

RESEARCH ARTICLE

Ameliorative Effects of Nanocurcumin on Cyclophosphamide Induced Immunosuppression in Male Rats

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Abstract

Curcumin, an active component of turmeric, possesses different protective functions due to its anti-inflammatory, anti-oxidative and immunomodulatory activities. Curcumin's medicinal use is restricted as a result of low solubility, instability, poor bioavailability and rapid metabolism. Nanocurcumin is an enhanced form with higher tissue distribution, improved internalization and reduced systemic elimination than curcumin. Cyclophosphamide (CP), the anticancer alkylating agent, is proved to be useful for managing a wide range of cancers. Its use is limited due to harmful effects including the induction of both oxidative stress and immunosuppression. The present study evaluated the immuno-enhancement and protective properties of nanocurcumin in an immunosuppressed rat model. The experimental rats were divided into four groups (control, immunosuppressed, treated and protected) of ten rats each. Both treatment and protection trials of immunosuppressed rats with nanocurcumin significantly increased indices of spleen (119.83 and 128.61%) and liver (104.95 and 109.74%), respectively as well as the levels of immunoglobulins (IgG, 148.85 and 197.62% and IgM, 144.09% and 211.51%, respectively) beside the enhancement of the phagocytic activity of macrophages (122.37 and 156.16% respectively), in comparison with immunosuppressed rats. Both approaches down-regulated the proinflammatory cytokines comprising TNF- α (15.91 and 13.23%, respectively) and IL-1 β (74.22 and 36.98%, respectively), while they upregulated the anti-inflammatory cytokines (IL-10, 130.59 and 184.68%, respectively) in splenic expression. In hepatic mRNA expression of TNF- α , both treatment and protection approaches significantly down-regulated their transcriptional level (19.17 and 46.38%, respectively), but no significant variations were reported in IL-1 β expression (100.12 and 103.42%, respectively), whereas both approaches significantly up-regulated IL-10 (170.13 and 264.51%, respectively). Also, nanocurcumin restored the integrity of DNA in hepatic tissue. The protected group had more ameliorative effects than the one being treated. It could be concluded that nanocurcumin has a potential or even a vital protective activity against immunodepression in CP administered rats and could be used as an immunomodulatory agent.

Keywords: Nanocurcumin, Cyclophosphamide, Immunosuppression, Cytokines.

Introduction

The immune system is a vital biological network that interacts with external antigens to protect the host and preserve homeostasis [1]. Immunosuppression, an immune system dysfunction, is caused by a number of biological, environmental and humoral (IgG and IgM) physical issues. Long-term exposure to these adverse factors can lead to health

deterioration, lower body weight gains, lower efficacy of preventive vaccination and increased susceptibility to cancer, parasitic and latent infections [2]. Most commonly used cancer drugs have impaired body defense resulting in immunosuppression and cytotoxicity regardless the major healing impact they possess [3]. Cyclophosphamide

(CP), an alkylating anti-cancer agent, has an extraordinary therapeutic index and a wide range of activities to treat the autoimmune diseases and cancer [4]. Cyclophosphamide use has deleterious side effects, including immunosuppression, myelosuppression and leukopenia, contributing to severe morbidity and mortality. However, CP, induced immunosuppression, is a significant limiting factor in clinical chemotherapy [5]. It is therefore necessary to evade normal cell damage from therapy.

It is essential to use medicinal herbs and plant extracts for chemo-preventive and immunomodulatory purposes in traditional therapy. Natural products have been shown to be an excellent and reliable source for the development of new medications and to be able to repair impaired immune systems and CP-induced immunodeficiency [6]. Consumption of plants and/or fruits with elevated levels of polyphenols is associated with enhanced immune function and disease resistance. Plants and fruits rich in polyphenolic compounds are turmeric (Curcumin), flax seeds, sesame seeds, whole grains, apples, onions, dark chocolate, red cabbage and oats [7].

Curcumin is the main phytochemical dietary plant with disease avoidance and immunomodulation functions. Curcumin, the active component of *Curcuma longa*, is a bright yellow compound with numerous beneficial properties, particularly, antioxidant, anti-inflammatory, immunomodulatory and anti-tumor activities [8]. Curcumin has been shown to be able to regulate cell responses and the production of different forms of cells specific for immune system, including T and B cells and phagocytic cells [9]. Curcumin has the ability to lower TNF- α [10] and IL-6 levels [11] and to modulate a number of different growth factors including kinases, transcription factors, cytokines and different enzymes [12]. It has been established that curcumin is effective in inhibiting T cell-mediated immune functions and could control various immune mechanisms such as the processing of cytokines, antigen presentation, cell-mediated and humoral immunity [13]. Curcumin has shown a strong anti-inflammatory function in which macrophages play a main role [14].

Curcumin is adversely affected by poor solubility, limited bioactivity and fast elimination that may decrease the efficacy of therapy [15]. Biodegradable polymer nanoparticles (nanocurcumin) approach has been developed to increase the stabilization, bioavailability and preservation of curcumin in the cells [16]. Once nanoparticles (NPs) enter the body, they are highly likely to interact with the innate immune system, resulting in an immune modulatory response [17].

