Biochemical effect of New Novel Composite on 7,12 dimethylbenz (a) anthracene Induced Mammary Carcinogenesis in Rats.

Omayma Abouzaid[1], Abdel Fatah M. Badwi[2], Hanaa .F.Gabr [2]

[1]*Department of Biochemistry, Faculty of Veterinary Medicine, Benha University*
[2]*Departement of health & Radiation Research, National Centre for Radiation Research and Technology (NCRRT), Atomic Energy Authority, Cairo Egypt.*

A B S T R A C T

Breast cancer is the second leading cause of cancer death in women, There are several ways to treat breast cancer .The effect of Lysine-Cetrimonium gallium Complex with zinc oxide nanopatricle and sodium ascorbate (NNC) on DMBA induced mammary carcinogenesis was investigated in female rats. Oral dose of DMBA induced a significant increase in ALT, AST, MDA and serum NO levels. On other hand marked depletion in CAT, GSH. On other hand oral administration of NNC was able to mitigate mammary carcinogenic damage induced by DMBA as evidence to pronounce curative effect against lipid peroxidation, serum NO level and deviated enzymatic variables and maintained glutathione status, antioxidant enzymes in addition histopathological changes directed toward control. We observed that the administration of (NNC) have potential to exert curative effects against mammary carcinogenesis.

Keywords: Breast cancer, gallium chloride, Zinc oxide nanoparticles,CTAB

(http://www.bvmj.bu.edu.eg)**(BVMJ-33(2): 375- 383,, 2017)**

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. At present, combination therapy (i.e., combinations of different chemotherapeutic drugs in a chemotherapy regime) is becoming a more popular attractive strategy for effective anticancer treatment because it generates synergistic anticancer effects, reduces individual drug related toxicity, and suppresses multi-drug resistance through different mechanisms of action (Parhi, 2012) .

Over the past two to three decades, gallium (Ga) compounds have gained a steady interest in the field of clinical medicine due to the proven ability of Gacations to inhibit tumor growth both in vitro and in vivo, on the one hand, and enhanced bioavailability and efficacy provided by the conversion of Ga into chelate complexes. (Collery, 2012). In recent years, nanotechnology-based combination drug delivery systems to the cancer tissue have emerged as an effective 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 suitable 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) . Surfactantsare wetting agents that lower the surface tension of a liquid, allowing easier spreading, and lower the interfacial tension between two liquids. 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 mitochondriotoxicity (Chen, 1988).

PH plays an important role in almost all steps of metastasis (Hashim et al., 2011) Lysine is α-amino acid that is used in the biosynthesis of proteins. It contains α-amino, α-carboxylic acid group, and a side chain lysyl ($(CH_2)_4NH_2$), classifying it as a charged (at physiological pH), aliphatic amino acid. It is essential in humans, meaning the body cannot synthesize it and thus it must be obtained from the diet. Free-base lysine (pKa = 10) have all been shown to be effective in reducing metastases in vivo (Robey et al., 2009) Reduction of metastasis is dependent upon buffering (Ribeiro 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)

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 and protein (Soheili et al., 2003).

The present study wasdesigned tointroduce a newly synthesized metal-based compound, namely Lysine-Cetrimonium gallium Complex with zinc oxide nanopatricle and sodium ascorbate (NNC), as achemotherapeutic agent withspeculatedreduced toxicity risk accompanied with higher potential in cancer treatment

2. Materials and methods

2.1. Experimental animals:

90 virgin Swiss albino rats with an average weight of (90-110 g) were used in this study. Rats were obtained from the Laboratory Animals Research Center, Faculty of Veterinary Medicine, Benha University, housed in separate wire mesh cages, exposed to good ventilation, humidity and to a 12-hr light -dark cycle, and provided with a constant supply of standard pellet diet and fresh, clean drinking water ad libitum

2.2. Drug and compounds:

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

-Gallium trichloride (≥99.9% trace metals basis): is a metal halide with the linear formula $GaCl_3$, was kindly purchased from Al-Dawlya Company in the form of anhydrous beads. It is colourless and virtually soluble in all solvents. It is the main precursor to most derivatives of gallium and a reagent in organic synthesis. The

molecular weight is 176.08 and the melting point is 77.9°C (anhydrous).

-Cetyltrimethyl ammonium bromiode (CTAB) (≥99.9%):

Aquaternary ammonium salt, was kindly purchased from Al-Dawlya Company .light beige odorless powder, molecular weight is 364.46, melting range (F) 459-469, Decomposition temperature (F) 459

-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

-Zinc oxide nanoparticle (≥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 kindely purchased from Al-Dawlya Company, the Empirical Formula C_6H_7 NaO_6, Molecular Weight (198.11).

