Biochemical effect of Lysine – cetrimonium zinc compound on 7,12dimethylbenz(a)anthracene Induced Mammary Carcinogenesis in Rats

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A B S T R A C T

Breast cancer is the second leading cause of cancer death in women in the world .The chemopreventive effect of Lysine-Cetrimonium Zinc Complex with zinc oxide nanoparticle and sodium ascorbate (L CZN) on 7,12-Dimethylbenz[*a*]anthracene(DMBA) induced mammary carcinogenesis was investigated in(60) female rats aged from (4-5)weeks and weighting from (90-110) gr . Oral dose of DMBA (100mg/k.gr.b.wt). induced a significant increase in serum ALT, AST, MDA, urea, Creatinine, and CD20 in serum or tissue .However were observed in DMBA induced mammary cancer in rats depletion in CAT,GSH and Annexin V were markedly decreased . Administration of (NNC) Novel Nano composite was able to mitigate mammary carcinogenic damage induced by DMBA as to directory pronounce chemo preventive effect against lipid peroxidation, sever enzymatic changes and maintained glutathione status and antioxidant enzymes in addition histopathological changes directed towards control. It could be conducted that administration of (NNC) have potential to exert therapeutic effect against mammary carcinogenesis.

Keywords: anthracene ,Mammary Carcinogenesis, Lysine – cetrimonium

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1-INTRODUCTION

Breast cancer is still today one of the leading causes of cancer mortality. despite .The development of improved diagnostic tools and novel therapeutic modalities. Presently, combination therapy (i.e., combinations of different chemotherapeutic drugs in a chemotherapy regime) is becoming a more common attractive strategy for effective anticancer treatment because it generates synergistic anticancer effects, reduces individual deregulated toxicity, and inhibit multi-drug resistance via different mechanisms of action (Parhi , 2012).One of the factor which formerly has been identified to be responsible for the development of neoplasia is the polycyclic aromatichydrogen7,12-dimethylbenz [a] anthr-acene (DMBA) , which is an immune depress also a powerful organ-specific carcinogen .(Miyata ,et al .,2001). producing reactive oxygen species , which consider the key role in initiating the cancer process ‘(Tab czar et al .,2012).

Over the past two to three decades, Zinc is an essential mineral that is integral to many enzymes and transcription factors that regulate key cellular functions such as the

response to oxidative stress, DNA replication, DNA damage repair, cell cycle progression, and apoptosis. In particular, several proteins involved in DNA damage signaling and repair, replicative enzymes such as DNA and RNA polymerases and transcription factors such as tumour protein p5317, require zinc for proper functions . (*Pavletich ,* et al .,1993). CTAB is a quaternary ammonium compound belonging to a group of small molecules known as delocalized lipophilic cations (DLCs). Because of their lipophilic nature and delocalized positive charge, DLCs can penetrate the hydrophobic barriers of plasma and mitochondrial membranes and accumulate in the mitochondria in response to the negative transmembrane potential, resulting in mitochondrial toxicity (Chen, 1988).

Sodium ascorbate is considered a powerful hydro-soluble antioxidant capable of deoxidizing the reaction of oxygen and nitrogen free radical species . Therefore sodium ascorbate is able to prevent important deleterious oxidative effects on biological macromolecules , such as DNA ,lipid to build human proteins. (Soheili Majed et al ., 2003).

PH plays an important role in almost all steps of metastasis (Hashim et al., 2011) . Lysine cannot be naturally synthesised within the body and must be sourced from food or dietary supplements. As a protein building block , lysine is required for normal growth and development; and all been shown to be effective in reducing metastases in vivo . Decreasing of metastasis is dependent upon buffering (S.A. Ribeiro MdLC et al ., 2012). Buffer therapy was initiated before inoculation to prevent progression to metastatic disease. Previous studies show buffer therapy has little effect on reducing primary tumor growth, but significantly reduces spontaneous metastasis formation (Robey et al ., 2009). In last years, nanotechnology-based combination drug delivery systems to the cancer tissue have unfold as an efficient strategy by overcoming many biological, biophysical, and biomedical barriers, largely due to the physical and chemical properties of these nanomaterials. Therefore, administration of different chemotherapeutic drugs in combination, with a compatible nanocarrier platform could be considered as an emerging approach for the treatment of cancer in near the future by offering smart drug delivery systems (Parhi , 2012).

Zinc oxide (ZnO) NPs belonging to a group of metal oxides are characterized by their photocatalytic and photo-oxidizing ability against chemical and biological species. In recent times, ZnO NPs have received much attention for their implications in cancer therapy (Zhang et al., 2011) . Several studies have shown that ZnO NPs induce cytotoxicity in a cell specific and proliferation-dependent manner, with rapidly dividing cancer cells being the most susceptible, and quiescent cells being the least sensitive (Premanathan et al., 2011).

Accordingly the present study was prepared to insert a newly synthesized metal–based composite ,namely lysine -cetrimonium –tri chloro –bromo-zincate (CLTCBZ), as a chemotherapeutic agent with prediction reduced toxicity risk accompanied with higher potency in cancer treatment. The long period term in vivo study designed to evaluate the antitumor effective of (Novel Nano Composite) on mammary gland-induced tumors. Experimental investigations following 1-month treatment with the tested compounds involved biochemical analysis of

whole blood antioxidant status , plasma lipid peroxidation levels , CD20, Annexin v activity in mammary gland , liver and kidney function levels .In parallel to biochemical changes histopathological variation were performed

2- MATERIALS AND METHODS :

2.1- Chemical studies :

-7,12-Dimethylbenz[*a*]anthracene (≥95%):was purchased from Sigma-Aldrich Company.