Thus, the ongoing experiment was designed to deal with the following question: Could nanocurcumin protect the host from CP adverse effects of immunosuppression and genotoxicity?

Materials and Methods

Chemicals and reagents

Cyclophosphamide was purchased from Multi Pharma Company, Egypt and was diluted following the manufacturer instructions. Nanocurcumin was obtained from Sanat product Ltd., Delhi, India and each 100 mg was diluted in 1 mL NaOH. ELISA kits for estimation of IgG and IgM were purchased from Cusabio Biotech Co, (Wuhan, China). Reagents/chemicals for phagocytic activity were bought from Sigma-Aldrich (USA). Kits and chemicals for real time-PCR and DNA fragmentation were purchased from Ambion (Thermo Scientific, USA). *Candida albicans* (*C. albicans*) was obtained from Department of Bacteriology, Mycology and Immunology, Animal Health Research Institute, Zagazig branch, Sharkia, Egypt.

Animal selection and housing

Forty adult Male Wister albino rats (180 \pm 20 g) were obtained from National Research Center to be included in this research. The rats were provided with normal standard diet and water. Animals were kept in clean, fumigated and well-ventilated units under safe environment (22 \pm 2 °C) with (12:12 h) light-dark cycles. They were acclimatized under control conditions at the laboratory for two weeks before the initiation of the experiment. Food and water were supplied in unique open containers attached to the cage walls. Water and food were changed day by day for all animals. This study was conducted in Scientific and Medical Research Center (ZSMRC) Zagazig University, Sharkia, Egypt.

Experimental design

Experimental rats were divided into four groups, 10 rats each as shown in Figure 1. The protocol of this study was accepted by the ethics committee at Zagazig University (ZU-IACUC/2/F/163/2019). The first group (G1) was a control that was treated with physiological saline once a day for 20 days. The other three groups of rats were immunosuppressed by intraperitoneal injection of CP (50 mg/kg) [18]. The second group (G2, immunosuppressed) was treated with physiological saline once a day for 20 days. The third group (G3, treated) was administered with an IP dose of nanocurcumin (20 mg/kg) daily. The fourth group (G4, protected) was injected with an intraperitoneal (IP) dose of nanocurcumin (20 mg/kg) daily for two weeks

before induction of immunosuppression. CP was administered in G2, G3 and G4 at days 1, 2, 3, and 20 following initial validation [19]. Twenty-four hours after administration of the last dosage, two separate blood samples were collected from the retro-orbital plexus from each rat; the first blood samples were collected in serum tubes and left to coagulate at room temperature for 20 min, the resultant serum was separated after centrifugation at 3000 rpm for 15 min and stored at -20 ° C until used in determining IgG and IgM concentration. The second blood samples were collected into heparinized tubes to isolate phagocytes to estimate bacterial activity. All rats from each group were then weighed and euthanized by cervical dislocation.

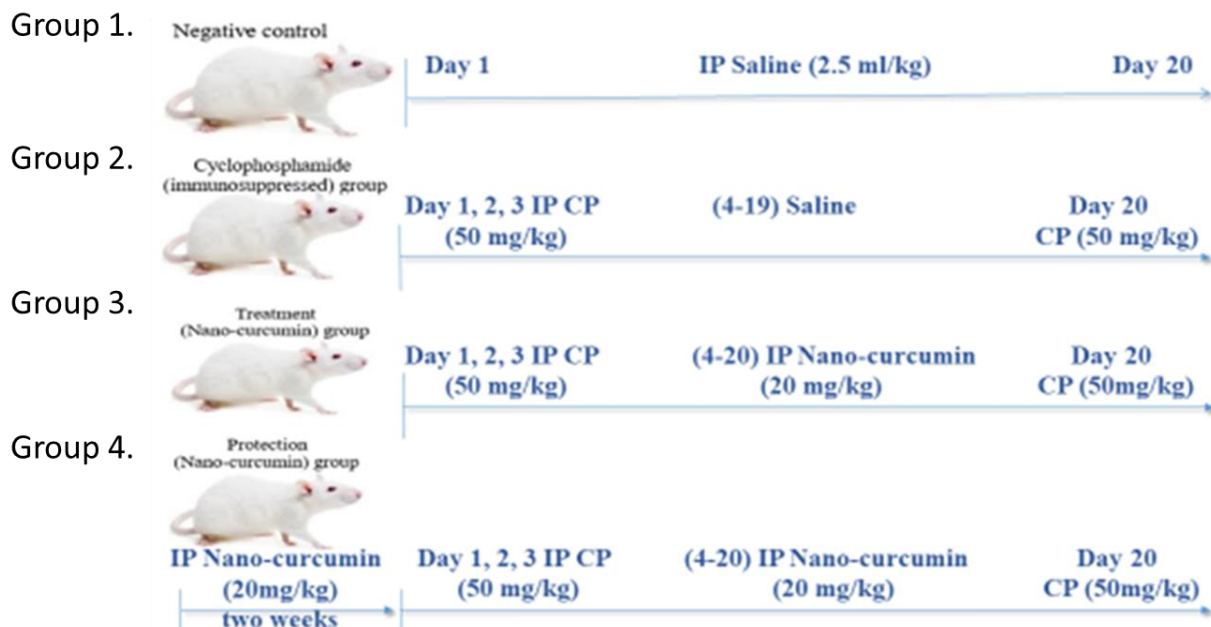


Figure 1: Experimental design of the investigation

The organs indices

The spleen and liver were gathered and weighed immediately to estimate indices of spleen and liver. The spleen and liver indices were calculated according to the following formula [20,21]:

$$\text{Organ index (\%)} = (\text{weight of organ} \times 100) / \text{body weight}$$

Serum IgG, IgM

The concentrations of IgG and IgM were determined using ELISA kits from Cusabio Biotech Co, (Wuhan, China) as instructed by the manufacturer. All samples were assayed in duplicates.

