Available online at www.scientiaresearchlibrary.com

Scientia Research Library

ISSN 2348-0408
USA CODEN: JACOGN

Journal of Applied Chemistry, 2015, 3 (2): 1-14
(*http://www.scientiaresearchlibrary.com/arhcive.php*)

Effect of N-acetyl cysteine , Zinc oxide and high pH ascorbatenanocomposite against 7,12- dimethylbenz[a]anthracene-induced mammary gland carcinoma in female rats on caspase 3 & 9 genes and TNF-α level

Omayma A.R. AbouZaid[1] ;Badawi A.F.[2] and SamehA.Elsayes[1]

1.Biochemistry Department, Faculty of Vet. Med. Moshtohor, Benha University, Egypt.
2.Petrochemical Department, Egyptian Petroleum Research Institute, Cairo, Egypt.

ABSTRACT

7,12- dimethylbenz[a]anthracene (DMBA) mediates carcinogenesis through formation of DNA adducts, DNA damage, generating excess reactive oxygen species and by producing chronic inflammation. N-acetyl cysteine , Zinc oxide and high pH ascorbate nanocomposite have been shown to confer various biological effects, anticancer, enhance immune system and antioxidant properties and induction of apoptosis. The present study was undertaken to evaluate the chemopreventive effect of N-acetyl cysteine , Zinc oxide and high pH ascorbate nanocomposite with dose 50mg/kg.b.w against 7,12- dimethylbenz[a]anthracene carcinogenesis with dose 100mg/kg.bw at the end of the second week (On day 16).Then, animals left until mammary gland carcinoma formed. The results indicated that DMBA induced mammary gland carcinoma in rat Also, significant increase in TNF-α and significant decrease incas3, cas9 activity. While, treating with Nanocomposite decreasing in TNF-α and significant increase incas3, cas9 activity.

Keywords: Nanocomposite (N-acetyl cysteine, Zinc oxide and high pH ascorbate), TNF-α, caspase3,caspase 9.

INTRODUCTION

Carcinogenesis is a multi-step process and the progression from the pre-malignancy stages to the symptomatic invasive stages may span 20 years or more.[1]Polycyclic aromatic hydrocarbons (PAHs) including 7,12-dimethylbenz[a]anthracene (DMBA) are environmental pollutants, which undergo metabolic activation to exert their carcinogenic effects.[2]Nanotechnology is broadly defined as the manipulation of matter on a molecular scale. This refers to the production of structures and even devices, called nanomaterials, in the size range of 1 to 100 nm in any dimension.[3] Antioxidants such as NAC have been known to be cytoprotective after exposure to cellular damaging agents such as reactive oxygen species. NAC is a precursor to the cellular antioxidant glutathione (GSH), a scavenger for cell and DNA-damaging oxygen species such as hydrogen peroxide, superoxide, and lipid peroxides. In numerous studies NAC has been shown to provide significant protection for stress-related cell and genomic damage.[4]Studies demonstrate that the cytotoxic properties of ZnO nanoparticles against cancerous cells is directly related to size,

with smaller nanoparticles exhibiting greater toxicity.[5]The external pH of solid tumors is acidic as a consequence of increased metabolism of glucose and poor perfusion. Acid pH has been shown to stimulate tumor cell invasion and metastasis in vitro and in cells before tail vein injection in vivo. The present study investigates whether inhibition of this tumor acidity will reduce the incidence of in vivo metastases. Here, we show that oral NaHCO3 selectively increased the pH of tumors and reduced the formation of spontaneous metastases in mouse models of metastatic breast cancer.[6] Ascorbate is a primary antioxidant in that it directly neutralizes radical species. Ascorbate is not very reactive with prevalent cellular oxidants such as hydrogen peroxide and probably reacts mostly with hydrogen peroxide breakdown products.[7]

MATERIALS AND METHODS

***Chemicals* : 7,12-dimethylbenz[a]anthracene (DMBA)**purchased from (Sigma, USA), **N-acetyl cysteine**purchased from (Sigma, USA), **Zinc oxide nanoparticals**purchased from (Sigma, USA),**VitaminC as Sodium ascorbate**purchased from (Sigma, USA), **Sodium bicarbonate**purchased from El Nasr Pharm. Chem. Co. 'ADWIC'and **β-Cyclodextrin** purchased from(Sigma, USA).