-Zinc oxide nanoparticles (≥99.9%): was purchased from Sigma-Aldrich Company, in the form of 40-100 nm APS powder. The molecular weight (FW) is 81.37 and the melting point is 1975°C.

-Sodium ascorbate (≥98%) : It was kindly purchased from Al-Dawlya Company , the Empirical Formula $C_6H_7NaO_6$, Molecular Weight (198.11) .

-L-Lysine hydrochloride (≥99.9%) : was purchased from Al-Dawlya Company light beige odorless powder the Empirical Formula $C_{20}H_{16}$ $C_6H_{15}ClN_2O_2$. Molecular Weight is 182.648 g/mol.

-Acetyl trimethyl ammonium bromide (CTAB) (≥99.9%) : A quaternary ammonium salt ,was purchased from Al-Dawlya Company .light beige odorless powder ,molecular weight is 364.46 , melting range (F) 459-469 ,Decomposition temperature (F) 459.

2.2- Biochemical investigations :

The present study was intended to comprise a set of in vitro and in vivo realization as follow :In Vitro study :Human breast cancer cell line (MCF-7) was obtained from VACSERA tissue culture units ,and maintained in the Regional Center for Mycology& Biotechnology , Al-Azhar University , Cairo, Egypt. The antitumor effect and inhibitory concentration 50(IC_{50}) of new novel composite (L CZN)was investigated on the viability of the human breast cancer cell line (MCF-7) using (DMEM) medium (Dulbecco's modified Eagle's medium) (Mosmann (1983) &(Gomha et al .,2015).

In Vivo study :A total number of 60 virgin Swiss albino female rats aged from(4-5) weeks and weighting from (90-110) g .were obtained from the Laboratory Animals Research Center , Faculty of veterinary Medicine Benha University . Animals were randomly housed in suitable steel mesh cages and kept at a constant temperature and nutritional condition throughout the period of experimental. Animals were fed on constant watering and fresh and clean drinking of water was supplied ad-libtium. All rats were adopted for laboratory conditions .for approximately two weeks before the beginning the experiment .

Determination of median lethal dose (LD_{50}) :Conventionally , acute oral toxicity testing concentrated on limiting the dose that kills half of the animals (the medium lethal dose or LD_{50}) , the timing of lethality following acute chemical exposure as long as observing the start, nature, riskiness, and reversibility of toxicity . (Hodgson , 2010). The optimum chosen dose for estimating the in vivo antitumor activity of the novel synthesized compound (LCZN) was calculated nearly as (1/12) of its LD_{50} value , which was 20mg/kg .b .wt Fresh solution of the test compound was prepared in sterile saline, according to their selected dose fully before their administration .

2.3- Induction of mammary carcinogenesis :

An experimental exemplar of chemical mammary tumors in virgin female Swiss albino rats was inducted by receiving a single oral dose of (100mg/kg b .wt) of the chemical carcinogen , 7,12-dimethylbenz [a] - anthracene (DMBA), diluted in sesame oil(100ml). and (1ml) given intragastrically by gavage according to the method of Barros et al.,2004).Physical examination were performed hebdomadal . Each had six pairs of mammary glands that were checked by examination , probing and palpation . For estimating of the induction manner, then after DMBA injection at the end of (16 –20)weeks a random sample was taken from mammary gland of rats groups for histopathological examination to assure tumor incidence .

2.4- Experimental animals design :

Animals were randomly divided into four groups (15 rats each g). as follow :

Group (1) (Normal Control) :Rats received oral dose of sesame oil (1ml)/kg. b. wt at the beginning of the experiment and were left untreated for 5 months.

Group (2) (DMBA –inducted mammary tumor rats) : Rats received a single oral dose of DMBA 100mg/kg b .wt. DMBA diluted in sesame oil(1ml) taken intragastrically by gavage . Animals were left until the end of the experiment for tumor incidence , and second oral dose of DMBA were administrated after (4 months) for enhancing tumor incidence Then they were killed at the end of six month.

Group (3) (DMBA+ Nano composite (LCZN) : After induction of cancer breast rats were treated with(LCZN) nanoparticles for one month three times per week .

Group(4) novel composite (LCZN) only: Rats in this group administrated with novel composite orally three times a week for a duration of one month.

2.5.1- Blood samples :Two blood samples were collected from all groups under light ether anesthesia by heart puncture: The first part was collected in heparinized tubes and used directly for the determination of the following biochemical markers: Reduced glutathione (GSH) , Catalase (CTA) and Malondialdehyde (MDA).The second part of blood samples were collected in vacuumed tubes or heparinized tubes and centrifuged at 3000rpm for 10 minute for separate the plasma or serum and used directly for determination of AST, ALT, Urea and Creatinine or quickly frozen in a deep freezer at (-20 °C) until used for analysis .

2.5.2- Tissue samples :

After collection blood sampling, rats of each group were sacrificed by cervical decapitation The abdomen was opened and the mammary gland was quickly removed and opened gently using a scrapper, cleaned by rinsing with ice-cold isotonic saline to remove any blood cells, then blotted between 2 filter papers to determine apoptosis and kept at -20°C until used for analysis . Another part of mammary tissues were dissected and kept in 10% formalin for histopathological examination (Banchroft et al., 1996).