Macrophage phagocytosis assay

To measure the macrophage phagocytosis assay, the Ficoll-Histopaque density gradient technique was used to isolate white blood cells from heparinized blood [22]. Briefly, the heparinized blood was diluted to 50% with heparinized phosphate buffered saline (PBS), added on top of Histopaque 1077 and centrifuged at 2400 rpm for 30 min. Phagocytes were collected from the middle layer, diluted with heparinized PBS, then centrifuged at 2400 rpm for 10 min at 4 °C to collect the pellet. After washing with heparinized PBS, Rose well park memorial institute 1640, (RPMI-1640, Sigma-

Aldrich, USA) media containing 1% of fetal calf serum (FCS) was added to the pellet. *C. albicans* was added to leukocytes suspension then incubated at 37°C for 30 minutes in CO₂ incubator. The leukocytes suspension was centrifuged at 2500 rpm for 5 minutes and the smears were prepared from the deposit, dried in air and stained with Leishman's stain. A total number of 100 phagocytic cells were counted randomly in about ten microscopic fields. The phagocytic rate was estimated by counting the number of macrophages phagocytosing *C. albicans* in a population of 100 macrophages. The phagocytic index was estimated by counting the number of phagocytosed *C. albicans* per 100 macrophages [23].

Real-time PCR analysis

Total RNA was extracted from spleen and hepatic tissues (40 mg) using PureLink® RNA Kit for animal tissue (Thermo Scientific, USA) following the rules of the manufacturer. Amount and purity of RNA were established using

NanoDrop® ND-1000 Spectrophotometer. Reverse transcription in a total volume of 20 µL using high capacity cDNA reverse transcription kit (Thermo Scientific, USA) as designated by the manufacturer was performed. cDNA products were subjected to quantitative real-time PCR (qRT-PCR) analysis and was performed in a Mx3005P Real-Time PCR System (Agilent Stratagene, USA) with 5x HOT FIRE Pol EvaGreen qPCR Mix Plus (Solis BioDyne, Tartu, Estonia) following the manufacturer's instructions using primers for the different genes of interest as shown in Table 1. The PCR cycling conditions included an initial denaturation at 95 °C for 12 min, followed by 40 cycles of denaturation at 95 °C for 15 s, annealing at 55 °C for 30 s, and extension at 72 °C for 30 s [20, 21]. The comparative rates of target gene expression were normalized to the *Gapdh* gene. The relative expression was expressed as fold change by $2^{-\Delta\Delta CT}$ relative to control [24].

Table 1: Oligonucleotide primer sequences used for real time PCR.

Gene	Forward primer (5'-3')	Reverse primer (5'-3')	Accession No	Product size (bp)	References
GAPDH	GGCACAGTCAAGGCTGAGAATG	ATGGTGGTGAAGACGCCAGTA	NM_017008.4	143	[25]
TNF-α	AGGGTCTGGGCCATAGAAC	CCACCACGCTCTTCTGTCTAC	NM_012675.3	103	[21]
IL-1β	CACCTCTCAAGCAGAGCACAGA	ACGGGTTCATGGTGAAGTC	NM_031512.2	81	[26]
IL-10	GTAGAAGTGATGCCCCAGGC	AGAAATCGATGACAGCGTCG	NM_012854.2	116	[26]

GAPDH; Glyceraldehyde 3-phosphate dehydrogenase, IL; interleukin, TNF-α; Tumor necrosis factor alpha

Estimation of DNA fragmentation

Small parts of liver tissue were taken, weighted and kept frozen in -80°C until the evaluation of DNA fragmentation by agarose gel electrophoresis. DNA was extracted from hepatic tissue using QIAamp® DNA Mini kit (Qiagen Company, Germany) for animal tissue by the guidelines of the manufacturer. Quantity and quality of the DNA was tested for purity using NanoDrop® ND-1000 Spectrophotometer (Thermos Scientific, USA). DNA pure samples with purity more than 1.8 were injected into a 1.0% (W/V) agarose gel. Electrophoresis separated the fragments of DNA at 50 V for 3 h at 4°C in Tris Acetate EDTA (TAE) buffer. The presence of DNA fragmentation was detected by the appearance of a ladder of oligonucleosomal

DNA fragments on the agarose gel. Using ethidium bromide, the DNA was interpreted and photographed using a digital camera.

Statistical analysis

All results were statistically analyzed by SPSS version 24 and then subjected to one-way analysis of variance (ANOVA), followed by Duncan's multiple comparisons. The results were expressed as mean ± standard error of mean (Mean ± SEM), and P < 0.05 was considered a significant difference.

