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Folic Acid Conjugated Graphene Oxide Graviola Nanoparticle for Sono-Photodynamic Leukemia Treatment: Up-To-Date Cancer Treatment Modality

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Abstract

Background: There is no doubt that one of the largest researcher dilemmas is cancer. Traditional therapeutic options such as chemotherapy, radiation, surgical and combinational treatment are widely accepted to cure or eradicate tumors. Despite chemotherapy being still a very potent weapon for cancer treatment, it is overwhelmingly associated with borders and serious side effects. There is consistently the probability of recurrence and these cancers can evolve resistance to chemotherapy and radiation treatments. Therefore, seeking a new therapeutic option of treatment is necessary to treat tumors accurately and prevent cancer metastasis. **Objective:** This work was directed at the evaluation of the efficacy of using activated Graviola nano-composite for leukemia targeted therapy. This work was conducted in vitro (two leukemia cell lines), and in vivo (90 leukemia induced mice). Laser and ultrasound were applied as an energy source. **Material and methods:** In this work, the biological effects of using activated Graviola nano-composite for leukemia targeted therapy were investigated through biophysical, biochemical, and hematological analysis. **Results:** The results revealed that Graviola nano-composite is a potential promising photo-sono sensitizer for cancer treatment and plays a critical role both in vitro and in vivo for inhibition and induction of cancer cell death. **Conclusion:** Our results revealed that activated Graviola nano-composite could be applied as a natural nano-sensitizer for sono-photodynamic therapy (SPDT) cancer targeting.

Keywords: leukemia, nano-Graviola, photodynamic therapy, sonodynamic therapy

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Introduction:

There is no doubt that one of the largest researcher dilemmas is cancer. Leukemia is one type of cancer that occurred due to bone marrow clonal disorder that originated during hematopoiesis and is characterized by the poorly white blood differentiated cell's unregulated proliferation. Based on the type and proliferation degree of affected cells, the disease is classified as myeloid or lymphoid and acute or chronic respectively [1]. The most prevalent type in adults and among pediatric patients is acute, myeloid, and lymphocytic leukemia (AML, ALL) respectively with incidence and mortality of (4.2, 1.7 cases) and (2.8, 0.4 cases)

respectively annually per 100,000 persons [2]. myeloproliferative disorder accounting for 15–20% of adults newly leukemia diagnosed cases is Chronic myeloid leukemia (CML) with incidence and mortality of 1.8 and 0.4 cases respectively annually per 100,000 persons [2.3].

Traditional therapeutic options such as chemotherapy, radiation, surgical and combinational treatment are widely accepted to cure or eradicate tumors. Despite chemotherapy being still a very potent weapon for cancer treatment, it is overwhelmingly associated with borders and serious side effects. There is consistently the probability of recurrence and these cancers can evolve resistance to chemotherapy and radiation treatments. Therefore, seeking a new therapeutic option of treatment is necessary to treat tumors accurately and prevent cancer metastasis. So to overcome the main obstacles involved in traditional cancer treatment, researchers are looking for effective treatments in alternative, complementary medicine and supplements [4,5].

Photodynamic therapy (PDT) treatment method has been established to treat different kinds of cancers. PDT involves a photo-sensitive drug intake and subsequently illuminating with light corresponding to the sensitizer absorbance wavelength of the target area. Factors that govern PDT cancer therapy, the photosensitizing agent localization in cancerous tissue, and an appropriate light dose delivered to that tissue [6-9].

Sonodynamic therapy (SDT) treatment method has been evolved from PDT to overcome light short infiltration deepness. SDT involves a sono-sensitive drug intake and the target area subsequently exposed to ultrasound. Using SDT enabled penetration of up to several tens of centimeters of tissue. Consequently, SDT put an end to PDT obstacles and major limitations [10-14].

Sono-photobiomodulation therapy (SPBMT) is a treatment method that includes the intake of photo- and sono- sensitive agents. PDT has been conducted separately away of SDT for years with variable success for cancer treatment. PDT has been directed for superficial cancer, while upon the combination with SDT, it showed effectiveness against leukemia, deep-seated, and metastatic tumors when spread to lung, liver, and bone in particular [15].