***Method and prepration of novel nano composite* :** Prepration of nanocomposide for biochemical investigation of antitumor activity as anti cancer was prepared through the cursty of prof. Dr .Abdel fattah Mohsen Badwi after preparing ,we get some analysis as TEM as following which represents TEM image of the novel nano composite(N-acetyl cysteine, Zinc oxide, High pH sodium ascorbate) ranging from 6.01 - 29.35 nm in diameter and has spherical shape.

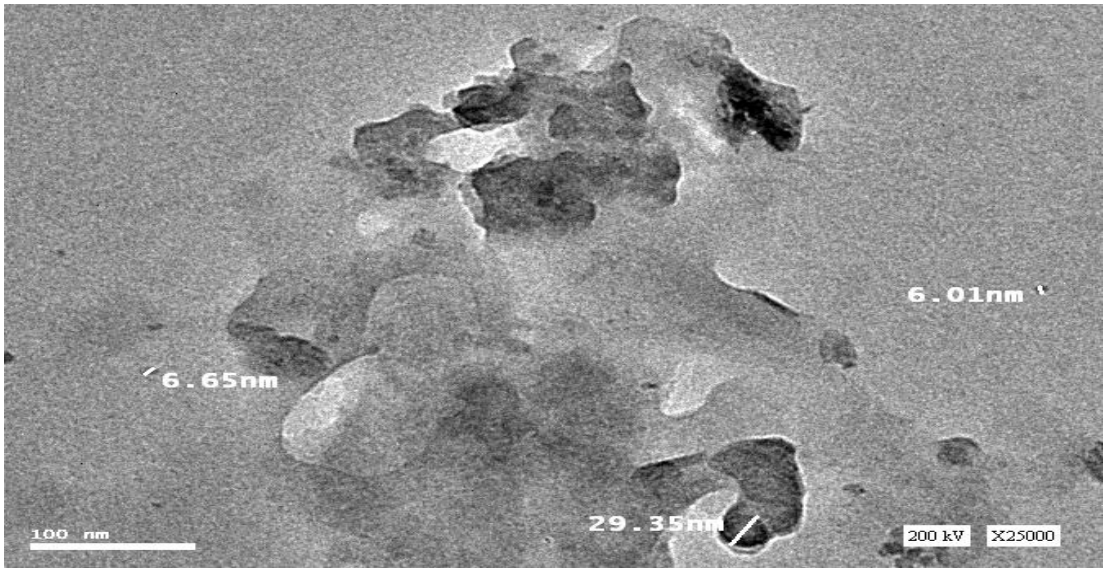

Fig.(1): TEM image of the Nano composite (N-acetyl cysteine, Zinc oxide, Sodium ascorbate and Sodium bicarbonate Loaded Cyclodextrin).

Induction of tumor : By 7, 12-dimethylbenz[a]anthracene (DMBA) was diluted in olive oil (1ml) and administrated once orally at dose of 100 mg/kg. b.wtat the end of the second week (On day 16). Then, animals left until mammary gland carcinoma formed.

In vivo Experiment : 60female swiss albino rat, 4-5 weeks old age and weighining about 100-120gm,recieved novel nanocomposite orally , mortality was reported.calculate LD_{50}.

Exprimental design:

The present studied was carried out on 60 Female swiss albino rat divided into 4 groups:
Group I(control normal group): Rats received single oral dose of vehicle oil (Olive oil) at the beginning of the experiment.
Group II(DMBA group): At the end of the second week [On the 16 thday at (51-58 days of age)] rats received single oral dose of DMBA diluted in Olive oil (1 mL) orally by stomach tube (100 mg/kg b.wt) **(GokhanOto*et al*.,2011).**Then the animals were left until mammary carcinoma formed.
Group III (DMBA + Nanocomposite Protected group): Rats was treated with Nano composite at a dose of (50 mg/kg b.wt.) dissolved in saline (0.9% NaCl solution) three times per week as in group IV, at the end of the second week (On the 16 thday) rats exposure to single oral dose of DMBA (100 mg/kg b.wt) as in the group II, and then repeat doses as in group (IV), until the end of experiment.
Group IV (Nanocomposite Treated group): Rats administrated with Nanocomposite orally (50 mg/kg b.wt.) dissolved in saline (0.9% NaCl solution) three times per week until the end of the experiment.