2.5.3-Biochemical analysis :

Whole blood MDA , Reduced glutathione (GSH) and Catalase (CAT) activity were determined according to the method described by(Yoshioka T et al ., 1970), (Beutler *et al.*, 1963) and (Aebi,H. (1984)& (Fossati, et. (1980) respectively. CD20 , Annexin v, AST, ALT, Urea and Creatinine activity were

determined according to the method described by (*Traganos F et al.,1977),* (Z. Klin; 1972), (Balleter et al .,1961), and (Murray R.L.(1984) respectively.

2.5.4- Histopathological examination :

Specimen of mammary tissue were fixed in 10% formalin for 24 hours then serial dilution of alcohol were used for dehydration and paraffin-wax embedding .Sections (4 µm) were cut in a microtome , adhered to glass slides and stained by hematoxylin and eosin stains (H&E) for histo-pathological examination.

2.6- Statistical analysis :

All statistical analyses were performed using SPSS software (Version 15.0). Data were analyzed using a one-way analysis of variance (ANOVA), followed by least significant difference (LSD) test. when an overall significant indicate $p<0.05$ in all cases and all data are reported as the mean ±S.D (Bailey .,1994).

3. RESULTS:

In vitro study :The 50% growth inhibitory concentration (IC_{50}) values was presented in table (1) .It was observed that (LCZ) NP-s exhibited the strongest cytotoxic effect against MCF-7 . The recorded data showed the lowest IC_{50} value represented (5.8 µg/ml) .

In vivo studies :

Biochemical analysis :

The obtained results shown a significant decrease of
tumor-induced rats (DMBA group) when compared to
increase in (DMBA treated group) with (NNC) or (NN
(DMBA) group. On the other hand the values of pla
Creatinine . Whole blood MDA and CD20for all studie
Table(2,,4) shown in (DMBA-inducted rats significantly
normal control group and significantly decrease in DMB
or (NNC) only when compared to (DMBA-administrated

3-2.Histopathological findings :

Histopathological examination of mammary gland tissues of tumor-inducted rats (DMBA)group shown variation of glands graded from dysplasia to anaplasia in the lining epithelium of the acini replacing the mammary acini and lymph gland of rats in showing metastatic cancer cells from the mammary parenchyma to the regional lymph gland Treatment of tumor –inducted rats treated with (NNC) showing intact normal histopathological structure of the acini .also shown normal .

Table (1) The 50% growth inhibitory concentration (IC_{50})values of the tested compounds on human breast cancer cell line(MCF-7).

IC_{50}		
Cell line	**Teat compound**	**Lysine-Cetrimonium zinc(NPs)**
Human breast cancer cell line		**5.8**

Table (2) Effect of (LCZN) on whole blood GSH , MDA concentration ,CAT activity and CD20 concentration in mammary tissue of different rat groups.

Animal groups/ Parameters	GSH (mg/dl)	CAT (mg/dl)	MDA (mol/L)	CD20 (Unit/mL)
Group(1) Control	39.04±0.19^{a}	188.25±0.43^{e}	7.73±0.13^{e}	28.10±1.62^{d}
Group(2) DMBA	19.10±0.06^{f}	162.80±0.69^{f}	35.68±0.28^{a}	55.50±1.10^{c}
Group(3)DMBA+NNC	28.78±0.25^{d}	261.85±0.37^{b}	11.55±0.20^{c}	15.75±0.89^{f}
Group(4) NNC	32.75±0.20^{b}	292.50±0.29^{a}	8.48±0.27^{d}	17.70±1.27^{ef}

*****Data are represented as (mean ±SD) . values with different superscript letters with same column are significantly different at **(P<0.05).**

Table(3) Flow cytometry of Annexin v of female inducted DMBA and treated with (NNC) or Nano only compared to normal control group(c).

%	Control	DMBA	DMBA+ NNC	Nano(NNC)
UL	0.3±0.06	1.55±0.2	31.75±0.06	25.10±0.98
UR	11.10±0.12	6.20±0.06	26.60±0.87	33.10±0.64
LL	86.10±0.12	91.35±0.26	26.10±0.58	23.70±0.35
LR	2.50±0.29	0.85±0.09	15.55±1.13	18.05±0.72
Apoptosis (UR+LR)	13.60±0.17	7.05±0.03	42.15±1.99	51.15±1.36

Abbreviations : (UL upper left , UR upper right , LL lower left , LR lower right) .
different letters denotes significant difference between (DMBA+NANO)and (Nano) only compared to control and Not significant in DMBA compared to control.

Table(4) effect of (LCZN) on plasma biochemical parameters in female rats of the different groups.