Annona muricata (Graviola) is a small erect evergreen, tropical fruit tree, growing 5–6m in height belonging to the family Annonaceae. Traditionally Graviola was employed in cancer treatment. Through altering cell metabolism the Graviola extract manifested promising selective tumorigenic inhibitory effect in vitro and in vivo [16].

The present work aims to study Graviola nano-composite role in leukemia treatment in vitro and in vivo. The following has been done:

Materials and methods:

Preparation of Graviola nano-composite: In current work, nano-Graviola was employed as SPS. Nano-Graviola was prepared according to Abd El-Kareem, et al; 2018 method [17]. To all treated leukemia cell lines and leukemia-induced mice groups, Graviola nano-composite was incubated or administered by intraperitoneal (ip) injection 9-12 hrs before exposure to PDT and/or SDT for two weeks.

Experimental protocol:

In vitro study: The present work involves two human leukemia cell lines. The two leukemia cell lines were maintained in RPMI supplemented the media with streptomycin, penicillin, and heat-inactivated fetal bovine serum (100mg/ml, 100 units/ml, and 10% respectively) at 37 °C in a 5% (v/v) CO_2 humidified atmosphere. The two leukemia cell lines were seeded on 96-well plates one day before treatment with different modalities and divided into the following groups: **Group I:** leukemia cell lines were maintained in a drug-free environment as untreated control. **Group II:** leukemia cell lines were treated with Graviola nano-composite only. **Group III:** leukemia cell lines were subjected to ultrasound. **Group IV:** leukemia cell lines were treated with Graviola nano-composite and subjected to the ultrasound as group III. **Group V:**

leukemia cell lines were subjected to a laser. **Group VI:** leukemia cell lines were treated with Graviola nano-composite and subjected to laser as group V. **Group VII:** leukemia cell lines were subjected to ultrasound followed by laser light. **Group VIII:** leukemia cell lines were treated with Graviola nano-composite, subjected to ultrasound, followed by laser as group VII.

In vivo study: The present work involved a total of 90 (20-25 gm) male and female Swiss Albino mice. The present study protocol was conducted following the ethical guidelines. **Group 1:** 10 mice received topical application of (0.1ml/mouse) thiophene-free benzene vehicle only twice weekly for 8 weeks and serve as a negative control. **Group 2:** 80 mice received topical application of (50µg 9,10-Dimethyl-1,2-Benzanthracene/0.1ml thiophene-free benzene/mouse) twice weekly for 8 weeks, leukemic mice were grouped into **Group 2a:** 10 leukemic mice were kept without any further treatment as a positive control. **Group 2b:** 10 leukemic mice were administered with Graviola nano-composite only. **Group 2c:** 10 leukemic mice were subjected to ultrasound. **Group 2d:** 10 leukemic mice were administered Graviola nano-composite, then subjected to the ultrasound as group 2c. **Group 2e:** 10 leukemic mice were subjected to laser light. **Group 2f:** 10 leukemic mice were administered Graviola nano-composite, then subjected to laser light as group 2e. **Group 2g:** 10 leukemic mice were subjected to ultrasound, followed by laser light. **Group 2h:** the leukemic 10 mice were administered Graviola nano-composite, then subjected to ultrasound followed by the laser as group 2g.

In vitro and in vivo Laser/ultrasound exposure and Graviola nano-composite activation: PDT was applied using red diode laser; model LAS-50 Germany and SDT were applied using an ultrasonic instrument; model F-801C China.

Characterizations of Graviola nano-composite: shape, size, absorbance, and photoluminescence using electron transmission and scanning microscope (TEM, SEM), X-ray diffraction and energy-dispersive X-ray (XRD, EDX scan), absorbance and photoluminescence (UV/Visible, PL scan) spectrometer, and Fourier transform infrared spectroscopy (FTIR scan).

Cytotoxicity and cell viability study: Graviola nano-composite effect on cell viability was evaluated through ab155902 WST-1 Abcam kit cell proliferation reagent (WST-1 assay). In 96-well plate cells, suspension aliquots (50 µl; 3x103 cells) were seeded and incubated in complete media for 24h, then 50 µl aliquots of drugs at serial concentration media containing cells were treated. At 450 nm after 1h absorbance was taken by Labtech-FLUOstar Omega BMG microplate reader after 24 h of drug exposure.

