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Biochemical effect of some novel nanocompsite on metabolic changes in experimentally induced tumor in female mice

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A B S T R A C T

Nanoparticles have one dimension that measures 100 nanometers or less. The properties of many conventional materials will be changed when they are in nanoparticle form. This is typically because nanoparticles have a greater surface area per weight than larger particles which make them to be more reactive to other molecules. The aim of this study to demonstrate the biochemical effect of basic curcumin – zinc oxide nanocompsite modified with vitamin C and surfactant induced experimentally in female mice. This study was carried out on 80 mice and weighted 25-30 gm. Mice were classified into four groups Group 1: Non tumor bearing mice (act as normal control) .Group 2: Tumor bearing mice (act as tumor bearing control group). Group 3: NTBM-treated with nanocompsite orally at dose of (1.850 g/kg /day) 6 weeks. Group 4: TBM-treated with nanocompsite orally at dose of (1.850 g/kg /day) 6 weeks. Blood samples were collected from all animals groups after 2, 4 and 6 weeks of treatment. Serum were separated and processed directly for AST and ALT activities, Urea, Creatinine, Nitric oxide concentration and Interleukin-6 (IL-6) and Interleukin-8 (IL-8) levels. The obtained results revealed that, these novel nanocompsite have a very important role in improving liver and kidney functions and pro inflammatory agents.

Keywords: Curcumin nanoparticles, ZnNP, CTAB, ALT, AST, Creatinine, Urea, NO, IL-6, IL-8

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1. INTRODUCTION

Ehrlich ascites carcinoma (EAC) is one of the experimental breast tumor derived from spontaneous mouse adenocarcinoma. Similar to other tumors developing in body cavities, EAC cells fill the peritoneal cavity by rapid division. During this rapid increase, besides the tumor cells, accumulation of a fluid named ascetic fluid is also observed With the growth of the tumor, the amount of the fluid increases as a consequence, due to the pressure induced by both tumor cells and the ascetic fluid and also the tumor's damage to the organism, animal dies following 17-18 days of EAC transplantation (G¨ulruh and Seyhan, 2004)

Nanotechnology allows the manipulation of materials at nanoscale level (1–100 nm), which enables precision engineering to control nanoparticles' (NPs') physicochemical properties, as well as their interactions with biological systems (Boisseau and Loubaton, 2011). Inorganic NPs, including metal oxides, are promising materials for applications in medicine, such as cell imaging, biosensing, drug/gene delivery, and cancer therapy (Sohair et al., 2014). Curcumin is an orange–yellow crystalline powder derived from the rhizome of (*Curcuma longa*), practically insoluble in water and ether but soluble in ethanol,

dimethylsulfoxide (DMSO) and acetone (Aggarwal *et al*., 2003). In spite of having such a broad range of therapeutic and antioxidant property, the major drawback associated with the use of curcumin is its low systemic bioavailability upon administration. Poor absorption from the intestine coupled with high degree of metabolism in the liver hinders the development of curcumin as a potent clinical agent (Yadav et al., 2012). Nanocurcumin is a novel approach to overcome the problem of low bioavailability. The natural phenolic compound curcumin has been shown to have anti-inflammatory, anti-oxidant and anti-proliferative effects on cells, thereby profoundly affecting their metabolism and proliferative potential (Aggarwal and Sung, 2009). Vitamin C, or ascorbic acid, has been a component of various dermatologic drugs and cosmetics for many years. It is known for its antioxidant properties and its applications in cancer therapy and inflammatory skin changes (Albert Szent- Györgyi, 2010). Ascorbate is a prooxidant anticancer therapy through the production of H_2O_2, pharmacologic a scorbate can induce some cancer cell death in vitro and inhibit a number of types of tumor growth in animal models. Cetrimonium bromide (CTAB) belongs to a group of quaternary ammonium compounds, which also includes benzethonium chloride and dequalinium chloride, both of which have demonstrated anticancer properties in vitro and in vivo by targeting tumor mitochondria (Yip et al., 2006). Quaternary ammonium derivatives have also been reported to enhance antitumor activity when compared with their parent compounds (Giraud et al., 2002), suggesting that molecules may be highly effective anticancer agents. So, this study was applied to investigate the antitumor effect of different types of nanoparticles on some blood parameters.