Groups/Parameters	ALT (U/L)	AST (U/L)	Urea (mg/dl)	Creatinine (g/dl)
Group (1) Control	35.50±0.35^{d}	94.56±0.31^{d}	37.45±0.38^{c}	0.93±0.03^{cd}
Group(2) DMBA	49.40±0.46^{c}	126.90±0.64^{c}	52.89±0.82^{a}	2.25±0.09^{b}
Group(3) DMBA+NNC	35.30±0.29^{d}	93.40±0.52^{de}	38.00±0.29^{c}	1.25±0.03^{c}

Group (4) NNC	34.30±0.29^{d}	92.25±0.43e	36.70±0.40^{c}	0.77±0.01^{d}

*****Data are represented as (mean ±SD) . values with different superscript letters with same column are significantly different at **(P<0.05).**

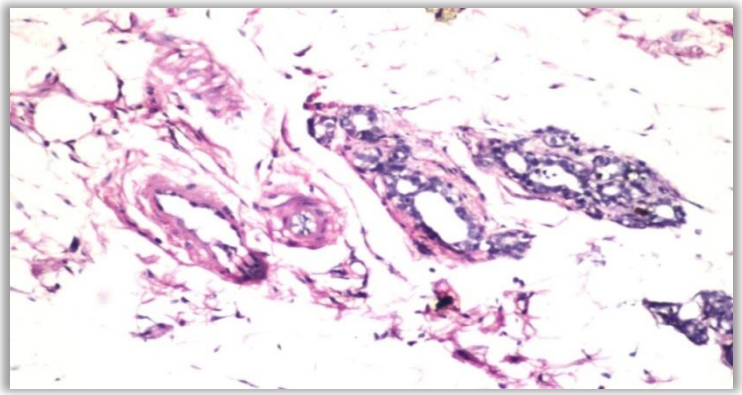
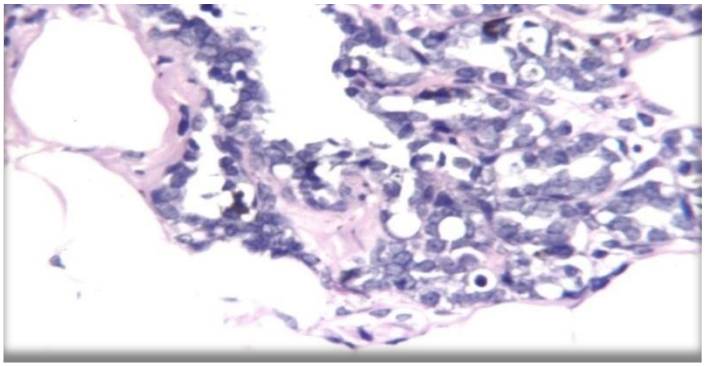
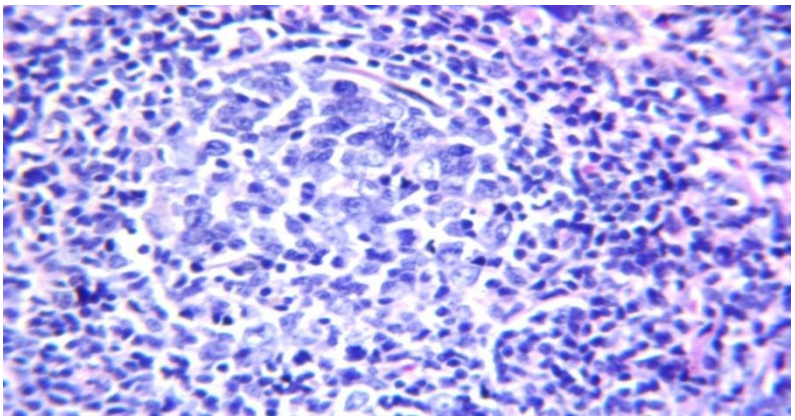


figure (1)mammary gland of rat in gp (1) showing normal histological structure of the acini and ducts system embedded in the adiopose tissue.



Figure(2) Mammary gland of rat after 4months gp(2) of DMBA –inducted group showing the polarity ,pleomorphism, hyperchromachia and mitosis in the anaplastic cell (H&E,80) .



Figure(3)Lymph gland of rat after 4 months in gp(2) of DMBA –inducted group showing the magnification of(fig.2)to identify the metastatic cancer cells from the mammary parenchyma to the regional lymph gland (H&E,80X).

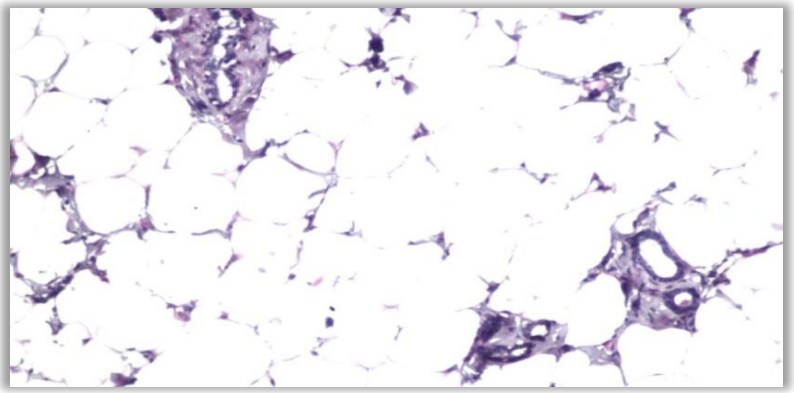


Figure (4)Mammary gland of tumor inducted rats treated with DMBA+ (Nano composite+ ZNO) in gp(3) showing the magnification of normal histological structure (H&E,X40).