Blood sampling:
Directly, after animals were anaesthised using diethyl ether, Blood samples were collected from the heart by heparinized syringes at 18 th week from the beginning of treatment.

Tissue specimens:
Fresh tissue specimens were transported to our laboratory in isotonic saline and preparedas follow: 1-The material was washed with isotone tris EDTA buffer, 3.029 gm of 0.1 M tris (hydroxymethylaminomethane, 1.022 gm of 0.07 M sodium chloride (ADWIC) and 0.47 gm of 0.005 M EDTA.2-They were dissolved in 250 ml of distilled water and then adjust the PH at 7.5 by using 1N Hcl. 3-Then, the cell suspension was centrifuged at 1800 rpm for 10 mins., where upon the supernatent was aspirated. If they was macroscopically contaminated with blood, it was then subjected to haemolysis with filtered tap water for 10 mins. 4- After centrifugation and aspiration of the supernatent the cell is fixed in ice-cold 96-100% ethanol in approximately 1 ml for each sample. These fixed cells can be stored indefinitely in a refrigerator and can also be mailed without running the sample. 5-Fixation of cells, Fixation with ice cold absolute alcohol 1ml for each tube and preserved in +4C forever until analysis.6- Staining method **,**This technique is applicable where the fluorochrome is directly linked to the primary antibody by FITC conjugates.

Biochemical analysis: Caspase 3,9 in mammary gland tissue were analyzed according to the methods described previously [8], and Serum TNF-α was determined according to [9],by solid phase Enzyme Linked Immuno Sorbent Assay (ELISA) using rat TNF-α kits (RayBiotech, USA) and a microtiter plate reader capable of reading at 450 nm.

Statistical Analysis:
Statistical analysis and correlations were performed using SPSS program version 16 [10] Data are presented as Mean ± standard error mean (SEM). Student "t" test and analysis of variance (ANOVA) followed by Bonferroni's post hoc analysis were used for comparisons between groups. The level of statistical significance was set at probability $P < 0.05$

RESULTS AND DISCUSSION

The obtained results demonstrated in (table 1),figures(1,2) revealed that, administration of DMBA induced mammary carcinoma in female rats exhibited a significant increase in TNF-α and decrease incas -3 and cas- 9 gene activity. While, Treatment with novel nanocomposite showed there was significant increase observed in cas -3 and cas- 9 gene activity but decrease in TNF-α.

In mammary carcinogenesis, apoptosis is mediated by several molecules such as tumor necrosis factor-alpha (TNF-α) and caspases. The former belongs to the death receptor gene superfamily. It is an inflammatory cytokine produced both bymacrophages and lymphocytes. It exerts cytolytic or cytostatic activity against tumor cells. It is also responsible for a diverse range of signalling events within cells leading to necrosis or apoptosis. Moreover, caspase-3 (cysteinyl aspartate proteinase) is one of the cysteine proteases which plays a major role in the execution of apoptosis under oxidative stress conditions[11] The increased levels of TNF-α in DMBA group, as compared to the control group, agrees with the findings of other studies.[12] This high level may be due to increased production by the tumor infiltrating lymphocytes and/or by the tumor cells.[13]Increased TNF-α promotes invasion and metastasis in ductal carcinomas in a scalar fashion.[14]TNF-α secreted by tumor-related macrophages can enhance tumor invasion by increasing the expression of MMPs in breast carcinoma.

The decrease in the tissue levels of caspase-3 activity in the DMBA group as compared to the control group is in line with previous reports.[15] This reduction may be due to over expression of caspase-3 inhibitors and survivin in tumour cells [16]Our results in agreement with[17]reported that the activation of caspase 3 and 9 were decreased in the buccal mucosa of hamsters treated with DMBA alone.