2. MATERIALS AND METHODS

2.1. Chemicals:

Basic nanocurcumin and ZnO nanocomposit, surfactant (CTAB) purchased from Sigma-Aldrich (Sigma, USA) Company. Sodium ascorbate purchased from El-Gomhoria Chemicals Company.

2.2. Nanocompsite compound:

Basic nanocurcumin 3.75 gm + ZnO-nanocomposit 1.25 gm modified with Vitamin C 1.0 gm + Surfactant (CTAB) 0.1 gm.

2.3. Preparations and Measurements of novel nanocomposite (Basic Curcumin & Zinc Oxide):

To prepare curcumin nanoparticles, 1M curcumin with low solubility in water was mixed with 4M sodium bicarbonate buffer, then grinded using mechanical ball mill (350 rounds/ Sec) for 8 hrs. The colour of curcumin changed from yellow to red as a result of the curcumin sodium salt formation. Curcumin nanoparticles were then dispersed into 50 ml of distilled water making aqueous solution which was filled in a reactor that was immersed in a water bath adjusted at 11ºC. Afterwards, this reactor was placed in an ultrasound apparatus (VCX-750 commercial sonicator) and sonication was applied in continuous mode at 100 Watt in a glass reaction vessel with thin and indented bottom for uniform and more efficient energy transmission. Zinc Oxide nps Modified with Basic Nano-curcumin Preparations: Modified ZnO NPs was achieved by sooking ZnO NPs for 24h in basic nano-curcumin 0.05 g in 50 ml distilled water and stirred overnight to allow complete complexation. The resulting solids were dried in evacuated desiccators to give Zinc oxide NPs modified with basic nano-curcumin. The novel formula drug was ball milled for 8 hours in the nanoscale to the corresponding nano drug as nano (Basic curcumin, Zinc Oxide) and we got transmission electron microscope (TEM).

From the TEM studies represents TEM image of the novel nano composide (Basic curcumin, Zinc oxide) ranging from 6.87-12.7 nm in diameter and has spherical shape.

Ultra sonication for nanocomposide drug by dissolving it in saline at concentrations of 5g/ L, then putting vitamin C and Cu-CTAB with Nanocompsit were performed.

2.4. In vitro study (Cytotoxic assay):

The antitumor effect and inhibitory concentration 50 (IC50) of this novel nanocompsite will be investigate against MPC-7 (breast carcinoma cell line).

2.5. In vivo study:

Acute Toxicity Study (Determination of LD50) for Nanocomposite according to (Finney, 1964).

2.6. Animals:

A total number of 80 Australian females' albino mice of 12-14 weeks old age and weighting 25-30 gm were used in the experimental investigation of this study. Mice were obtained from the Research Institutes of Ophthalmology, Giza, Egypt. Animals were housed in separate metal cages, fresh and clean drinking water was supplied ad-libtium through specific nipple. Mice were kept at a constant environmental and nutritional condition throughout the period of the experiment.

2.7. Tumor induction:

The experimental induction of tumor in female mice was carried out at the National Cancer Institute Egypt. Every 1 ml of Ehrlich ascites adenocarcinoma was diluted with 4 ml of normal saline. Each mouse was injected subcutaneously (S/C) in the medial aspect of the right thigh with 0.2 ml of Ehrlich ascites adenocarcinoma (2.5 × 106 tumor cells with single cell suspension) (Zeinab, 2009). The tumor developed and become palpable in all injected animals at 5-7 days post tumor inoculation.

2.8. Experimental design:

Mice were randomly divided into four groups each one comprises of 20 mice kept in separate metal cages: Group 1: (NTBM) normal mice act as (Normal control). Group 2: it was tumor bearing control (TBM). Group 3: Mice treated with nanocompsite at dose of (1.850 g/kg b. wt/day) 6 weeks orally. Group 4: tumor bearing mice treated with nanocompsite at dose of (1.850 g/kg b.wt/day) 6 weeks orally.