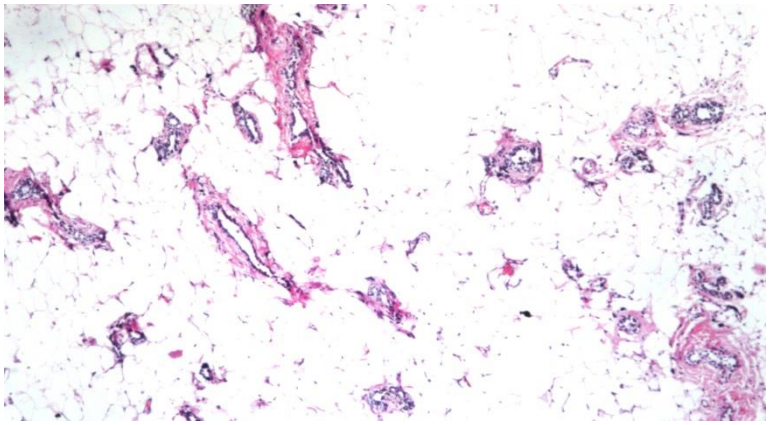


figure (5) mammary gland of rat in gp(4) showing normal histological structure.

4-Discussion :

Breast cancer is the most common cancer and pioneer cause of cancer related deaths among women. Worldwide, over 1.3 million cases of breast cancer are diagnosed, and yearly more than 4.5 women die from breast cancer (Manisha 2017).The present study was prepared aiming to synthesize and estimate the activity of the new novel composite (LCZN) lysine- cetrimonium zinc complex against breast tumor in animal model . The in vitro study diagnosed to estimate the cytotoxic effect of the tested compounds on MCF-7 human breast cancer cell line using Dulbecco's modified Eagle's medium (DMEM) assay . The results revealed that (novel composite) affect cell viability of MCF-7 cancer cell line .These results are in unison with first studies which observed the in vitro anticancer activity of zinc compound against many cancer cell lines , involving those for breast cancer, head and neck cancer (HNC)(Wang et al .,2000).

The in vivo study conducted in female rats experimental administrated (DMBA) as a powerful mammary carcinogen , showed a significant decrease in blood antioxidants noteworthy by a significant decrease in (GSH, CAT and Annexin v) activities associated with a significantly increase in plasma or whole blood (MDA, CD20, ALT, AST ,Urea and Creatinine) compared to normal control group proven that DMBA treatment excite just significant decrease in the antioxidant status . The antioxidant protection of human cells includes enzyme mediated and non-enzymatic defense mechanisms. The affinity of CAT for H_2O_2 is relatively low, therefore, some H_2O_2 remains in the cell. GSH-px is capable of detoxifying the remaining H_2O_2 (Arrigoni & De Tullio , 2002). In recent years, using MDA as a marker of oxidative stress, there has been a growing interest in studying the role played by lipid peroxidation in cancer progression. MDA is low-molecular weight aldehydes that can be produced from free radical attack on polyunsaturated fatty acids. Increased plasma MDA level have been reported in breast cancer (Kumaraguruparan *et al*.,2002). The observed result in table (2) demonstrated the fact that DMBA induced neoplastic process by forming adducts of active metabolism from DNA , exciting oxidative stress and by decreasing the activity of natural killer cells ((Cao *et al.*, 2001 and Muqbil & Banu, 2006). Moreover, the study of Soujanya *et al.*, (2011) inferred that, the biochemical alterations observed in cancer bearing animals may be due to the reduction of antioxidants levels following carcinogen

(DMBA) administration; which may be due to the utilization of antioxidants to scavenge the free radicals. The reduction of GSH level was a function of oxidative stress and/or intoxication of DMBA and this is in harmony with (Cheng ,2017).

Soujanya *et al.,* (2011) reported that a decrease level of CAT activity observed in cancer-bearing animals may be due to the utilization of antioxidant enzymes in the removal of H_2O_2 by DMBA. Moreover, decreased CAT activity was measured in patients with breast cancer and benign breast disease conditions (Gonenc *et al*., 2006). Our results showed increase in MDA level in mammary gland carcinoma as compared to controls this agree with the previous studies that suggested increased lipid peroxidation in breast cancer patients. On the contrary, gallium-induced increase in cellular ROS precedes the increase in MT and HO-1. It is known that both these genes are activated in response to oxidative stress (Chitambar 2012). In (DMBA+ Nano) treatment showed A significant increase in (GSH and CAT) and significant decrease in (MDA) level was observed when compared to (DMBA) group . Hashemi et al .,2007) reported that ,zinc supplementation was proven to inhibit cancer development . Thus it perhaps sound that, zinc supplementation applied in those former studies should , at least by competing with iron and copper , effectively weaken the process of cell proliferation . Furthermore, , zinc is critical for cellular antioxidant defenses as part of Cu/Zn SOD, enabling the conversion of superoxide radicals to the non-radical hydrogen peroxide, which is also known to induce MT (Metalloproeins) . The structural integrity of Cu/Zn SOD is reliant upon zinc, although its catalytic function necessitates the use of copper. (Dalton et al .,1996)& *(Bleday* et al .,1986) reported that, CTAB is a part of the group of quaternary ammonium compounds. It is used as a topical antiseptic and part of a group of molecules that (potentially) plays a role in cancer treatment in diverse ways. The most common complications in cancer patients are malnutrition, gastrointestinal disturbance, and liver dysfunction (Lee ,2005).The results obtained revealed a significant increase in liver marker enzymes in DMBA model compared to normal control rats. In this regard, consistent with the current results, it was found that DMBA injected to rats lead to marked significant elevation in the activity of serum ALT and AST which are markers of hepatocellular damage (Bedi and Pyrianka,2012) .The elevation of these enzymes could be attributed to their release from the hepatocytes' cytoplasm to blood circulation upon rupture of the plasma membrane and cellular damage (Said ,2014). The current study revealed marked decrease in plasma ALT and AST activities with respect to DMBA-treated rats supporting that zinc has ameliorated hepatocellular injury and protected against necrosis (Krecic-Shepard ME,1999). Also sodium ascorbate is considered a powerful hydro-soluble antioxidant capable of deoxidizing the reaction of oxygen and nitrogen free radical species. Therefore, it able to prevent important deleterious oxidative effects on biological macromolecules , such as DNA ,lipid, and proteins (Soheili Majed,2003) .