2.3. In vivo Experiment

sixty adult female rats weighing around (90-110 g) recived oral dose of novel nanocomposite at different doses, mortality was reported and LD50 was calculated .

2.4. Experimental design

Rats were randomly divided into four groups (15 rats each):

Group 1: rats in this group received a single oral dose of 1ml sesame oil. This group served as control normal group.

Group 2: rats in this group received a single oral dose of 100 mg/kg b.wt. DMBA diluted in sesame oil (1mL) given intragastrically by gavage.

Group 3: rats in this group received a single oral dose of 100 mg/kg b.wt. DMBA diluted in sesame oil (1mL) given intragastrically by gavage, animals were left for five month for tumor induction. Then, at the last month, they were treated with (NNC) each was injected with a daily oral dose of (NNC) (343.75 ±10 mg/kg b.wt.) for approximately one month, and then they were sacrificed.

Group 4: Rats in this group were normal rats. During the treatment period, sixth month, each was injected with a daily oral dose of (NNC)(343.75 ±10 mg/kg b.wt.) for approximately one month, then they were sacrificed.

2.5. Sampling

2.*5.1. Blood samples*

After overnight fasting, blood samples were divided into two parts one part was used immediately for measuring the activity of the following biochemical parameters:

Nitric oxide (NO) levels, Catalase (CAT), GSH, and L-MDA, The second part of blood samples were collected in dry, clean test tubes and allowed to clot for 30 min and serum was separated by centrifugation at 3000 rpm for 15 min at 4ºc and quickly frozen in a deep freezer at (-20 °C) for subsequent biochemical analyses ALT and AST.

2.5.2. Tissue specimen (mammary tissue)

After finishing blood sampling, rats of each group were sacrificed by cervical decapitation The abdomen was opened and the mammary specimen 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. Mammary tissues were dissected and kept in 10% formalin for histopathological examination (Banchroft et al., 1996).

2.6.1 Biochemical analysis:

Reduced glutathione (GSH) content was measured in whole blood according to the colorimetric method of (Beutler *et al.,* 1963) , catalase (CAT) activity the method described by (Sinha, 1972), Serum nitric oxide concentration was colorimetrically determined according to method described by (Montgomery and Dymock, 1961) , lipid peroxidation (LPx) The method described by (Yoshioka *et al.,* 1970) . ALT andAST the method described by (Reitman and Frankel,

1957), Biochemical tests were studied for each group.

2.6.2. Histopathological examination

Determined according to the method described by (Banchroft et al., 1996).

3.7. 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.

3. RESULTS

The obtained data in table (1) revealed a significant increase in L-MDA, NO, ALT, AST level and significant decreases in, CAT activities and GSH level tissue in group 2 (DMBA), when compared with control normal group. Treatment with (NNC) resulted in significant decrease L-MDA, NO, ALT, AST level and significant increase in CAT activities and GSH level in comparison with (DMBA) group 2. The obtained data in figures (1, 2, 3, 4 and 5) revealed histopathological changes that occur at the end of the treatment period as the following: the mammary sections of control rats showing normal histological structure of the acini and ducts system embedded in the adipose tissue. Mammary gland of rats in DMBA group showing group of anaplastic cells replacing the mammary acini and lymph gland of rats in showing metastatic cancer cells from the mammary parenchyma to the regional lymph gland. Mammary gland of rat in (NNC) treated group showing cystic dilatation of some duct.

Table (1):Data expressed as the mean±SD of some biochemical findings

Groups/ Parameter	**GSH (mg/dl)**	**CAT(µg/ml)**	**No (µmol/L)**	**ALT (U/L)**	**AST (U/L)**	**MDA(nmol/ml)**
1-group 1 Control	39.73±0.66	187.50±3.5	37.31±1.54	35.50±0.84	94.50±0.70	7.95±1.34
2- group 2 DMBA	18.99±1.71	161.60±1.97	126.56±1.95	49.40±1.13	120.83±1.64	36.16±1.78
3-group 3 DMBA+ (NNC)	28.35±1.62	262.49±3.52	65.70±1.97	37.70±0.84	96.65±1.20	11.90±0.56
4-group 4 (NNC)	33.10±2.12	293.0±5.6	45.78±1.44	36.90±0.42	95.83±1.03	8.9.±1.20