2.9. Sampling:

Blood samples were collected in the morning after overnight fasting from all mice by decapitation after 2, 4 and 6 weeks from the onset of treatment, blood samples were collected in dry and clean tubes then centrifugation at 3000 r.p.m for 15 minutes. The clear serum were aspirated by Pasteur pipette and received in dry sterile sample tube, processed directly for enzymes determination, then kept in a deep freeze at -20℃ until used for subsequent biochemical analysis, including serum (AST and ALT) activity, (urea, creatinine, Nitric oxide) concentration were analyzed colorimeterically according to the methods described by Reitman and Frankel, (1957), Searcy et al., (1967), Schirmeister et al., (1984), (Miranda et al., 2001) respectively. (IL-6 and IL-8) levels were analyzed by ELISA (Enzyme-linked Immunosorbent Assay) according to the methods described by Ferrari et al., (2003) and Fujishima et al., (1996) respectively.

2.10. Statistical analysis:

Statistical analysis of the obtained results was carried out using student's F-test according to Snedecor and Cochran (1969).

3. RESULTS

The results in table (1) revealed the followings.

3.1. Serum alanine amino transferase (ALT) activity:

Serum Alanine transferase (ALT) activity showed a very highly significant increase in tumor bearing mice (TBM) all over the experimental period of tumor induction as compared to NTBM group. Moreover, administration of nanocompsite to NTBM showed a significant increase in 2 and 4 weeks followed by a significant decrease in 6 weeks as compared to NTBM group. While, administration of nanocompsite to (TBM) showed a highly significant increase observed all over the experimental period as compared to (NTBM) administrated nanocompsite. Moreover, a non-significant decrease in 2 weeks followed a highly significant decrease in 4 and 6 weeks in (ALT) activity as compared to (TBM) group.

3.2. Serum aspartate transferase (AST) activity:

Serum Aspartate transferase (AST) activity showed a very highly significant increase in tumor bearing mice (TBM) all over the experimental period of tumor induction as compared to NTBM group. Moreover, Administration of nanocompsite to NTBM showed a highly significant decrease AST activity all over the experimental period as compared to NTBM group, while administration of nanocompsite to TBM showed a non significant decrease in 2 week followed by a highly significant decrease in 4 and 6 weeks as compared to TBM group. Moreover, a highly significant increase showed all over the experimental period in AST activity as compared to NTBM administrated nanocompsite.

3.3. Serum creatinine concentration:

Serum creatinine concentration showed a non significant change in 2 week followed by a highly significant increase in 4 and 6 weeks in tumor bearing mice (TBM) as compared to NTBM group. Moreover, Administration of nanocompsite to NTBM showed a significant decrease in 2 week followed by non significant changes in 4 and 6 weeks in creatinine concentration as compared to NTBM group, while administration of nanocompsite to TBM showed a non significant decrease in 2 week, this decrease became a very highly significant after 4 and 6 weeks as compared to TBM group, while a non significant increase in 2 week followed by a non significant changes in 4 and 6 weeks in creatinine concentration as compared to NTBM administrated nanocompsite.

3.4. Serum urea concentration:

Serum Urea concentration showed a significant decrease in 2 week followed by a highly significant increases after 4 and 6 weeks in tumor bearing mice (TBM) as compared to NTBM group. Moreover, Administration of nanocompsite to (NTBM) showed a significant decrease in 2 week, this decrease became a non significant after 4 and 6 weeks as compared to (NTBM) group while, Administration of nanocompsite to (TBM) showed a non significant change in 2 week followed by a highly significant decrease after 4 and 6 weeks as compared to (TBM) group. While, a non-significant increase in 2 week, this increase became a significant after 4 and 6 weeks in urea concentration as compared to (NTBM) administrated nanocompsite.

1.1. Serum Nitric Oxide levels:

Serum Nitric Oxide level showed very highly significant increases in tumor bearing mice TBM all over the experimental period of tumor induction as compared to NTBM group. Moreover, Administration of nanocompsite to NTBM showed a non significant increase in 2 week. This increase became a significant after 4 and 6 weeks when compared to NTBM group. Administration of nanocompsite to TBM showed a non significant decrease in 2 weeks followed by a non significant change in 4

weeks while, a highly significant decrease in 6 weeks as compared to TBM group.