On the other hand , zinc is a part of liver enzymes as cofactor involving (AST, ALT and GGT) as well it typically found in large quantities in liver enzymes (Bennett et al .,2001).While , zinc is required for the activity of above from (250-300) enzymes and participate in many enzymatic and metabolic functions in the body of animals (Prasad &

Kucuk ,2002). So the result showed that (DMBA –induced group treated with Nano composite cause significant decrease in liver markers when compared to normal control group. but (DMBA + Nano) group showed marked significant decrease in liver markers when compared to (DMBA –induced group).

Furthermore ,Creatinine is a break down product of creatine , which is an important part of the muscle . Creatinine and urea levels increase in blood , If the kidney function has been disturbed . (Bedi & Periyanka ,(2012) reported that the level of plasma (Creatinine and Urea) were significantly increased in (DMBA-treated group) when compared to Control group which lead to kidney damage . So the result of kidney markers observed in (DMBA- administrated group) were significantly increased when compared to normal control group . However (DMBA+ Nano) showed a significant decrease when respect to (DMBA-induced group) .

Apoptosis is a factor in many human disease , ranging from neurodegenerative disorders to malignancy (Elmore , 2007). Deregulated apoptosis may also be the cause of cancer therapy (Anderson 1984 & Stashenko et al .,1980). CD20 antigen expression on malignant B cells corresponds to the ontogenetic stage at which the malignancy is assigned. So the result showed that significant increase inCD20 in (DMBA-induced rat) group when compared with normal control group. However, a significant decrease in CD20 was observed in (DMBA+ Nano) when compared to (DMBA-induced rat) group.

D'Arceuil et al .,2000 reported that , (annexin v) is a protein binds to phosphatidylserine (PS), which is typically in abundance of the inner leaflet of live cells. annexin v shown ;(live cell or viable cell) negative for both (annexin v and Pi (propidium iodide). Early apoptosis indicate positive for annexin and negative of propidium iodide, while late apoptosis and necrosis positive for both annexin and propidium iodide .So the result of showed in (DNBA-induced rat) resulted in significant decrease Annexin v when compared to normal control group. and marked significant increase in (DMBA+ Nano) when compared to (DMBA- treated rat)and (normal control group).

Histopathological investigations of the mammary gland tissues of DMBA group showed variation in the architecture of the glands graded from hyperplasia to anaplasia in the lining epithelium of the acini .when compared to the normal control group . These results partially agreed with those of others (Barros et al .,2004) who reported that administration of DMBA into virgin Sprague-Dawley female rats induced carcinogen that were particularly adenocarcinomas with several morphological types . Epithelial and myoepithelial cell proliferation were observed in most of the induced tumors in their experiment. Other study showed that DMBA cellular changes occur within 24 hours of carcinogen exposure (Russo and Russo, 1979). Depending upon the state of development of the mammary tissue at the time of initiation, multiple histological pathways of tumorigenesis can be identified (Russo and Russo, 1996). Carcinogen exposure to mammary lobules results in the formation of lobular hyperplasia. So the result showed hyperplasia and dysplasia in the lining epithelium of the acini. On the other hand after treatment of rats with(LCZN) nanocomposite for one month (DMBA+ NANO) treated registered a huge recurrence

of normal histological structure appearance of few sections of tissue of the acini.

Conclusion

The findings of the present study demonstrated that (NNC) provided an effective protection against mammary carcinogenesis induced by DMBA in rats, since these compound was able to ameliorate serum biochemical parameters ,enzymatic and non-enzymatic antioxidant defense system and to prevent the lipid peroxidation in these tissues. We recommended that, administration of diet rich in the antioxidant and high pH is very important for protection of different body tissue against oxidative stress or even cancer.

References :

Aebi,H . (1984) Methods Enzymol 105,121-126& Fossati, P.M et al .(1980) Clin .Chem.26,227-231.

Arrigoni, Oand De Tullio, M.C. (2002): Ascorbic acid: much more than just an antioxidant. Biochim Biophys Acta 1569: 1-9.

Anderson K, Bates M, Slaughenhoup B, Pinkus GS, Schlossman SF, Nadler LM: Expression of human B cell-associated antigens on leukemias and lymphomas: a model of human B cell differentation Blood 1984 63: 1424–1433.

Barros ACSD, Muranaka ENK, Mori LJ, Pelizon CHT, Iriya k, Giocondo G, et al (2004). Induction of experimental mammary carcinogenesis in rats with 7,12-dimethylbenz[a] anthracene . Rev Hosp Clin Fac Med Sao Paulo.59(5):257-61.

