	132.4±6.31 ^c	47.5±8.23 ^b
EAC+LOX	29.2±2.41 ^a	59.54±6.21 ^a	78.3±5.14 ^a	17.1±4.7 ^a

Data are presented as mean ±S.E. Within each column, means with different superscript letters are significantly different ($P \leq 0.05$) using one way (Tukey tests) ANOVA test. -LOX (L-Lysine Alpha-Oxidase), EAC (Ehrlich Ascites Carcinoma)

Histopathological results of liver.

Livers from group (1) & (2) showed normal histological structure; group (3) perinephric fat and the capsule of liver were infiltrated by malignant cells, which showed pleomorphism, hyperchromatic nuclei, increased nucleo/cytoplasmic ratio, prominent eosinophilic nuclei and many abnormal mitotic figures. In group (4), the malignant cells were markedly reduced in number and were infiltrated by inflammatory cells.

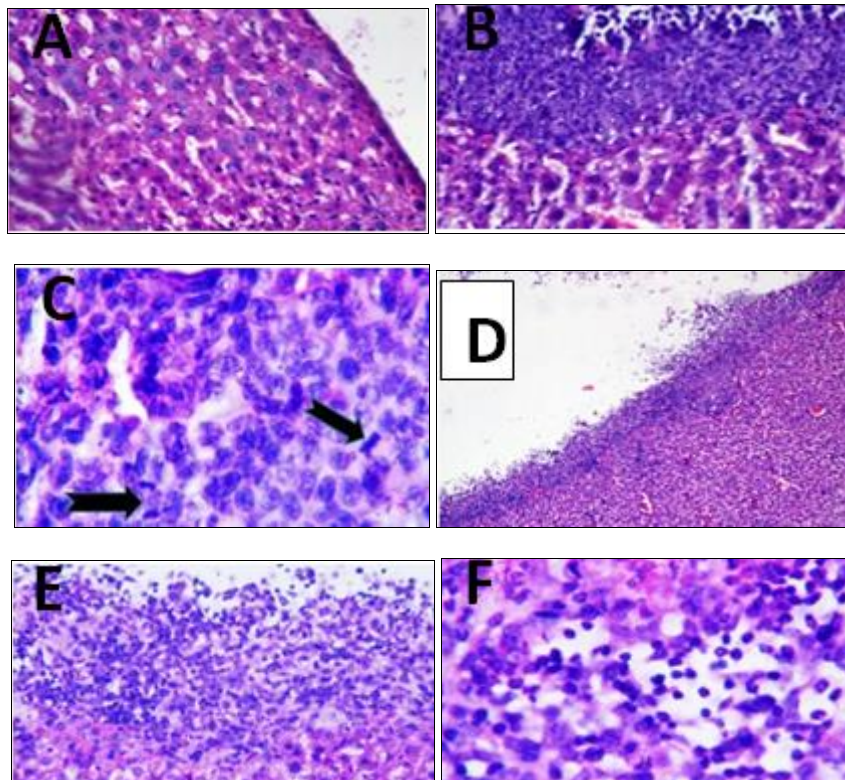


Fig 1 A: Microscopic micrograph show normal liver architecture (group 1&2) (100x). **B:** Microscopic picture showing liver with surrounding tumor cells (group 3) (400x). **C:** Microscopic picture showing pleomorphic tumor cells with mitosis (arrows) (group 3) (1000x). **D, E&F:** Microscopic picture showing liver with decreased tumor cells surrounding it than group 3 and infiltration of tumor by inflammatory cells (group 4) (100x, 400x & 1000x respectively).

Discussion

The application of enzyme technologies to pharmaceutical research, development and manufacturing is a growing field and is the subject of many articles, reviews and books. We will limit the scope of this review to recent articles on the use of enzymes as drugs. Majority of medically important microbial enzymes are obtained from a limited number of fungi, yeast and bacteria. These organisms are also considered when a new enzyme is required [37]. Medically important enzymes are required in very less quantity as compared to the industrially important enzymes. But they should have a high degree of purity and specificity. The Regular consumption of enzymes and enzyme-rich foods contributes to vibrant health, prevention of disease, and antiageing process. Each cell in our body needs enzymes for its biochemical functions, and a deficiency of these enzymes will accelerate the aging process. Some of the important functions of enzymes are regulation of the growth of the body from a single cell to a mature organism, conversion of food to energy to fulfill the bodys needs, and break down or buildup of certain substances within the cell [38]. Medically important enzymes produced by microorganisms find their application in removal of cytotoxic substances within the blood circulation, treatment of life threatening disorders as oncolytics, thrombolytics, anti-coagulants and as replacements for metabolic deficiencies [38]. There is very less information about the utilization of microbial enzymes for therapeutic purposes except for some anticancer enzymes and the enzymes active against cystic fibrosis [39]. There major application is in the treatment of cancer (prodrug activator enzymes and antineoplastic enzymes) and various other diseases as genetic diseases.