Table (1): Effect of nanocompsite treatment on serum ALT, AST, Urea, Creatinine, NOX, IL-6 and IL-8 Levels induced experimentally in mice after 2, 4 and 6 weeks

Parameters		NTBM	TBM	NTBM + NP	TBM + NP
S.ALT (U/ml)	2	81.25^{a} ±3.64	192.00^{c} ±3.97	97.25^{b} ±6.85	189.50^{c} ±6.86
	4	76.75^{a} ± 1.65	196.50^{d} ±5.76	94.25^{b} ±4.33	135.25^{c} ±2.90
	6	110.75^{b} ±4.61	199.75^{c} ±4.13	88.75^{a} ±1.11	111.75^{b} ±4.82
S.AST (U/ml)	2	85.75^{b} ±2.72	196.50^{d} ±2.53	67.25^{a} ±2.87	185.75^{d} ±2.02
	4	86.75^{b} ± 2.66	237.75^{d} ±6.29	79.50^{a} ±1.44	148.75^{c} ±8.65
	6	148.75^{c} ±8.65	237.75^{d} ±6.29	83.00^{a} ±3.11	136.50^{b} ±6.34
S. Urea (mg/ml)	2	46.25^{b} ±1.31	35.25^{a} ±4.48	33.75^{a} ±1.65	35.50^{a} ±4.03
	4	31.00^{a} ± 1.08	52.75^{c} ±3.01	29.50^{a} ±1.26	36.50^{b} ±3.30
	6	42.50^{ab} ±3.97	82.50^{c} ±3.75	38.00^{a} ±1.47	43.25^{b} ±3.47
S. Creatinine (mg/dl)	2	0.84^{b} ±0.11	0.81^{b} ±0.04	0.56^{a} ±0.08	0.76^{ab} ±0.11
	4	0.71^{a} ± 0.04	1.41^{b} ±0.27	0.66^{a} ±0.09	0.73^{a} ±0.07
	6	0.93^{a} ±0.12	1.78^{b} ±0.09	1.04^{a} ±0.16	0.83^{a} ±0.12
S. NO_X (μM)	2	8.57^{a} ±0.44	16.40^{b} ±1.72	11.58^{a} ±1.03	13.70^{ab} ±1.93
	4	8.31^{a} ± 0.85	21.09^{b} ±0.83	17.75^{b} ±5.00	21.07^{b} ±3.90
	6	8.35^{a} ±0.29	59.75^{d} ±6.5	21.97^{b} ±3.13	28.43^{c} ±3.65
S. IL-6 (Pg/ml)	2	26.15^{a} ±1.83	58.04^{c} ±1.70	33.88^{b} ±2.74	57.31^{c} ±7.03
	4	20.95^{a} ± 2.10	75.01^{c} ±3.65	46.53^{b} ±6.60	73.03^{c} ±5.19
	6	25.48^{a} ±3.40	100.95^{d} ±1.56	43.86^{b} ±3.85	84.64^{c} ±3.23
S.IL- 8 (Pg/ml)	2	8.34^{a} ±0.23	25.06^{b} ±2.86	9.18^{a} ±0.91	22.84^{b} ±6.66
	4	8.24^{a} ± 0.45	50.99^{c} ±11.3	8.21^{a} ±1.10	30.80^{b} ±5.67
	6	7.45^{a} ±0.32	58.99^{c} ±8.42	5.58^{a} ±0.47	29.07^{b} ±2.64

a, b & c: There is no significant difference *(P> 0.05*) between any two means, within the same column have the same superscript letter. Data are presented as (mean ± S.E) & S.E= standard error.

Moreover, a non significant increase in 2 and 4 weeks, this increase became a significant after 6 weeks in Nitric Oxide level NO when compared to NTBM group received nanocompsite.

1.2. Serum IL-6 levels:

Serum Interleukin-6 (IL-6) level showed a very highly significant increase in tumor bearing mice TBM all over the experimental period of tumor induction as compared to NTBM group. Moreover, administration of nanocompsite to NTBM showed a significant increase all over the experimental period as compared to NTBM group. While, Administration of nanocompsite to TBM showed a non significant decrease in 2 and 4 weeks, this decrease became a significant after 6 weeks as compared to TBM group. While, a highly significant increase showed all over the experimental period in Serum IL-6 level as compared to NTBM administrated nanocompsite.