Banchroft, J.D.; Stwvens ,A. and Turner, D.R. Theory and practice of histological techniques. 4th ed. New York: Churchil Livingstone; 1996.

Beutler E., Duron O., Kelly MB .J. Lab Clin. Med . (1963) ,61,882.

Balleter, W.G.; Bushman, C.J and Tidwell , P.W. :Anol. Chem .33 (1961) , 592.

Bailey TC.A review of statistical spatial analysis in geographical information systems . In : Formation S, Rogerson P, editors . Spatial analysis and GIS .London: CRC Press,2013,Ltd.; Taylor and Francis ;1994.p.13-44.

Bedi PS and Priyanka S (2012): Effects of garlic against 7,12- dimethyl benzanthracene induced toxicity in Wistar albino rats. Asian J Pharm Clin Res.;5(4):170–3.

BledayR, Weiss MJ, Salem RR, Wilson RE, Chen LB and Steele G, Jr (*1986*) : Inhibition of rat colon tumor isograft growth with dequalinium chloride. *Arch Surg121*:*1272*–1275.

Bennet, D.M.; Jepson, P.D.; Law, R.J .; ones, B.R.; Kuiken, T. ; Baker, J.R.; Rogan ,E. and Kirkwood, J.K.(2001):Exposure to heavy metals and infectious disease mortality in harbor porpoises from England and Wales . Environmental pollution;112:33-40.

Barros ACSD , Muranaka ENK, Mori LJ, Pelizon CHT, Iriya K, Giocondo G, et al : Induction of experimental mammary carcinogenesis in rats with 7,12-dimethylbenz(a)anthracene . Rev Hosp Clin Fac Med Sao Paulo . 2004;59(5): 257-61.

Chen, L.B; 1988. Mitochondrial membrane potential in living cells. Annu Rev Cell Biol. 4:155–181.

Cao Y, Wang J, Henry-Tillman R and Klimberg VS (2001): Effect of 7, 12-dimethylbenz[a]anthracene (DMBA) on gut glutathione metabolism. *J Surg Res*. 100(1): 135–140.

Cheng SB , Liu HT, Chen SY , Lin PT , Lai CY and Huang YC Affiliations (2017) :Changes of Oxidative Stress, Glutathione, and Its Dependent Antioxidant Enzyme Activities in Patients with Hepatocellular Carcinoma before and after Tumor Resection .10.1371/journal.pone. 12(1):e0170016].

Chitambar C. R. (2012): Gallium-containing anticancer compounds. *Future Med Chem*. 4(10): 1257–1272.

Dalton T.P., Li Q., Bittel D., Liang L., Andrews G.K: Oxidative stress activates metal-responsive transcription factor-1 binding activity. Occupancy *in vivo* of metal response elements in the metallothionein-I gene promoter. J. Biol. Chem. 1996;271:26233–26241.

D'Arceuil H., Rhine W., de Crespigny A., Yenari M., Tait J. F., Strauss W. H., Engelhorn T., Kastrup A., Moseley M., Blankenberg F. G. ^{99m}Tc: Annexin V imaging of neonatal hypoxic brain injury. *Stroke*, 31*:* 2692-2700, 2000.

Elmore S. (2007): Apoptosis : A review of programmed cell death . toxicol pathol .,35(4) : 495-516.

Gomha, S.M.; Mahmmoud, E.A. and Elaasser, M.M. (2015): Synthesis and Anticancer Activities of Thiazoles, 1,3-Thiazines, and Thiazolidine Using Chitosan-Grafted-poly (vinylpyridine) as Basic Catalyst .Heterocycles ; 91(6):1227-1243.

Gonenc A, Erten D, Aslan S, Akyncy M, Sximsxek B and Torun M. (2006): Lipid peroxidation and antioxidant status in blood and tissue of malignant breast tumor and benign breast disease. *Cell Biol Int*. 30: 376–380.

Hashim AI, Zhang X, Wojtkowiak JW, Martinez GV and Gillies RJ.(2011): Imaging pH and metastasis. NMR in biomedicine.;24:582–591. [PubMed]. Hodgson E.A textbook of modern toxicology .4th ed. Hoboken: John Wiley& Sons, Inc; 2010.p.672.

Hashemi M., Ghavami S., E Shraghi M., Booy E.P .and LosM. (2007):Cytotoxic effects of intra and extracellular zinc chelation on human breast cancer cells. EyrJPharmacol., 557:9-192.

Krecic-Shepard ME, Shepard DR, Mullet D, Apseloff G, Weisbrode SE and Gerber N. (1999): Galliumnitrate suppresses the production of nitric oxide and liver damage in a murine model of LPS- induced septic shock. Life Sci.;65(13):1359–71.

Kumaraguruparan, R., Subapriya, R.,Viswanathan, P and Nagini, S.(2002): Tissue lipid peroxidation andantioxidant status in patients with adenocarcinoma of the breast, Clin Chim Acta, 325(1–2) 165–170.

Lee A, Levine M. (2005): Treatment of venous thromboemobolism in cancer patients. Cancer Control.;12:17–21.

Miyata M, Furukawa M, Takahashi K, and Gonzalez FJ, Y (2001). Mechanism of 7,12-dimethyl benz[a] anthracene –induced immunotoxicity :role of metabolic activation at the target organ . Jpn Jpharmacol ;86(3):302-9.