Results

Effect of nanocurcumin on organ indices

The organ indices represent the immune function of the organ and the prognosis of the immune system. Splenic and hepatic somatic indices were studied on the day of sacrifice in

CP immunosuppressed rats and the results are shown in Figure 2 (A&B). The icnd hepatic somatic indices in the CP immunosuppressed group resulted in significant reduction in comparison with the control group ($P < 0.001$). Nanocurcumin treatment and protection approaches induced a significant increase ($P < 0.001$) in splenic (119.83 and 128.61%, respectively) and hepatic (104.95 and 109.74%, respectively) somatic indices in comparison with the immunosuppressed group, while a higher ameliorative impact was observed in the protective group (G3).

Effect of nanocurcumin on immunoglobulin IgG and IgM

The concentrations of IgG and IgM in the sera of CP immunosuppressed rats were calculated to estimate the effects of nanocurcumin on humoral immunity. As shown in Figure 2 (C & D), CP immunosuppression significantly ($P < 0.001$) reduced serum IgG and IgM levels, in comparison with the control group. However, significant boost ($P < 0.001$) in total serum IgG (148.85 and 197.62%, respectively) and IgM (144.09 and 211.51%, respectively) levels were observed in rats treated (G4) and protected (G3) with nanocurcumin in comparison with the CP immunosuppressed rats. A higher ameliorative effect was observed in the protective group.

Effect of nanocurcumin on macrophage phagocytosis

Macrophage phagocytosis capability, the most essential indices for assessing innate immune mechanism, was examined and shown in Figure 2 (E and F). A significant inhibition ($P < 0.001$) of macrophage phagocytosis was observed in CP immunosuppressed rats in comparison with the control phagocytosis ratio. However, in comparison with CP immunosuppressed group, the phagocytic activity of the treatment and protection with nanocurcumin groups was significantly ($P < 0.001$) enhanced (122.37 and 156.16%, respectively). In the protective group, a higher ameliorative effect was observed.

Effect of nanocurcumin on the relative gene expression of TNF- α , IL-1 β and IL-10 in spleen and hepatic tissue

The mRNA expression levels of TNF- α , IL-1 β and IL-10 were analyzed in spleen (Figure 3 - A, B and C) and hepatic tissues (Figure 3- D, E and F). In spleen tissues, the results indicated that the mRNA expression levels of TNF- α (1.86 ± 0.11), IL-1 β (1.7 ± 0.12) were up-regulated in the CP immunosuppressed rats as in comparison with control group ($P < 0.001$). The mRNA expression levels of TNF- α in the treated (0.3 ± 0.12) and protected (0.25 ± 0.18) groups and IL-1 β in the treated (1.26 ± 0.08) and protected (0.63 ± 0.14) groups were down-regulated in comparison with one immunosuppressed by CP ($P < 0.001$). The mRNA expression level of IL-10 was up-regulated (2.83, 3.69 and 5.2) in all groups in comparison with the control group ($P < 0.001$).

In hepatic tissues, the results indicated that the mRNA expression level of TNF- α (5.61 ± 0.29) was up-regulated in the CP immunosuppressed rats in comparison with the control group ($P < 0.001$). TNF- α level of mRNA expression in the treated (1.08 ± 0.19) and protected (2.60 ± 0.14) groups was down-regulated in comparison with the immunosuppressed one by CP ($P < 0.001$). Neither immunosuppression with CP nor nanocurcumin treatment or protection caused significant changes in IL-1 β level of mRNA expression in comparison with control ($P > 0.05$). Although CP immunosuppression did not induce significant down-regulation in the mRNA expression level of IL-10 (0.86 ± 0.14) (numeric downregulate exists) in comparison with the control group, both treated (1.46 ± 0.08) and protected (2.27 ± 0.12) groups with nanocurcumin induced significant up-regulation in the mRNA expression level of IL-10 in comparison with CP immunosuppressed group ($P < 0.001$).