In the present study, oral administration of nanocomposite to normal rats resulted in a non-significant increase inserum tumor necrosis factor alpha (TNF-α) activity when compared with normal control group.

Moreover, showed that increased in caspase-3 and caspase-9 Compared to control group, our result in agreement with.[18] reported that the higher activity of caspase-3 enzyme along with the DNA fragmentation in liver cancer cells treated with ZnO NPs. Consequently, ZnONPs were shown to selectively induce apoptosis in cancer cells, ZnO NPs show much promise as new anticancer agents, given the specific apoptotic response of cancer cells.

In the present study the administration of N-acetyl cysteine /Zinc oxide Nanocomposite and high pH ascorbate after dosing of DMBA showed slightly increase in TNF- α activity compared to control group and very highly significant decrease compared to DMBA induction group at $P < 0.05$ as showed in fig (3) & table (1). NAC therapy will block the TNF-α and NF-KB activation, The antioxidant properties of NAC also induced structural modification which will reduce the TNF-α affinity receptor.[19] NAC also affectively decreases TNF-α level.[20]And showed slightly increase in Caspase -3 &Caspase -9 compared to control group and very highly significant decrease compared to Nano supplement group and very highly significant increase compared to DMBA induction group at $P < 0.05$ as showed in fig (1&2) & table (1).

Our results are in accordance to[21] who finding that caspase-3 was activated on sodium ascorbate treatment, sodium ascorbate induced apoptosis via the mitochondria-dependent pathway in melanoma cells.

All these events led to a decreased activation of caspase-9 and caspase-3 compared to Nano supplement group with resultant inhibition of apoptosis induced by hypoxia–reperfusion. These findings suggest that mitochondrial vitamin C is a major component in the maintenance of the mitochondrial membrane potential, and that vitamin C exerts its anti-apoptotic effect through its ability to scavenge ROS.[22]

Table (1): Activity of Caspase-3 &-9 and level of TNF-α in studied groups.

Control = vehicle (oil-treated) control; DMBA= 7,12-dimethylbenz(a)anthracene;Nanocomposite=

Parameters / Groups	TNF-α	Caspase-3	Caspase-9
Control	12.99 ± 0.69 [a,c]	22.38 ± 0.31[c]	23.97 ± 0.43 [c]
DMBA	35.59 ± 4.69 [a,b]	13.21 ± 0.45 [a,b]	11.86 ± 0.18 [a,b]
Nanocomposite+DMBA	15.91 ±0.98 [b]	26.17 ± 0.63 [b]	31.36 ± 0.92 [b]
Nanocomposite	14.37 ± 1.00 [c]	37.83 ± 0.49 [a]	43.40 ± 2.96 [a]

N-acetyl cysteine +Zinc oxide + High pH sodium ascorbate.

Data represent means ± SEM.Mean values with different superscript letters in the same column are significantly different at (P≤0.05).