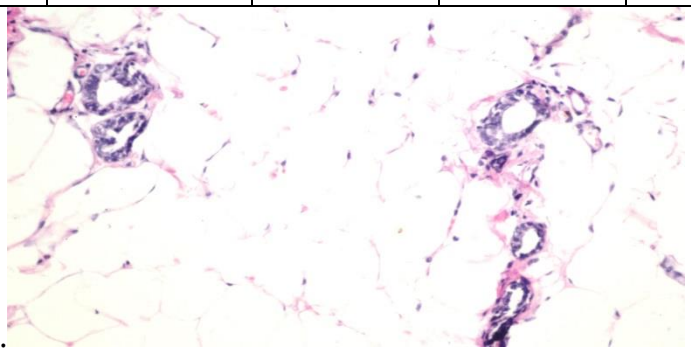

Fig 1. Mammary gland of rat in gp (1) showing normal histological structure of theacini and ducts system embedded in the adipose tissue

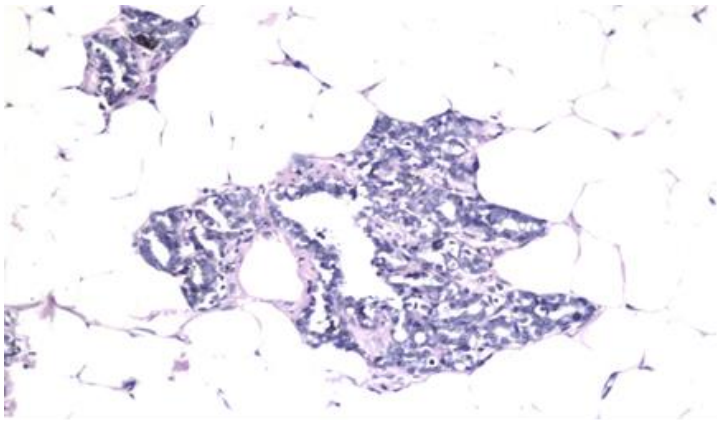

Fig 2. Mammary gland of rat in gp (2)showing group of anaplastic cells replacing the mammary acini

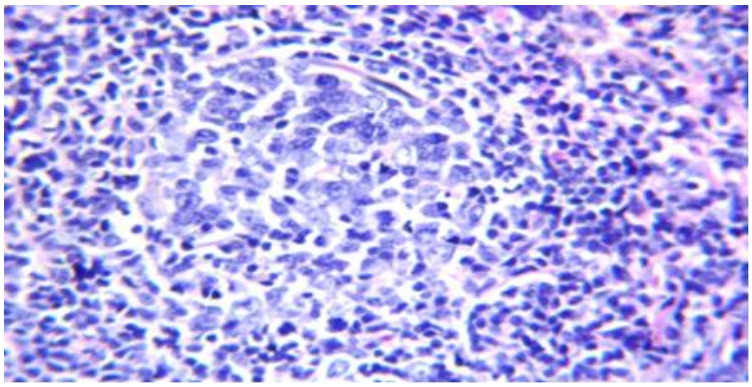

Fig 3.Lymph gland of rat in gp(2) showing magnification of (fig. 2) to identify metastatic cancer cells from the mammary parenchyma to the regional lymph gland