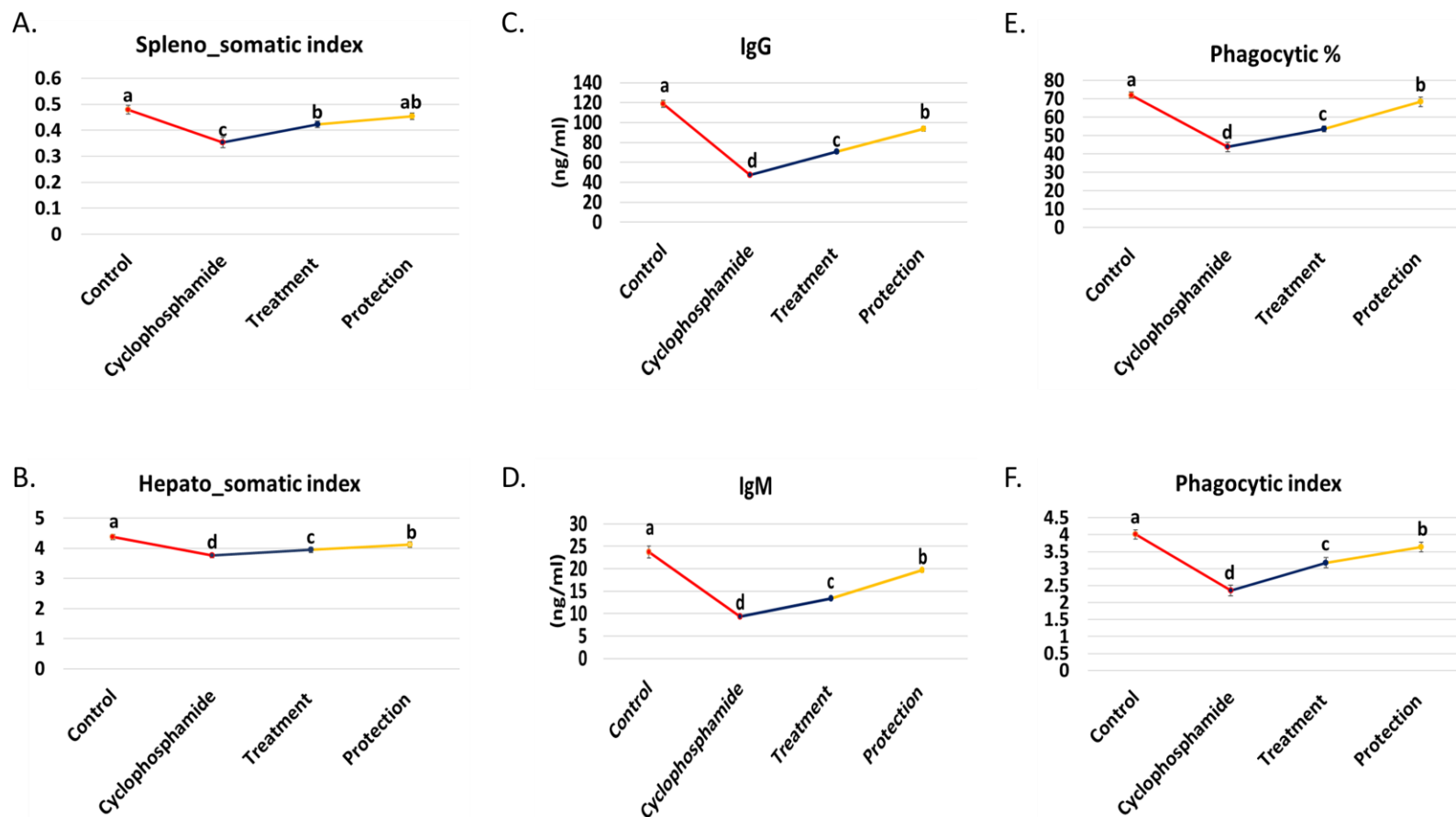


Figure 2: Spleno and hepato somatic indices and serum level of IgG, IgM and macrophage phagocytosis in immunosuppressed, treated or protected with nanocurcumin in rats. Values are mean \pm SEM of 8-10 animals per experimental group. Means with different superscript are statistically different according to Duncan's multiple range test at $P < 0.05$.