1.3. Serum IL-8 levels:

Serum Interleukin-8 (IL-8) level showed a very highly significant increase in tumor bearing mice TBM all over the experimental period of tumor induction when compared to NTBM group. Moreover administration of nanocompsite to NTBM showed a non significant increase in 2 week followed by a non significant change after 4 weeks anon a non significant decrease after 6 weeks when compared to NTBM group while, administration of nanocompsite to TBM showed a non significant decrease in 2 weeks, this decrease became a highly significant after 4 and 6 weeks as compared to TBM group. While, a highly significant increase showed all over the experimental period in Serum IL-8 level as compared to NTBM administrated nanocompsite.

4. DISCUSSION

The presented data in tables (1) revealed that, a significant increase in serum (AST and ALT) activities,(urea, creatinine) concentration and (IL-6 and IL-8) levels were observed in tumor-bearing female mice all over the experimental period of tumor induction when compared to control.

Similary, a rise in plasma bilirubin and hepatic enzyme activities were observed in tumor bearing rats is the results of changes in the liver indicated by the presence of tumor (Rafei et al., 1993). The recorded increase in plasma ALT and AST activities in tumor bearing mice of the present study might be due to generalized destruction of liver cells and release of AST into plasma after tumor induction. On the other hand, a significant increase in serum urea concentration in TBM was confirmed by the results observed by (Hussein and Azab, 1997): who observed that, there was a highly significant increase in plasma urea concentration in tumor-bearing mice. The author attributed such increase in blood urea concentration to the increase in urea production as a result of catabolic effect of tumor.

Our results demonstrated a very highly significant increase in serum creatinine concentration in tumor bearing mice. These results were similar to (Hussein, 2003) who observed that, serum creatinine level showed a significant increase in mice-bearing Ehrlich ascites carcinoma due to muscle necrosis.

Our results demonstrated a highly significant increase in serum nitric oxide (NO) concentration in tumor bearing mice .these results agree with results of (Prazma et al., 1995) who attributed that, Excessive and unregulated NO synthesis has been implicated as causal or contributing to patho-physiological conditions including cancer, Expression of NOS has been detected in various cancers such as cervical, breast, central nervous system, laryngeal, and head and neck cancers. NO seems to promote tumor growth and proliferation.

Also, (IL-6 and IL-8) levels showed a highly significant increase in tumor bearing mice were similar results reported by (Fayad et al., 2001) who reported that, patients with lymphoma have high levels of IL-6 and IL-8. Serum levels of IL-6 correlate with the presence of B-symptoms and IL-8, and both IL-6 and IL-8 have been found to be prognostic factors in low-grade lymphomas, Hodgkin disease, and chronic lymphocytic leukemia.

Administration of nanocompsite to (TBM) showed a highly significant increase observed all over the experimental period as compared to (NTBM) administrated nanocompsite. While, a non significant decrease in 2 weeks followed a highly significant decrease in 4 and 6 weeks in (ALT) activity (U/ml) as compared to (TBM) group. Also, administration of nanocompsite to (TBM) showed a non significant decrease in 2 week followed by a highly significant decrease in 4 and 6 weeks as compared to (TBM) group. Moreover, a highly significant increases showed all over the experimental

period in (AST) activity (U/ml) as compared to (NTBM) administrated nanocompsite , these results were similar results reported by (Mokhtar et al., 2008) who showed that curcumin decreased the induction of (AST and ALT) activity of rats treated with Sodium arsenite. The author attributed such decrease in transaminase enzymes to administration of curcumin preserved the structural integrity of the hepatocellular membrane. This was evident from the reduction in the enzyme activities against the arsenic induced rise in the enzyme levels in plasma. It could be suggested that the leakage of enzymes because of liver injury is prevented by the liver cell membrane stabilizing action of curcumin.

(Sohair et al., 2014) reported that, exposure to ZnO NPs (7 and 35 μg/ml) for three consecutive weeks elicited a significant decrease in total protein and albumin contents coinciding with enhancement of total lipids and cholesterol levels as well as activities of aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase in hemolymph and soft tissues of treated snails.

Administration of ZnO NPs (400 mg/L) to wistar rats for 14 days increased all three liver enzymes (ALT, AST and ALP) significantly in a dose dependent manner as compared to the control group, but presence of vitamin C prevents damage to the liver cells Band normalized this enzymes. We know vitamin C is an antioxidant agent so results showed damaging effect of ZnO nanoparticle is oxidative destruction (Bakhshiani and Fazilati, 2014). In contrary, (Wang et al., 2010) reported that, the amount of ALT, AST, ALP, total protein, creatine kinase and lactate dehydrogenase (LDH) were significantly decreased at 2.5 mg/kg body weight of inhalation exposure to ZnO nanoparticles in rats that was associated with increasing zinc content and tissue disorders in the liver.