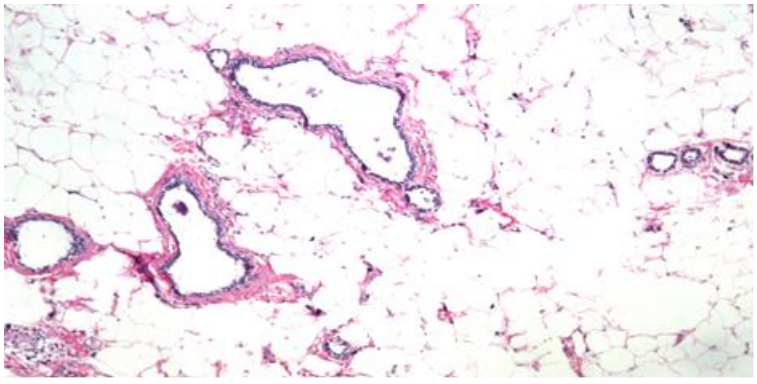

Fig 4.Mammary gland of rat in gp(3) showing cystic dilatation of some duct

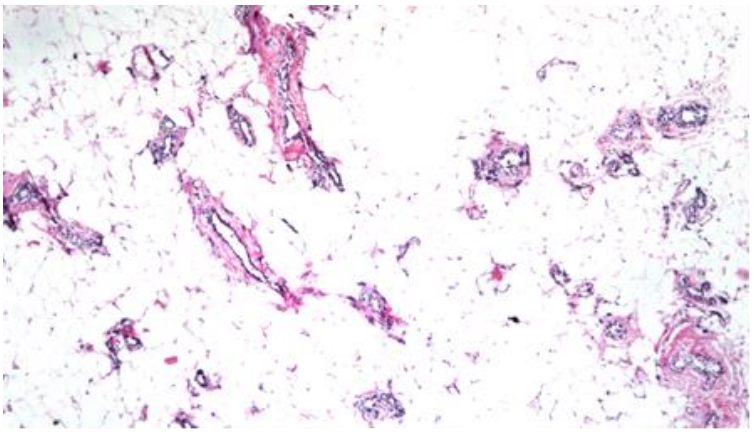

Fig 5.Mammary gland of rat in gp(4) showing normal histological structure

4.DISCUSSION

Breast cancer is the most common cancer and major cause of cancer related deaths among women. Worldwide, over 1.3 million cases of breast cancer are diagnosed, and annually more than 4.5 lakhs women die from breast cancer (Manisha, 2017). Antioxidants are the first source of protection of the body against free radicals and other oxidants, being the compounds that halt the attack and the formation of radical species within cells. The group of antioxidants inside the organism is known as the total antioxidant state (TAS) (Teixeira *et al*., 2013). The antioxidant protection of human cells includes enzyme mediated and non-enzymatic defense mechanisms such as catalase (CAT) and glutathione enzyme, CAT converts H_2O_2 into water and oxygen. 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, 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 aldehyde that can be produced from free radical attack on polyunsaturated fatty acids. Increased plasma MDA levels have been reported in breast cancer (Kumaraguruparan*et al*., 2002).

Our result in table 1 demonstrated significant increase in L-MDA, NO, ALT, AST level and significant decreases in CAT activities and GSH level In (DMBA) group, when compared with control normal Group. These findings are in agreement with some studies which reported that the DMBA-induced neoplastic process is based on many mechanisms; firstly, carcinogenesis is induced by forming adducts of active metabolites from DNA, secondly, by generating the state of oxidative stress which is manifested by a decrease of the level of antioxidative enzymes such as catalase and block liberation from the intestine of glutathione, an important antioxidant; or else by decreasing the activity of natural killer (NK) cells (Cao *et al.*, 2001 and Muqbil, 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 decreased level of CAT 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 level of CAT activity was measured in patients with breast cancer and benign breast disease conditions (Gonenc*et al*., 2006). NO is a multifunctional molecule in cancer. (Panis, 2015) has demonstrated that the presence of the primary tumor mass is determinant for the sustained proinflammatory systemic status found in women with breast cancer, which included high NO. 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 aldehyde that can be produced from free radical attack on polyunsaturated fatty acids. Increased plasma MDA levels have been reported in breast cancer (Kumaraguruparan *et al*., 2002). Our results showed increase in MDA level in mammary gland carcinoma as compared to controls thus agreeing 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 this regard, our results could be appreciated by studies made by (Yang and Chitambar, 2008) they have shown that Ga is implicated in intracellular oxidative stress through the generation of reactive oxygen species, with a decrease in the ratio of cellular glutathione reduced form (GSH) on glutathione oxidized form (GSSG). Cells exposed to inorganic gallium salts, such as gallium nitrate, displayed increased generation of ROS (Joseph, 2005).in addition Nano ZnO is able to protect cell membrane integrity against oxidative stress damage, increase antioxidant enzyme levels and decrease MDA level (Dawei *et al.,* 2009). Similarly,(vijayavel et al., 2006) reported that, ascorbic acid is a good scavenger of free radicals and it protect cellular membranes their by preventing degenerative disease like cancer.

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 levels of serum ALT and AST which are markers of hepatocellular damage (Bedi and Priyanka, 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). Our study revealed decrease in both ALT and AST enzyme activities with respect to DMBA-treated rats supporting that gallium has ameliorated hepatocellular injury and protected against necrosis (Krecic-Shepard, 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, 2003).

Collectively, these observations suggested that novel synthetic gallium derivatives may potentially present new hope for the development of breast cancer therapeutics, which should attract further scientific and pharmaceutical interest.

5. CONCLUSION

The findings of the present study demonstrated that (NNC) provided an

effective protection against mammary carcinogenesis induced by DMBA in rats, since this 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.

6. EFERENCES

Arrigoni,O. and De Tullio, M.C. 2002. Ascorbic acid: much more than just an antioxidant. BiochimBiophysActa 1569: 1-9.

Banchroft, J.D., Stevens, A. and Turner, D.R. 1996. Theory and practice of histological techniques. Fourth Ed. Churchill Livingstone, New York, London, SanFrancisco, Tokyo.

Bedi,P.S. 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.

Beutler, E., Duron, O. and Kelly,B.M.1963.Improved method for the determination of blood glutathione. J Lab Clin Med.; 61:882–8.