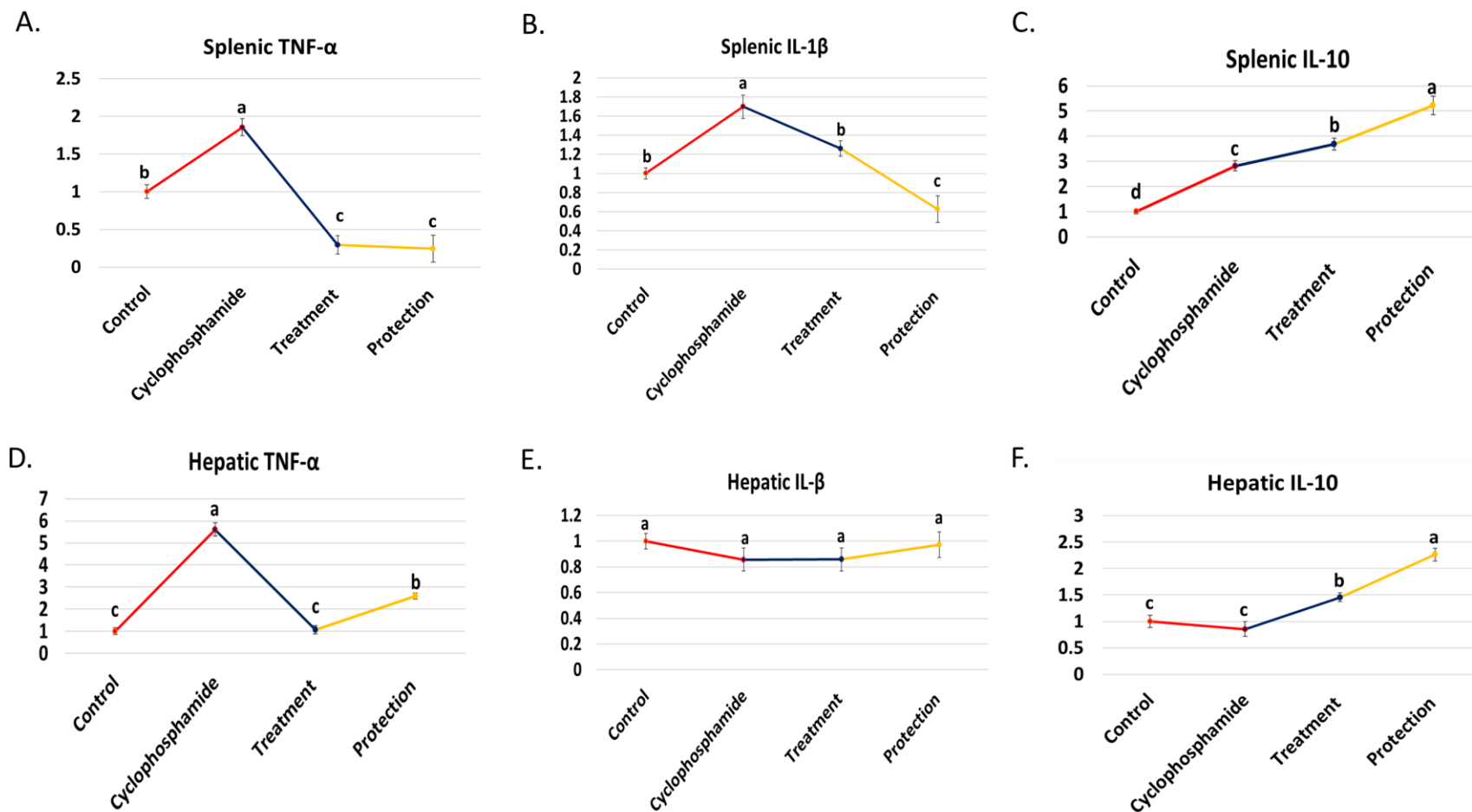


Figure 3: The mRNA expression levels of A) TNF- α , B) IL-1 β , C) IL-10 in spleen tissue, D) TNF- α , E) IL-1 β and F) IL-10 in hepatic tissue of immunosuppressed, treated or protected with nanocurcumin in rats. Values are mean \pm SEM. Means with different superscript are statistically different according to Duncan's multiple range test at P < 0.05.

Effects of nanocurcumin on genotoxicity

DNA damage was detected in the bands of Figure 4. The results showed that there was a significant DNA fragmentation in the CP immunosuppressed group compared with the

control one. There was no fragmentation in the treated and protected groups of nanocurcumin in comparison with the CP immunosuppressed group. Nanocurcumin pretreatment has restored the integrity of DNA in the liver.

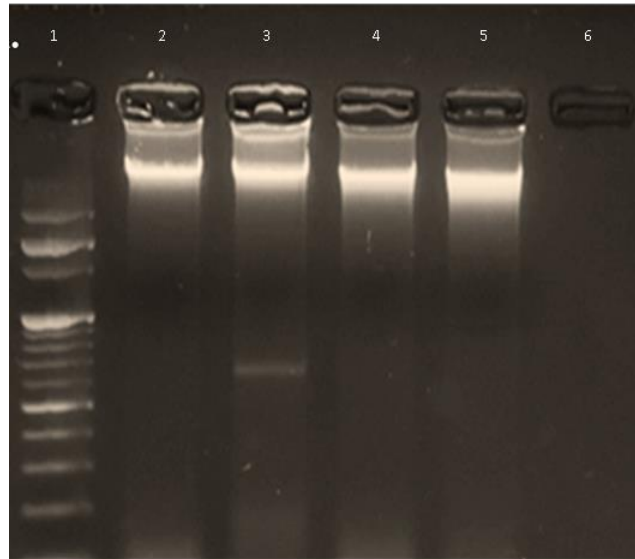


Figure 4: Gel electrophoresis of DNA obtained from rat liver. Lane1: ladder, Lane2: control group (G1), Lane 3: CP treated group (G2), Lane 4: nanocurcumin treatment group (G4), Lane 5: nanocurcumin protection group (G3), Lane 6: Negative control. CP injection in G2 introduced DNA fragmentation by appearance for DNA laddering when compared to control. Nucleosomal DNA was fragmented into oligonucleosome. No DNA laddering was noticed in treated and protected groups when compared to immunosuppressed one.

Discussion

The immune system defends the body against diseases and tumors by a highly specific response [27]. The function of the immune system and its significance in the treatment of many diseases is prominent during chemotherapy [28]. Using immunoenhancing agents to boost cell protection is thought to be one of the more effective lines to current drug treatment [29]. Cyclophosphamide is an effective anticancer agent used for cancer therapy; however, it is harmful to normal cells and induces adverse effects like myelo-depression and immune-assault that often puts lives at risk [30]. In this analysis, the immunosuppressed rats by CP were used as a compromised immune system animal template for evaluation of the immunomodulatory beneficial and protective effects of nanocurcumin against them by biochemical and molecular assays. It was

found that nanocurcumin had the ability to boost the host immunity.

Nanocurcumin significantly increased the immune organs indices, serum immunoglobulin levels (IgG and IgM) and phagocytic activity. It down-regulated the mRNA gene expression of IL-1 β and TNF- α . However, it upregulated the mRNA expression of IL-10 in spleen and liver tissues.