Mosmann,T. (1983) : Rapid colorimetric assay for cellular growth and survival :application to proliferation and cytotoxicity assays .J. Immunol. Methods;65:55-63.

Murray R.L. Creatinine .Kaplan A et al . Clin Chem The C.V. Mosby Co. St Louis . Toronto . Princeton 1984; 1261-1266 and 418.

Manish G, Anil Kumar Badana and Rama Rao Malla.(2017) : Emerging Diagnostic and Prognostic Biomarkers of Triple Negative Breast Cancer . Biomed J Sci & Tech Res.

Muqbil I and Banu N.(2006): Enhancement of pro-oxidant effect of 7,12-dimethylbenz(a)anthracene (DMBA) in rats by preexposure to restraintstress. Cancer Lett.;240(2):213–20.

Parhi P, Mohanty C, Sahoo SK (2012):. Nanotechnology-based on combinational drug delivery: an emerging approach for cancer therapy. Drug Discov Today. 2012;17:1044–52.

Pavletich NP, Chambers KA and Pabo CO.. 1993 *Genes Dev*. 7: 2556–2564.

Premanathan, M., Karthikeyan, K.,Jeyasubramanian, K and Manivannan, G. 2011: Selective toxicity of ZnO nanoparticles toward Gram positive bacteria and cancer cells by apoptosis through lipid peroxidation. *Nanomedicine: NBM*. 7: 184–192.

Prasad A.S. and Kucuk, O.(2002):zinc in cancer prevention .Cancer metastasis Rev.;21:291-295.

Parhi P, Mohanty C, Sahoo SK (2012):. Nanotechnology-based combinational drug delivery: an emerging approach for cancer therapy. Drug Discov Today. 2012;17:1044–52.

Robey I.F., B.K. Baggett, N.D. Kirkpatrick, D.J. Roe, J. Dosescu, B.F. Sloane, A.I. Hashim, D.L. Morse, N. Raghunand, R.A. Gatenby, *et al*(2009) :Bicarbonate increases tumor pH and inhibits spontaneous metastases.Cancer Res, 69, pp. 2260-2268.

Russo, J., Wilgus , G. and Russo, I.H.(1979) : Susceptibility of the mammary gland to carcinogenesis .I. differentiation of the mammary gland as determinant of tumor incidence and type of lesion . American Journal of Pathology ; 96:721-736.

Russo, I.H. and J. Russo (1996): Mammary gland neoplasia in lung . term rodent studies . Environmental Health Perspectives ; 104:938-967.

Soheili Majed,E,;Goldberg,M.and Stanislawski,L.(2003): In vitro effects of ascorbate and trolox on the biocompatibility of dental restorative materials .Biomaterials;24:3-9.

S.A. Ribeiro MdLC, K.M. Bailey, N.B. Kumar, T.A. Sellers, R.A. Gatenby and A. Ibrahim-Hashim, R.J(2012): GilliesBuffer therapy for cancer J Nutr Food Sci, S2 Sinha AK. Colorimetric assay of catalase. Anal Biochem.1972;47(2):389–94.

Soujanya J., Silambujanaki P. and Krishna V. L. (2011): Anticancer efficacy of holoptelea integrifolia, planch. against 7,12-dimethylbenz(a) anthracene induced breast carcinoma in experimental rats. *Int J Pharm Pharm Sci*. 3: 103-106.

Said UZ, Ahmed NH, Medhat AM, Mustafa MM .(2014): Effects of omega-3 fatty acids against Ehrlich carcinoma-induced hepatic dysfunction. J Cancer Res Exp Oncol.;6(2):20–8.

Soheili Majed,E,;Goldberg,M.and Stanislawski,L.(2003): In vitro effects of ascorbate and trolox on the biocompatibility of dental restorative materials .Biomaterials;24:3-9.

Stashenko P, Nadler LM, Hardy R, Schlossman SF: *Characterization of a human B lymphocyte-specific antigen* J Immunol 1980 125: 1678–1685.

Tab czar S, Koceva-Chyla A, Czepas J, Pieniazek A, Piasecka –Zelga J,Gwozdzinski K . Nitroxide pirolin reduces oxidative stress generated by doxorubicin and docetaxel in blood plasma of rats bearing mammary tumor. J Physiol Pharmacol . 2012;63(2):153-63.

Traganos F, Darzynkiewicz Z , Sharpless T, Melamed MR. Simultaneous Staining of ribonucleic and deoxyribonucleic in unfixed cells using acridine orange

in a flow cytofluorometric System .J. Histochem .Cytochem .1977;25:46.

Wang F, Jiang X , Yang DC , Elliott RL , Head LF . Doxorubicin –zin complexs conjugate overcomes multidrug resistance :evidence for drug accumulation in the nucleus of drug resistant MCF-7 /ADR cells . Anticancer Res .2000;20(2A): 799-808.

Yoshioka T, Kawada K, Shimada T, Mori M . Lipid peroxidation in maternal and ord blood and protective mechanism against activated in the blood. Am J Obstet Gynecol.1970;135(3):372-6.

Zhang, H., Chen, B., Jiang, H., Wang, C.,Wang, H and Wang, X. 2011. A strategy for ZnO nanorod mediated multi-mode cancer treatment. *Biomaterials*. 32:1906–1914

Z.Klin. Chem.4.Klim. Biochem .8 (1970) 658 or (1972) , 182.