Hematological study: At the end of the experiment mice were sacrificed. Hematological parameters (a) complete blood count (CBC) [18]: blood was analyzed automatically by hematology analyzer for total red, white blood cell (RBCs, WBCs), hemoglobin concentration (Hb), hematocrit (HCT), RBC indices as mean corpuscular hemoglobin, concentration volume (MCH, MCHC, and MCV). (b) Differential leukocyte count (DLC): A blood film was prepared manually, blood films stained by Leishman staining technique, where the films were air-dried followed by flooding the slides with the stain for 2 minutes, then washed twice with water and then restain the films for 5-7 minutes. This is followed by washing with a water buffer till the films acquire a pinkish color; then the slides back were cleaned, and then upright laid to dry.

Biochemical analysis: from all experimental animals' blood, samples were withdrawn for obtaining sera for examinations. Malondialdehyde, antioxidant enzymes, hepatic and renal function tests were analyzed [19,20] in accordance with the manufacturer's kit instructions (BioVision Catalog # K739-100, #K274-100, #K761-100, #K263-100, #K335-100 and #K773-100, Sigma Catalog # MAK179, # MAK080, # MAK052, #MAK055, #MAK089 and #MAK089respectively) using Auto-

analyzer.

Molecular detection of [p53, Bax, Bcl-2, Caspase (3,9), NFkB, TNF alpha, and VEGF] RT-PCR expression: from the leukemic mice RNA was extraction and full-length cDNA preparation from RNA according to the manufacturer's instruction of iNtRON, Biotechnology, Inc. [21] The following were added in all PCR tube 5 µl Template p53, Bax, Bcl-2, Caspase (3,9), NFkB, TNFalpha, and VEGF-cDNAs, 10 µl RealMODTM Green W2 (2X) qRT-PCR Master Mix, iNTRON, Biotechnology, Inc., 1.5 µl forward and reverse primers and final volume 20 µl completed by RNase free deionized water. In all PCR tubes, the mixtures were vortexed gently, centrifuged briefly for all drops collection in tubes, then in PR0241401024 thermal cycler, all PCR tubes were placed. For verification of mRNA preparation +ve control Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as also -ve controls reaction tube without cDNA sample addition and containing no cDNA control template was included.

Statistical analysis: The data were processed using the SPSS program.

Results

Graviola nano-composite Characterization: Shape, size, absorbance, and photoluminescence were determined using SEM, TEM, X-ray diffraction and energy dispersive (XRD, EDX scan), UV/Visible scan spectrometer, PL scan spectrometer, and Fourier transform infrared spectroscopy (FTIR scan) (**Figure**: 1a-1h).