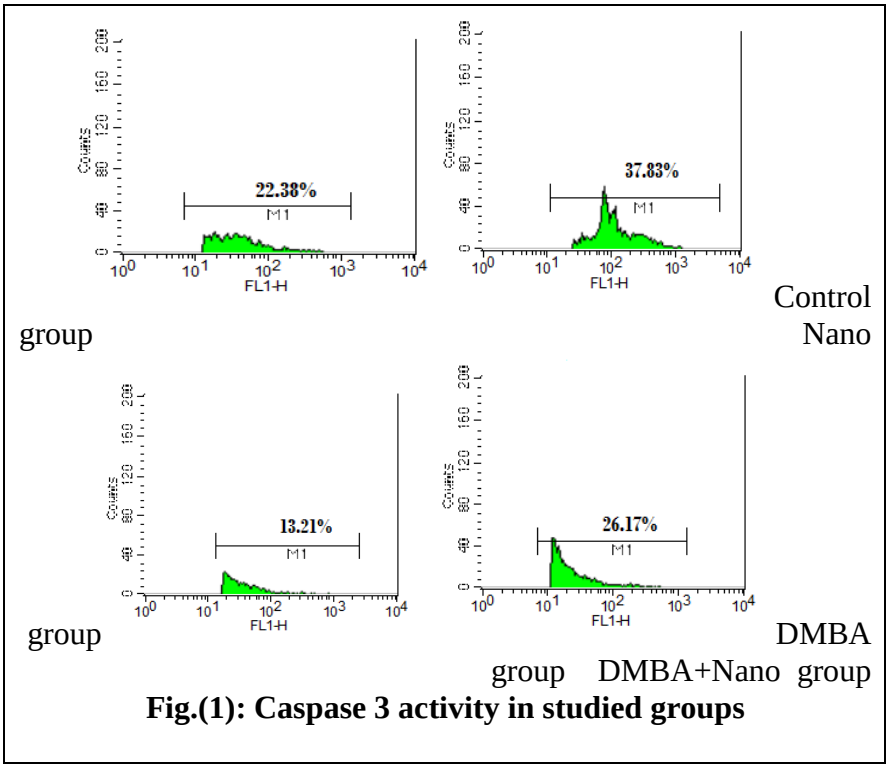

Fig.(1): Caspase 3 activity in studied groups

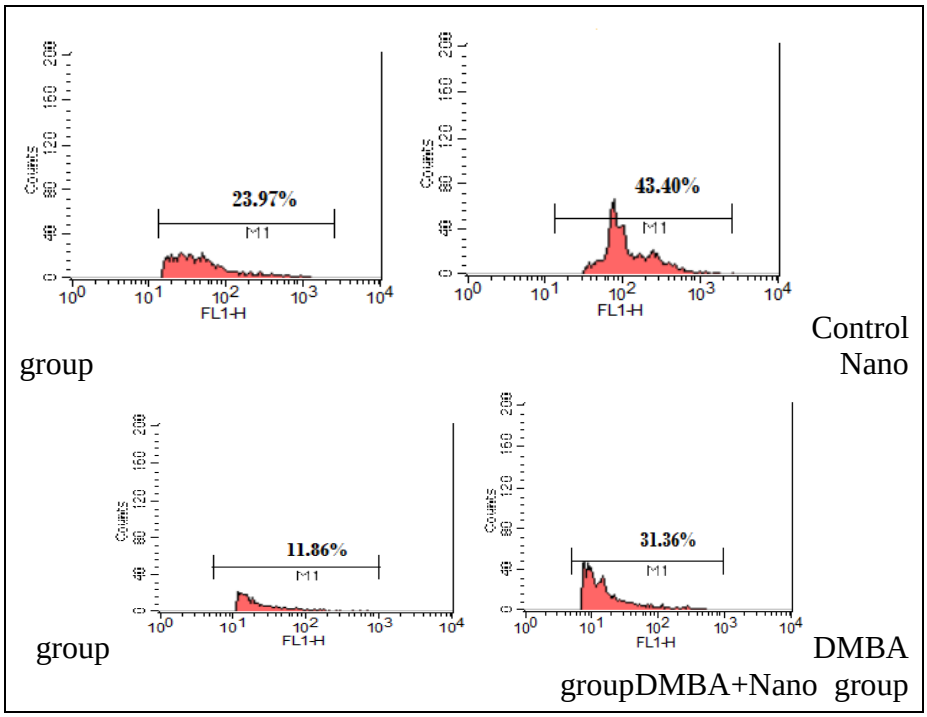

Fig.(2): Caspase 9 activity in studied groups

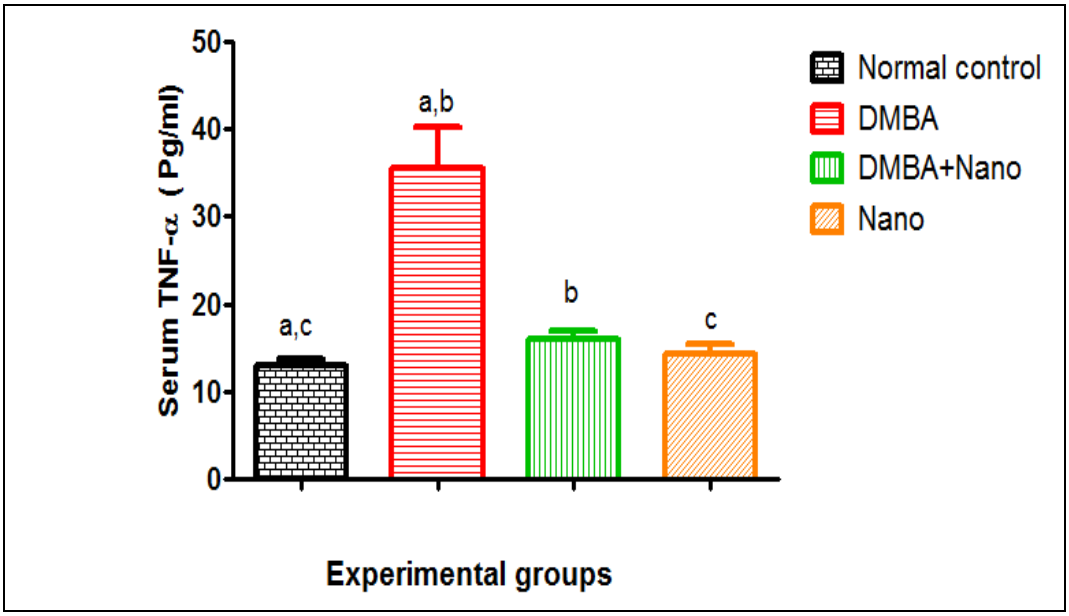

Fig.(3): TNF-α in studied groups

CONCLUSION

In the light of the foregoing results, it can be concluded that:

1. DMBA play a role in the development of mammary gland carcinoma and this effect may be mediated by inhibitionof Casepase-3 and Casepase-9 resulting in inhibition of apoptosis and cause carcinogenesis.
2. *N-acetyl cysteine /Zinc oxide Nano composite and high pH ascorbate*attenuates carcinogenic effects of DMBA on rat mammary glands and reverses DMBA-induced suppression of caspases -3 and -9 activity. Collectively, these observations suggested that novel synthetic Nano composite may potentially presents new hope for the development of breast cancer prevention, which should attract further scientific & pharmaceutical interest.

REFERENCES

[1]. Go, V., Butrum, R., Wong, D. (**2003**): Diet, nutrition, and cancer prevention:thepostgenomic era. J Nutrition, 133(11), 3830S.

[2]. Szaefer, H., Krajka-Kuźniak, V., Baer-Dubowska,W. (**2008**): *Toxicology*, 29, vol./is. 251/1-3(28-34), 0300-483X.