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

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

Cheng, S.B.,Liu, H.T., Chen, S.Y., Lin, P.T., Lai, C.Y. and Huang, Y.C.A. 2017.Changes of Oxidative Stress, Glutathione, and Its Dependent Antioxidant Enzyme Activities in Patients with Hepatocellular Carcinoma before and after Tumor Resection .journal.pone. 12(1):1-5

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

Collery, P., Mohsen, A., Kermagoret, A., D'Angelo, J., Morgant, G. and Desmaele, D. 2012. Combination of three metals for the treatment of cancer: gallium, rhenium and platinum. 1. Determination of the optimal schedule of treatment. Anticancer Res. 2012; 32:2769–82.

Dawei, A.I., Zhisheng, W. and Angu, Z. 2009. Protective Effects of Nano-ZnO on the Primary Culture Mice Intestinal Epithelial Cells in in vitro Against Oxidative Injury. Int J Nanotechnol App: 3: 1-6.

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, A.I., Zhang, X., Wojtkowiak, J.W., Martinez, G.V. and Gillies, R.J.2011.Imaging pH and metastasis. NMR in biomedicine.; 24:582–591.

Joseph, T.P., Janine, P.W. and Chitambar, C.R, 2005. Gallium nitrate as a novel agent for the treatment of mantle cell lymphoma: target and mechanisms of action. Proc Am Assoc Cancer Res.; 46:1383–4.

Krecic-Shepard, M.E., Shepard, D.R., Mullet, D., Apseloff, G., Weisbrode, S.E. 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, ClinChimActa, 325(1–2) 165–170.

Lee, A. and Levine, M. 2005.Treatment of venous thromboemobolism in cancerpatients.Cancer Control. 12:17–21.

Manish, G. Anil, K. B. and Rama, R. M. 2017. Emerging Diagnostic and Prognostic Biomarkers of Triple Negative Breast Cancer. Volume 1- Issue 3: J Med Chem 45: 2116-2119

Montgomery, H.A.C and Dymock, J.F.1961. Colorimetric determination of nitric oxide.Analyst.86:414.

Muqbil, I .and Banu, N.2006. Enhancement of pro-oxidant effect of 7, 12-

omega-3 fatty acids against Ehrlich carcinoma-induced hepatic dysfunction. J Cancer Res ExpOncol.; 6(2):20–8.

Panis ,C. V., Victorino, A. C., Herrera, A. L., Cecchini, A. N., Simão, L. Y., Tomita, and R. Cecchini.2015. Can Breast Tumors Affect the Oxidative Status of the Surrounding Environment? A Comparative Analysis among Cancerous Breast, Mammary Adjacent Tissue, and Plasma .Oxidative Medicine and Cellular Longevity , Article ID 6429812, 9 pages

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

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.

Reitman, S, and Frankel, S.1957.A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases.Am J ClinPathol. 1957; 28(1):56–63.

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

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

Said, U.Z., Ahmed, N.H., Medhat, A.M. and Mustafa, M.M. 2014. Effects of dimethylbenz(a)anthracene (DMBA) in rats by preexposure to restraint RadicBiol Med. 2008;45:763–72.

Singh, R., Beriault, R., Middaugh, J., Hamel, R., Chenier, D., Appanna, V. D., and Kalyuzhnyi, S. 2005. Aluminum tolerant Pseudomonas fluorescens: ROS toxicity and enhancedbNADPH production. *Extremophiles*.9: 367-373.

Soheili, M. 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

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

Teixeira, V., Valente, H., Casal, S.,Marques, F. and Moreira, P. 2013.Blood antioxidant and oxidative stress biomarkers acute responses to a 1000-m kayak sprint in elite male kayakers.*J Sports Med physical fitness*; 53(1):71–79.

Vijayavel,K., Gopalakrishnan,S., Thilagam,H. and Balasubramanian, M.P.2006. Dietary ascorbic acid and atocopherol mitigates oxidative stress induced by copper in the thorn fish teraponjarbua .Sci. Total Environ.; 372; 157-63

Yang,M. and Chitambar, C.R.2008.Role of oxidative stress in the induction of metallothionein-2A and heme oxygenase-1 gene expression by the antineoplastic agent gallium nitrate in human lymphoma cells.Free stress. Cancer Lett.;240(2):213–20

Yoshioka,T., Kawada, K., Shimada, T. and Mori, M.1970.Lipid peroxidation in maternal and cord blood and protective mechanism against activated-oxygen toxicity 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 ZnOnanorod mediated multi-mode cancer treatment. *Biomaterials*. 32:1906–1914.