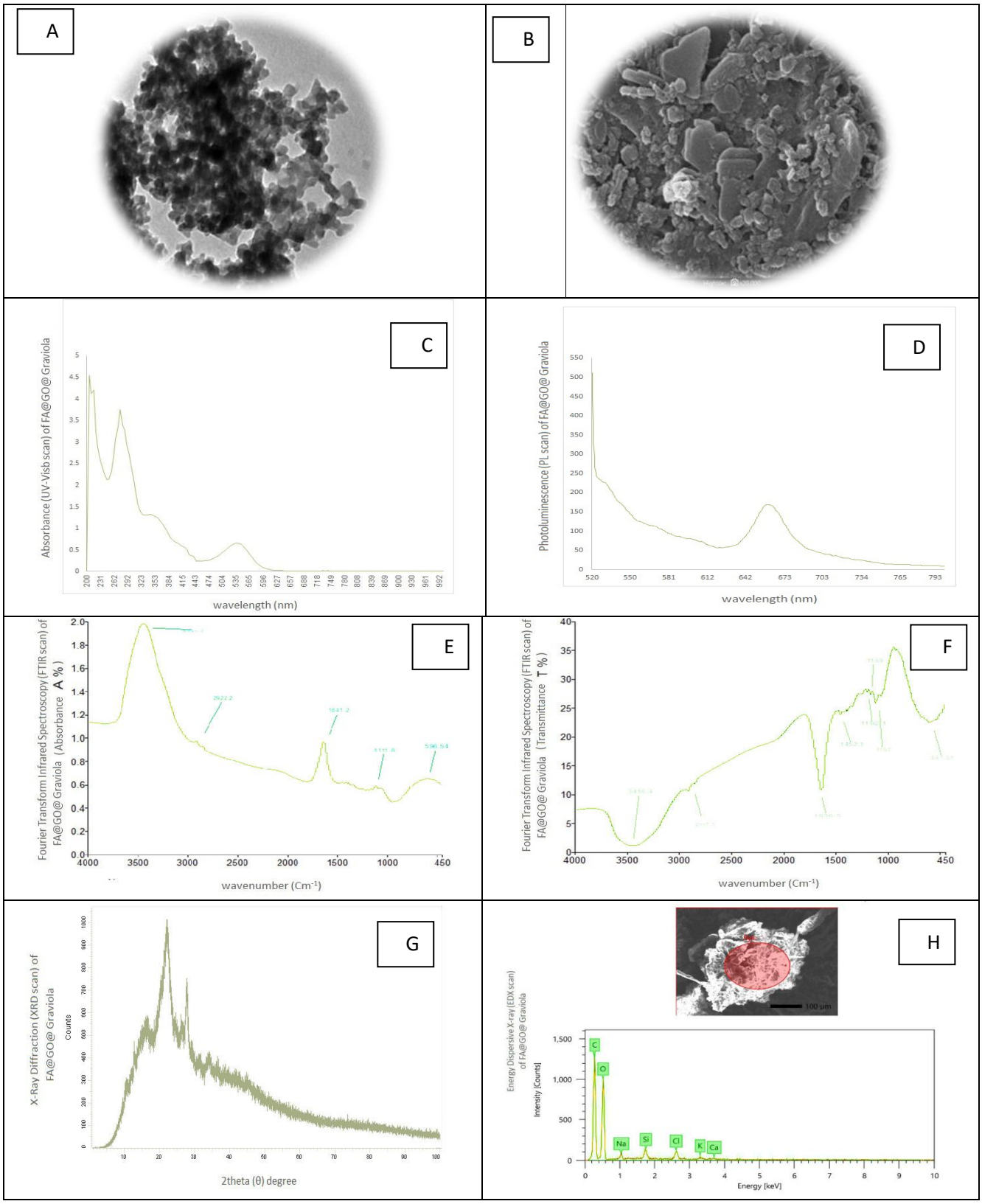


Figure (1): Graviola nano-composite; **A.**TEM, **B.** SEM, **C.** UV-vis spectra **D.** PL, **E.** FTIR absorbance, **F.** FTIR transmittance, **G.** XRD, **H.** EDX.

In vitro results.

In the present study cytotoxicity findings represented that after 24h incubation and treatment with Graviola nano-composite with no activation on the two human leukemia cell lines has slight or no effect. The use of laser and pulse or continuous US waves without Graviola nano-composite has little effect on the two human leukemia cell lines. The presence of Graviola nano-composite increases both the laser (PDT) and the US (SDT) effectiveness. The obtained results elucidated that puls US is more efficient than cont US in the presence and absence of Graviola nano-composite. Pulsed ultrasound waves were selected for integration with a red laser at high frequency. The combined therapeutic method (SPDT) is more efficient on the two human leukemia cell lines than using red laser or ultrasound alone (**Figure**: 2a,b).