Reports showed that vitamin C and some agent's synergistically normalized diabetes induce hepatotoxicity. This can be seen from the work of (Eze et al., 2012). They reported that Co-administered vitamin C and Zinc 100 and 50 mg/kg respectively restored alanine aminotransferase and aspartate aminotransferase function in diabetes induced hepatotoxicity. This showed that vitamin C and Zinc may play an important role in the prevention of hepatocellular injury that may occur in diabetes. This is further supported by the findings of (Hamden et al. 2009). He and co-researchers showed that vitamin C co- administration with vitamin E ameliorate oxidative stress, pancreatic and hepatic injury in alloxan diabetic rats.

Hepatic enzymes like alanine transaminase and aspartate transaminase were significantly increased on surfactant exposure. Alkaline phosphatase also showed significant increase compared to control. (Yip et al., 2006)

The Cu-CTAB, Cu-CTAB inoculated EAC, CD-CTAB and CD-CTAB inoculated EAC groups showed a moderate elevation of serum total protein and liver function enzymes which indicates presence of a moderate damage of liver (Abdelftah Badawi et al., 2012)

Tumor bearing mice treated with nanocompsite showed a non significant change in 2 week followed by a highly significant decrease after 4 and 6 weeks as compared to (TBM) group. While, a non significant increase in 2 week, this increase became a significant after 4 and 6 weeks in urea concentration (mg/dl) as compared to (NTBM) administrated nanocompsite. Also, Administration of nanocompsite to (TBM) showed a non significant decrease in 2 week, this decrease became a very highly significant after 4 and 6 weeks as compared to (TBM) group. While, a non significant increase in 2 week followed by a non significant changes in 4 and 6 weeks in

creatinine concentration (mg/dl) as compared to (NTBM) administrated nanocompsite, these results were confirmed by (Tirkey et al., 2005) who showed in studies with cyclosporine that, treatment with curcumin was significantly decreased the level of urea and creatinine because of its role as potent antioxidant.

These suggestion was confirmed by (Farombi and Ekor, 2006) who found that, the preventive effect of curcumin on the gentamicin-induced decrease in the activity of glutathione peroxidase (GSHPx) and CAT could contribute to the restoration of markers of renal tubular injury. It seems reasonable to assume that curcumin is able to suppress nephrotoxicity in kidney, as it was demonstrated in studies with adriamycin (Venkatesan et al., 2000)

Ali et al., (2014) reported that, the level of urea and creatinine increased just in group received 300 mg/kg while the uric acid decreased in all groups even who received 50 mg/kg. Moreover, ZnO NPs caused histopathological alterations in the mice kidneys. Other researchers indicated the histopathological changes such as glomeruli segmentation, hydropic degeneration in epithelial cells, necrosis and swelling of epithelial cells in the kidney tissues of mice treated with ZnO nanoparticles (Esmaeillou *et al*., 2013).

Kanter et al., (2005) demonstrates that, Vit C treatment decreases serum urea and creatinine concentrations and increase antioxidant enzyme activities, and also prevents renal damage in experimentally-induced endotoxemic rats. Similar results, antioxidant supplementation has proven to be beneficial in decreasing the oxidative stress induced by endotoxin in a variety of tissues. For example, endotoxin administration to guinea pigs increased oxidative damage to liver protein. This increase is totally prevented in animals supplemented with Vit C, a treatment that considerably increases liver ascorbate.

Administration of nanocompsite to (TBM) showed a non significant decrease in 2 weeks followed by a non significant change in 4 weeks .while, a highly significant decrease in 6 weeks as compared to (TBM) group. Moreover, a non significant increases in 2 and 4 weeks, this increase became a significant after 6 weeks in Nitric Oxide concentration (NO) (μM) when compared to (NTBM) group received nanocompsite. these results were similar results reported by (Pae et al, 2008) who observed that, mouse macrophage cell line has shown that LPS stimulation of NFkB is inhibited by curcumin and leads to decreased inducible nitric oxide synthase (iNOS or NOS2) activity. Moreover, Curcumin has shown anti-proliferative effect in multiple cancers, and is an inhibitor of the transcription factor NF-κB and downstream gene products (including c-myc, Bcl-2, COX-2, NOS, Cyclin D1, TNF-α, interleukins and MMP-9). In addition, curcumin affects a variety of growth factor receptors and cell adhesion molecules involved in tumor growth, angiogenesis and metastasis (Reason et al., 2011)