The antitumor enzymes in particular, can affect the metabolic pathways of tumor cells and promote apoptosis since the malignant cells generally cannot repair themselves effectively [40]. Therefore, the present study clearly indicated that microbial enzyme of purified L-Lysine alpha-oxidase enzyme showed a significant therapeutic activity against ehrlich ascites carcinoma.

The obtained data showed that intraperitoneal injection of female mice with the LOX enzyme didn't significantly change AFP, TNF- α and CEA; ALAT, ASAT, GGT and ALP activities, hepatic MDA, NO, SOD & GSH compared to the control group, while EAC-induced animals revealed a significant increases in these measurements when compared to healthy group. In addition, treatment of EAC-induced animals with LOX enzyme resulted in a significant reduction in tumor cell count, AFP, TNF-alpha and CEA; ALAT, ASAT, GGT and ALP activities, hepatic MDA & NO levels coupled with increased SOD & GSH in compared to the untreated EAC-induced animals group. These findings are in constituent with the reports of Reddy *et al.* [41], Abd El-Aziz *et al.* [42], Kapoor *et al.* [43], Ferlay *et al.* [44] Talaviya *et al.* [45]

The elevation in liver enzymes of mice treated with EAC has already been reported in earlier studies and is still consistent with more recent results [46]. Most experimental solid tumors have elevated levels of ROS and consequently oxidative stress [47]. Oxidative stress has been suggested to represent an important contributory factor to liver injury, and to enhanced morbidity related to liver hypo-function [48]. Therefore, accumulation of ROS and GSH depletion in tumor cells could induce unsaturated fatty acid to undertake lipid oxidation, there by disordering the transport and

storage of Ca²⁺ in mitochondria, endoplasmic reticulum and cell membrane, increasing the content of plasmic Ca²⁺, and ultimately causing death of cell. ALT, AST, ALP and GGT are then released into the blood [49].

Liver damage induced by tumor cells generally reflects disturbances in liver cell metabolism, which lead to characteristic changes in serum enzyme activities. The increased levels of ASAT, ALAT, ALP coupled with decrease albumin in serum may be interpreted as a result of liver damage or as changes in membrane permeability indicating the severity of hepatocellular damage by EAC. Serum liver enzymes (ALT, AST and ALP) showed significant increase in EAC bearing mice. The increment in serum enzymatic activities is related to hepatic parenchymal damage since ALT and AST are released from mitochondrial and cytosolic localization and GGT from membranal sites, and cellular rupture allows the enzyme to escape into the blood [50].

The free radicals formed by the reaction of metabolites of carcinoma with oxygen or by the interaction of superoxide radicals with H₂O₂, seem to initiate peroxidative degradation of membrane lipids and endoplasmic reticulum rich in polyunsaturated fatty acids. This leads to formation of lipid peroxides which in turn give products like MDA that cause loss of integrity of hepatocyte membrane consequently damage to hepatic tissue.

The free radical scavenging system, SOD and catalase are present in all oxygen metabolizing cells and their function is to provide a defense against the potentially damaging reactions of superoxide and hydrogen peroxide. Sun *et al.* [51] reported a decrease in SOD activity in EAC bearing mice which might be due to loss of MnSOD activity in EAC cells and the loss of mitochondria, leading to a decrease in total SOD activity in the liver. The inhibition of SOD and CAT activities as a result of tumor growth was also reported [52]. Similar findings were observed in the present investigation with EAC bearing mice.

Favorably, rat's ip with the microbial enzyme for two week didn't affect serum level total proteins, albumin and globulin. These results point to the safe effects of enzyme on the synthesis and excretion capacity of hepatic. This finding is in accordance with [53]

In contrast, animal's ip with EAC only revealed a significant decrease in the level of serum total protein and albumin levels as compared to normal animals. The decrease of serum total protein and albumin herein could be due to the direct inhibitory effect of carcinoma induced radicals on protein synthesis or cell necrosis of the liver. This result is consistent with the report of Said *et al.* [54] and Talaviya *et al.* [45]

Treatment with L-Lysine alpha-oxidase enzyme caused improvement in which ameliorating hepatic enzyme levels which related to the free radical scavenging activity, so LOX enzyme protect liver membrane from free radicals and decrease lipid peroxidation level.

In the present study, the presence of EC tumor in female Swiss albino mice was accompanied by a systemic inflammation as manifested by a significant increases in tumor necrosis factor-alpha (TNF- α) reflecting systemic inflammation in the liver. All of these could come from the tumor sensitive systemic marker of inflammation and liver tissue damage [55].