[3]. Nel, A., Xia, T., Madler, L., Li, N. Toxic potential of materials at the nanolevel. Science **2006**;311:622–7. Thorough review of experimental toxicological effects from nanoparticle treated assays including ZnO nanoparticles**.**

[4]. Albini, A., *et al*. (**2001**): Inhibition of angiogenesis-driven Kaposi's sarcoma tumor growth in nude mice by oral N-acetylcysteine. *Cancer Research*, 61, 8171-8178.

[5]. Nair, S., Sasidharan, A., Divya, Rani, V., et al. (**2009**):Role of size scale of ZnO nanoparticles and microparticles on toxicity toward bacteria and osteoblast cancer cells. J Mater Sci Mater Med;20:235–41.

[6]. Robey, I. F., Baggett, B. K., Sloane, B. F., et al (**2009**):Bicarbonate Increases Tumor pH and Inhibits Spontaneous Metastases, *Cancer Res;* 69(6): 2260–2268.

[7]. Aguirre, R., May, J.M., (**2008**): **"**Inflammation in the vascular bed: Importance of vitamin C" *Pharmacol.Ther*., 119, 96-103.

[8]. Tribukait,B. (**1984**): Flow cytometry in surgical pathology and cytology of tumours of the genito-urinary tract. In: Koss LG, Coleman DV,eds. Advances in clinical cytology. Vol 2. New York: Masson, PP. 89-163.

[9]. Chen, W.; Jin, W.; Cook, M.; Weiner, H.L. and Wahl, S.M. (**1998**):Oral delivery of group A streptococcal cell walls augments circulating TGF-beta and suppresses streptococcal cell wall arthritis.J.Immunol.; 161: 6297-6304.