(Kim et al., 2014) showed that, a significant decrease in NO level occurred after administration of ZnONPs fed mice as compared with the control. (Sohair et al., 2014) reported that, exposure to ZnONPs (7 and 35 μg/ml) for three consecutive weeks significantly induced malondialdehyde and nitric oxide with concomitant decreases in glutathione and glutathione-S-transferase levels in hemolymph and soft tissues of treated snails.

"Ascorbic acid [vitamin C] has been shown to stimulate endothelial nitric oxide (NO) synthesis in a time- and concentration-dependent fashion." (Regine et al., 2001).This same study even found how Vitamin C works to raise nitric oxide

levels: it protects (from oxidation) a cofactor eNOS called tetrahydrobiopterin. A cofactor is "a substance that acts with another substance to bring about certain effects - especially a coenzyme." So, assuming you have the baseline nitric oxide in the first place, which you can likely boost with either citrulline or nitrates (in food), then Vitamin C should raise nitric oxide levels for you. Moreover, how nitrate in food is converted to nitrite by bacteria in the mouth and later to nitric oxide in the gut. Vitamin C is a similar miracle worker and converts nitrites to nitric oxide. ""Vitamin C is an effective scavenger of nitrite, reducing it to nitric oxide and preventing nitrosamine formation in vitro and in vivo. Thus the protection of tetrahydrobiopterin is not the only way that Vitamin C can increase a man's baseline nitric oxide levels. TBM treated with nanocompsite showed a non-significant decrease in 2 and 4 weeks, this decrease became a highly significant after 6 weeks as compared to (TBM) group. While, a highly significant increase showed all over the experimental period in Serum Interleukin-6 (IL-6) levels (Pg/ml) as compared to (NTBM) administrated nanocompsite. Also, Administration of nanocompsite to (TBM) showed a non significant decrease in 2 weeks, this decrease became a highly significant after 4 and 6 weeks as compared to (TBM) group. While, a highly significant increase showed all over the experimental period in Serum Interleukin-8 (IL-8) levels (Pg/ml) as compared to (NTBM) administrated nanocompsite. Bharti et al., (2003) demonstrated that curcumin inhibited interleukin (IL) 6-induced STAT3 phosphorylation and consequent STAT3 nuclear translocation. Curcumin inhibits production of proinflammatory chemokines, including IL-8, by tumor cells. curcumin contributed not only to the inhibition of IL-8 production but also to signal transduction through IL-8 receptors. These data suggest that curcumin reduces numerous IL-8 bioactivities that contribute to tumor growth and carcinoma cell viability. From this point of view, curcumin is a potent anticancer agent that inhibits the production of proinflammatory cytokines, including IL-8, by tumor cells (Hidaka et al., 2002). Mononuclear inflammation of aortic walls was reduced in a mouse model of cardiovascular disease with curcumin treatment and corresponded with lower tissue concentrations of IL-8, IL-6, and monocyte chemoattractant protein (MCP)-1 as well as decreased NFkB DNA-binding (Parodi et al, 2006). (Kim et al., 2014) showed that, a significant decrease in interleukin -6 and IL-8 levels occurred after administration of ZnO nano particles fed mice as compared with the control.

Conclusion: we observed that a novel nanocompsite (Basic nanocurcumin + ZnO NP + Vitamin C + Cu-CTAB) have distinct effects on mammalian cell viability via killing cancer cells while posing no effect on normal cells. Cancer had its harmful effect on liver functions that appeared in the rising of (AST and ALT) enzymes activities. It also affected kidney functions which appeared in the rising of (Urea and Creatinine) concentrations and pro inflammatory agents that appeared in the rising of (NO_X, IL-6, IL-8) levels Hence, these a novel nanocompsite have a very important role in improving liver & kidney functions and pro inflammatory agents through decreasing these ratios and that indicated the ability of these compounds in protect kidney and liver against harmful of cancer effect.

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