Spleen is a main immune organ in which lymphocytes differentiate, develop as well as inducing resistant responses. The condition of immune cells influences the immune role immediately, beside the disease resistance [31]. Liver is the first major organ to identify both toxic chemicals and drugs. Many medications have been documented to cause severe human hepatic damage [32]. In immunosuppressed group, spleen and liver shrunk and the size reduced as the immune role was weakened. The indices are therefore representative for the immune role and

diagnosis and treatment of the immune system. In treated plus protected groups, there were significant increase in the size and weight of spleen and liver, which indicate that nanocurcumin stimulate organs cells and overcome CP induced immunosuppression, boosting the immune system. Splenocyte replication plays a key role in the immune response, as lymphocyte division, that lead to the progression of B and T cells [33]. There was a notable reduction in liver index in CP-immunosuppressed mice and it was modulated by phytochemical polysaccharide [30].

Immunoglobulins are more specifically, secreted by B lymphocytes and are key components of humoral immunity. The main types of immunoglobulins are IgG and IgM. Such immunoglobulins have an essential role in eliminating pathogens by immediately attaching to them, transforming them into new proper phagocytic forms, and improve the destruction of pathogens by chemicals [34]. In this experiment, nanocurcumin administration caused marked elevation in the serum level of IgG and IgM in the CP immunosuppressed rats which mean that nanocurcumin able to improve humoral immune function in immune depressed rats. It was documented that CP decreased the synthesis of IgG and IgM immunoglobulins [3]. Also, serum IgG and IgM levels in a CP induced immunosuppression model were extremely decreased [30].

Macrophage, is the most essential phagocytes, performs an integral and crucial role in the innate protection of the host combating infectious diseases and environmental factors [35]. Phagocyte is an important contributor to the innate immune response, and this function is widely used to evaluate vertebrates' un-specific immune response [31]. Macrophages protect the body by the engulfing mechanism, throughout acting as antigen molecules to lymphocytes, and in production of different cytokines for example IL-6, IL-1 β and TNF- α [36]. In this experiment, nanocurcumin significantly improved the activities of the macrophage in CP immunosuppressed rats. Immunosuppression by CP remarkably

decreased the phagocytic activity by decreasing the production of nitric oxide (NO) level of macrophage [30]. Nano-curcumin had been improved phagocytosis by down regulation of pro-inflammatory cytokines like TNF- α and IL-1 β [16]. Jageita and Aggarwal [37], had indicated that curcumin increased the phagocytic activity of macrophages and the production of cytokines, which play key roles in regulating immune responses. These findings indicate that nanocurcumin has an immunomodulatory effect as reported by our current research.

Cytokines play a vital role in the cell activity of the immune system and participate in the maintenance and recovery of cell function by cooperation of lymphocytes, cells of inflammation, and cells of hematopoietic. The various cytokine forms of Th1 and Th2 cells with different roles are essential determinants of variations in cell functions [31].

TNF- α is cytokine of inflammation that is secreted from various types of cells, especially activated phagocytic and T cells [38]. IL-1 β produced by all cells with nucleation, mainly macrophages that recruit additional immune cells for inflammation [39]. IL-10 is a prototypic anti-inflammatory cytokine which works through several pathways to reduce inflammation, both within the periphery and the central nervous system and is one of the most potent anti-inflammatory cytokines [40]. The results in spleen tissue indicated that TNF- α and IL-1 β expression rates in the treatment and protection with nanocurcumin were down-regulated in comparison with immunosuppression by CP as previously reported [16]. The expression rate of the mRNA for IL-10 was up-regulated in all groups in comparison with the control group. In hepatic tissues, TNF- α level of mRNA expression in the treated and protected groups was down-regulated in comparison with the immunosuppressed group by CP. Neither immunosuppression with CP nor nanocurcumin treatment or protection caused significant changes in IL-1 β level of mRNA expression in comparison with control.

Akçay *et al.*, [41] revealed that drug induced liver injury is associated with increased production of inflammatory mediators produced by injured or immune cells induced infiltration of leukocytes into the site of injury [41]. Studies have demonstrated that reactive oxygen species (ROS) augment gene expression of inflammatory mediators and NF- κ B and increase production of TNF- α from Kupffer cells [42]. Nanocurcumin induced a significant up-regulation in the mRNA expression level of IL-10 in comparison with CP immunosuppressed group.

Tokaç *et al.*, [43] observed that curcumin treatment significantly decreased the hepatic TNF- α level in the bile duct ligation rats, indicating that curcumin might reduce the inflammation induced by biliary obstruction [43]. It was showed that curcumin inhibited the production of IL-8, IL-1 β and TNF- α induced by inflammatory stimuli in human peripheral blood monocytes and alveolar macrophages [44].

Cyclophosphamide induces healthy cells to become cytotoxic despite its effectiveness against cancer [45]. The effective metabolites of cyclophosphamide, acrolein and phosphoramidate mustard are related to the accumulation of reactive forms of oxygen, contributing to degradation of the DNA strand and rising altered DNA impact [46]. Curcumin has been shown to be an effective antioxidant, which has improved oxidative stress so that it has been shown to significantly reduce DNA damage. Forty-day curcumin therapy has been shown to significantly reduce DNA damage to lymphocytes and liver and kidney cells in bile duct ligation rats [43]. The results indicated that nanocurcumin has repaired the integrity of DNA in hepatic tissue, and no fragmentation was observed. Curcumin has protective effects against drugs which induce genotoxicity [47].