[10]. Renno, W.M.; Alkhalaf, M.; Afsari, Z.; Abd-El-Basset, E. and Mousa, A. (**2008**):Consumption of green tea alters glial fibriliary acidic protein immunoreactivity in the spinal cord astrocytes of STZ-diabetic rats.*Nutr. Neurosci.; 11: 32-40.*

[11]. Hussein, M.R., Abu-Dief E.E., Abd El-Reheem M.H., Abd- Elrahman A. (**2005**):Ultrastructural evaluation of the radio protective effects of melatonin against X-ray-induced skin damage in Albino rats. Int. J. Exp. Pathol. 86, 45–55.

[12]. Bower, J.E., Ganz, P.A., Aziz, N., Fahey, J.L. (**2002**): Fatigue and proinflammatory cytokine activity in breast cancer survivors. Psychosom. Med. 64, 604–611.

[13]. Kopreski, M.S., Lipton, A., Harvey, H.A., Kumar, R. (**1996**): Growth inhibition of breast cancer cell lines by combinations of anti-P185HER2 monoclonal antibody and cytokines. Anticancer Res. 16, 433–436.

[14]. Gong, Y., Sun, X., Huo, L. (**2005**): Expression of cell adhesion molecules, CIM 4 s and Ecadherin, and microvessel density in invasive micropapillary carcinoma of the breast. Histopathology;46(1):24-30.

[15]. Huigsloot, M., Tijdens, I.B., Mulder, G.J., van de Water, B. (**2001**): Differential regulation of phosphatidylserine externalization and DNA fragmentation by caspases in anticancer drug induced apoptosis of rat mammary adenocarcinoma MTLn3 cells. *Biochem. Pharmacol*. 62, 1087–1097.
[16]. Krajewski, S., Krajewska, M., Turner, B.C. et al. (**1999**): Prognostic significance of apoptosis regulators in breast cancer. Endocr. Relat. Cancer 6, 29–40.
[17]. Rajasekaran, D., ShanmugamManoharan et al., (**2013**):Proapoptotic, anti-cell proliferative , anti-inflammatory and anti angiogenses potential of carnosic acid during 7,12- dimethyl benz [a]anthracene induced hamster buccal pouch carcinogenesis; *Afr J Tradit Complement Altern Med.*10(1):102-112.
[18]. MohdJavedAkhtar , MaqusoodAhamed et al (**2012**): Zinc oxide nanoparticles selectively induce apoptosis in human cancer cells through reactive oxygen species. International Journal of Nanomedicine 2012:7 845–857.
[19]. Hayakawa, H., Ishibashi T, Sekiguchi M. (**2003**) A novel mechanism for preventing mutations caused by oxidation of guanine nucleotides. EMBO.;4(5): 479-83.
[20]. Bambang, P. Efekpemberian; (**2011**): N-acetylcysteine oral terhadapkadar IL-6, TNF-α danhitungleukositpada continuous ambulatory peritoneal dialysis. In: Siregar P, Effendi I, Dharmeizar, Lydia A, Marbun MBH, Hustrini NM, eds. Naskahlengkapdanabstrakmakalahbebas. The 11th National Congress of INACN Annual Meeting of Nephrology. p. 162.
[21]. Shuw-Yuan Lin, Wan-Wen Lai et al., (**2006**): Sodium ascorbate inhibits growth via the induction of cell cycle arrest and apoptosis in human malignant melanoma A375.S2 cells . *Melanoma Research,* 16:509–519.
[22]. Kc, S., Cárcamo, J.M., Golde, D.W. (**2005**): Vitamin C enters mitochondria via facilitative glucose transporter 1 (Glut1) and confers mitochondrial protection against oxidative injury. *FASEB J;* 19: 1657–1667.
[23]. Lee, W.Y., Lee, J.S., Lee, S.M. (**2007**): Protective effects of combined ischemic preconditioning and ascorbic acid on mitochondrial injury in hepatic ischemia/reperfusion. *J Surg Res;* 142: 45–52.