Significant increases in proinflammatory cytokines (as TNF- α), in the present study might be attributed to the fact

that ehrlich solid tumors can cause a series of deleterious side-effects, including oxidative stress that may triggers lipid peroxidation which in turn initiates release of malondialdehyde and binds to hepatocyte proteins initiating a potentially harmful immune response and stimulate neutrophil chemotaxis or activates transcriptional factor NF κ B which in turn increases the production of proinflammatory cytokines as TNF- α , promoting hepatocyte injury and apoptosis and hepatic stellate cell activation [56].

Treatment with LOX attributed that inhibitory mechanism on Ras-mediated transformation is due to activation of signal pathways that lead to activation of NF- κ B (Nuclear factor of kappa light polypeptide gene enhancer in B cells) [57]. The decrease in the level of the essential amino acid L-lysine and the formation of hydrogen peroxide during the reaction possibly provide the basis for the unique properties of L-lysine alpha-oxidase: cytotoxic and antitumor [53].

In the present study, the levels of total cholesterol were higher in carcinoma ip mice, indicating that induce hypercholesterolemic condition; this is in agreement with the report of Habib *et al.*, [58] how attributed that to increased uptake of LDL from the blood by the tissues. The abnormal cholesterol deposition is favored by the dangerous tendency of cholesterol to undergo passive exchange between the plasma lipoproteins and the cell membranes [58]. The elevation of triglycerides (TG) level as a consequence to carcinoma induction may be due to impaired removal and destruction of TG rich in lipoproteins such as VLDL, LDL, IDL and remnants; this could be confirmed by the elevated level of LDL-c, or to the increased hepatic synthesis of fatty acids, precursors of TG formability [58].

In comparison with normal mice, the data showed administration of mice with L-Lysine alpha-oxidase enzyme didn't record any harm changes in the renal function, monitored from serum levels of creatinine, urea and uric acid, i.e microbial enzyme haven't any nephrotoxicological effects reflecting their safety; on the other hand, induction of carcinoma alone led to kind of nephrotoxicity evidenced with the significant increase in serum levels of creatinine, urea, and uric acid. Nephrotoxicity is serious characterized by high blood urea and serum creatinine levels. Carcinoma induced lipid peroxidation and oxygen free radical generation in kidney and these effects damaged the kidney. Oxidative stress and ROS accumulation are among the main mechanisms of carcinoma-induced nephrotoxicity [59].

Tumor cells have the capacity of up regulating antioxidant systems to protect themselves against reactive oxygen species induced cell damage [60]. L-Lysine alpha-oxidase enzyme exerts its anticancer property in part by increasing H₂O₂ which leads to induction of apoptosis in cancer cells [53].

As programmed cell death may follow either apoptosis or necrosis. Evidence suggests that apoptosis and necrosis represent morphological expression of shared biochemical changes known as "apoptosis-necrosis continuum" some factors as decrease availability of caspases or intracellular ATP may change an ongoing apoptosis into necrosis [61] and this may explain the inflammatory cells we found in the treated mice. This goes in agreement with Mukherjee *et al.*, [62] who found that breast cancer cells showed some hallmark of apoptosis after treatment with L-amino acid oxidase, however they showed morphological phenotype of necrosis after prolonged incubation.

Histopathological changes identified in the present study were perinephric fat and the capsule of liver were infiltrated by malignant cells, which showed pleomorphism, hyperchromatic nuclei, increased nucleo/cytoplasmic ratio, prominent eosinophilic nuclei and many abnormal mitotic in liver tissue of EAC mice. It is realized that the treatment of mice with purified LOX enzyme, in this work, alleviated the deleterious effect induced by EAC on liver. In addition, LOX may act as cofactor in the synthesis of biological endogenous antioxidant material such as glutathione and/or activate endogenous antioxidant enzymes such as glutathione-s-transferase (GST) [63]. Histopathological investigations are in a good agreement with biochemical changes; findings of the present study indicated that LOX showed a significant hepatoprotective and curative activity against EAC induced hepatic injury.

Conclusion

The present study was carried out to evaluate the antitumor activity of L-Lysine alpha-oxidase enzyme on EAC bearing mice. The L-Lysine alpha-oxidase enzyme treatment at the dose of 300U/kg inhibited the tumor cell count, tumor markers, serum biochemical as well as pathological investigation towards normal levels. The L-Lysine alpha-oxidase enzyme also restored the hepatic lipid peroxidation and free radical scavenging enzyme GSH as well as other antioxidant enzymes such as SOD in tumor bearing mice to near normal levels.

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