Conclusion

The obtained results showed that nanocurcumin boosts the immune system. Its polyphenol powerful component induced protection against immunosuppression triggered by CP in rat model by counteracting the adverse effects of cancer drugs.

Nanocurcumin is a strong natural agent with anti-inflammatory and immunomodulatory properties. It has improved therapeutic effectiveness with better solubility and bioavailability in the intended tissues. Our results provided experimental evidences for the medical use of nanocurcumin in immunosuppressed individuals and cancer fighters.

Acknowledgments

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Conflict of Interest

The authors declare no conflicts of interest, financial or otherwise.

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المخلص العربي

التأثير التحسيني لجزيئات الكركمين النانوية على تثبيط المناعة الناتج عن عقار السيكلوفوسفاميد في ذكور الفئران

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يعتبر الكركمين من اهم العناصر الفعالة بيولوجيا في جذور الكركم تم استخدامه في العديد من الثقافات الشعبية، واستخدم بشكل واسع في الطب التقليدي لعلاج مجموعة واسعة من الأمراض. أثبتت الدراسات والبحوث الاكلينيكية ان الكركمين له تأثير فعال وأمن في علاج الكثير من الأمراض مثل امراض المناعة الذاتية وامراض القلب والأعصاب والأورام واضطرابات الجهاز التنفسي والتمثيل الغذائي. ترجع الوظائف الوقائية المختلفة للكركمين إلى ثلاثة اسباب رئيسية حيث يعمل كمضاد للالتهابات والاكسدة ويعمل على تحسين الجهاز المناعي. هناك بعض العوامل التي تؤدي الى ضعف كفاءة الاستخدام الطبى للكركمين مثل قلة الذوبان، عدم البقاء لفترة طويلة في الخلايا، ضعف التفاعل البيولوجي وسرعة التمثيل الغذائي. تعتبر جزيئات الكركمين النانوية (Nanocurcumin) شكل محسن من الكركمين وذلك لزيادة خواصه الطبية حيث انه يحسن من توزيع الكركمين داخل الانسجة وامتصاصها له وتحسين توزيعه، والبقاء لمدة أطول داخل الخلايا. وبالتالي يزيد من مقاومته لعمليات التمثيل الغذائي، وتفاعله السريع مع الجهاز المناعي، وزيادة تأثيره العلاجي ضد العديد من الأمراض. يعد السيكلوفوسفاميد وسيلة للعلاج الكيميائي المستخدم لعلاج عدد كبير من الأورام السرطانية. يتم غالبا الحد من استخدام السيكلوفوسفاميد بسبب بعض اثاره الجانبية الضارة مثل الاكسدة وتثبيط الجهاز المناعي. الهدف من الدراسة الحالية تقييم التحسين المناعي والوقائي لجزيئات الكركمين النانوية على ذكور الجرذان البيضاء المثبطة مناعيا. تم تقسيم الجرذان الى أربع مجموعات (مجموعة ضابطة، مجموعة مثبطة مناعيا، مجموعة معالجة ومجموعة وقائية) واحتوت كل مجموعة على 10 من الجرذان. ظهرت سرعة الاستجابة والتحسين في كلا من المجموعتين المعالجة والوقائية بجزيئات الكركمين النانوية، وتبين ذلك بقدرة الطحال (128.61%, 119.83%) والكبد (109.74% and 104.95%) على القيام بالوظائف الحيوية ($P < 0.001$)، البروتين المناعي (IgG (197.62% and 148.85%) و IgM (211.51% and 144.09%) ($P < 0.001$), ظاهرة البلعمة لخلايا الدم وحيدة النواة المحيطية ($P < 0.001$) (156.16% and 122.37%) مقارنة بالجرذان المثبطة مناعيا. في خلايا الطحال ظهر ايضا لنفس المجموعتين انخفاض ملحوظ في التعبير الجيني للجينات الخاصة بالالتهابات مثل ($TNF-\alpha$) (13.23% and 15.91%)، وارتفاع ملحوظ في التعبير الجيني للجين الخاص بمضاد الالتهابات ($IL-10$) (184.68% and 130.59%; $P < 0.001$). اما في خلايا الكبد للمجموعتين المعالجة والوقائية وجد انخفاض ملحوظ في مستوى التعبير الجيني ل $TNF-\alpha$ (46.38% and 19.17%; $P < 0.001$) وعدم وجود أي تغيير ملحوظ في التعبير الجيني ل $IL-1\beta$ (100.12% and 103.42%; $P > 0.05$). كما ظهر ارتفاع ملحوظ في مستوى التعبير الجيني ل $IL-10$ (264.51% and 170.13%; $P < 0.001$). اظهرت المجموعة الوقائية بجزيئات الكركمين النانوية سرعة استجابة وتحسن عن المجموعة المعالجة. مما سبق نستنتج ان جزيئات الكركمين النانوية تلعب دورا كبيرا وهام في تقوية وظائف الجهاز المناعي) الاستجابة المناعية الفطرية، المناعة الخلية والخلاوية وخاصة عند العلاج من انواع السرطانات المختلفة، كما ان لها تأثير مهم في الحفاظ على المادة الوراثية DNA من العوامل الضارة المختلفة.