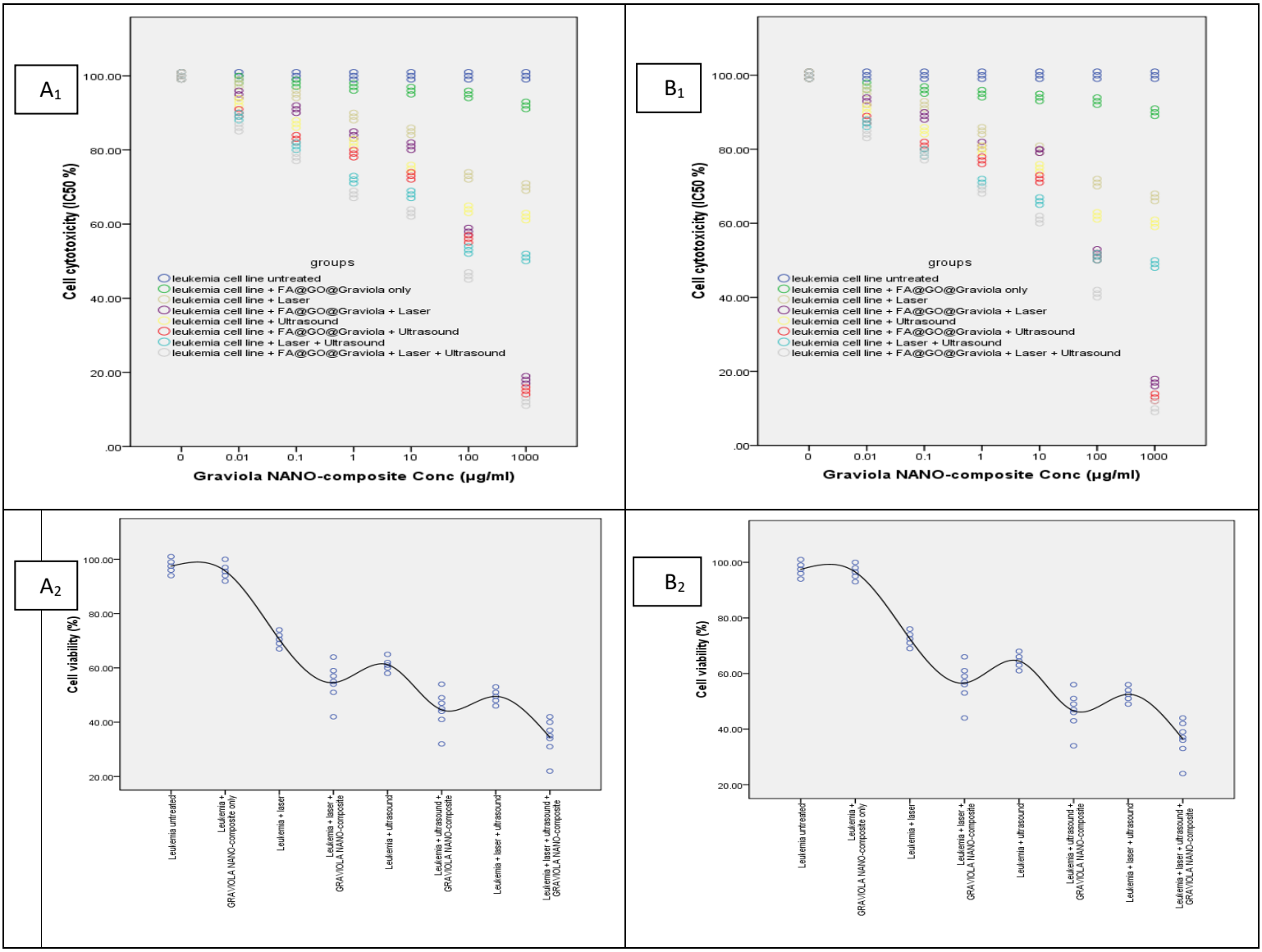
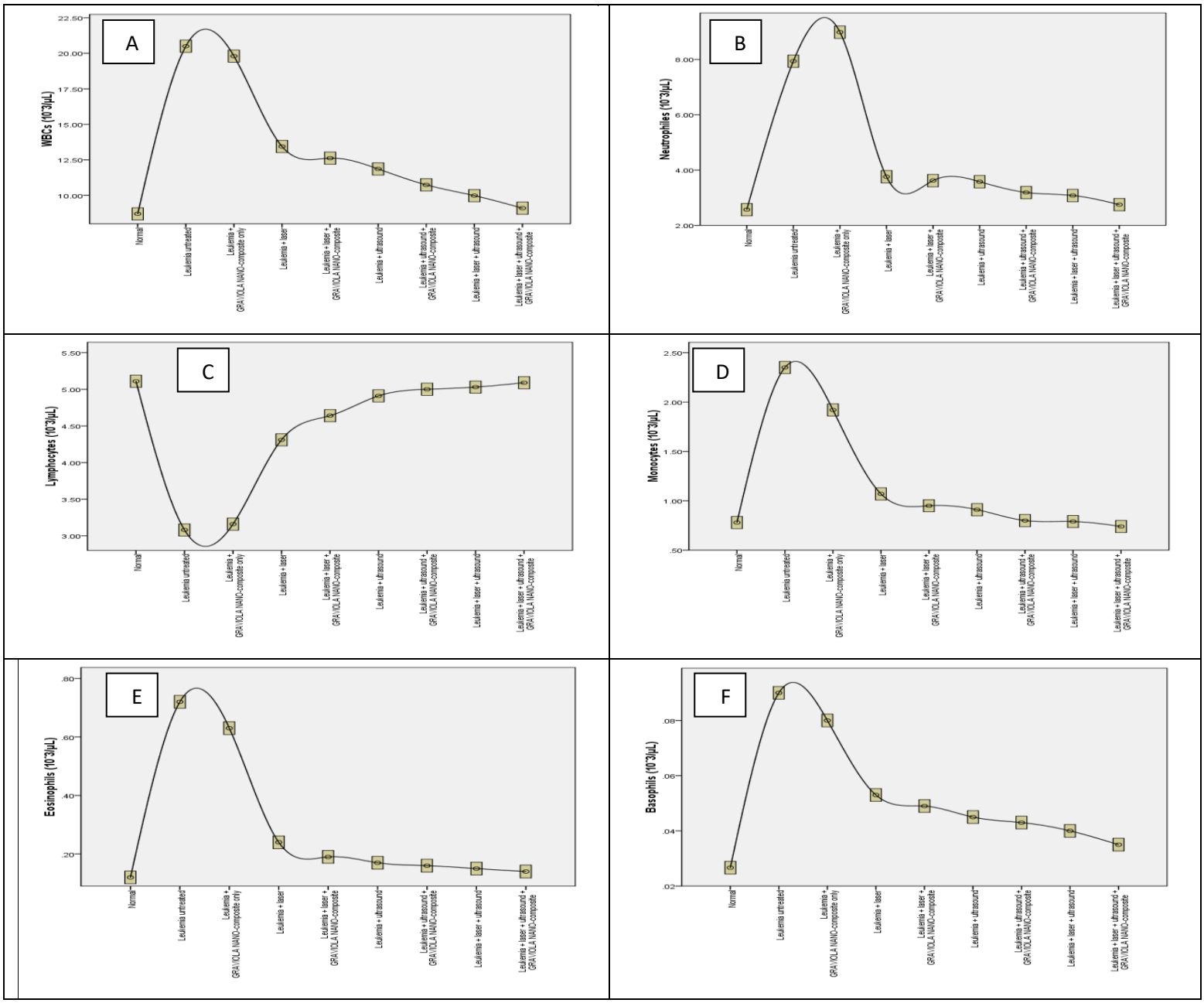


Figure (2): dose-response curve by WST-1 assay results to determine cytotoxicity, IC50, and cell viability value of Graviola nano-composite in the **two** leukemia cell lines treated with different modalities (leukemia untreated cell lines.

In vivo results;

In the current study compared with 9,10-Dimethyl-1,2-Benzanthracene/thiophene-free benzene -induced group, all treated groups manifested prominent decline of WBCs, neutrophils, eosinophils, monocytes, and basophil while a prominent elevation of RBCs, Hb, HCT, MCV, MCH, MCHC, platelet count and lymphocytes compared with the leukemia group. Also, they have shown restoration of normal differential WBCs count with normal RBCs in blood smear. Based on the previous results, it can be illustrated that the 9,10-Dimethyl-1,2-Benzanthracene leukemia-induction approach has been proven to be effective and successful in inducing leukemia. Moreover, our results emphasized the restoration of almost all the hematological parameters (blood analyses & differential leukocyte count) after treatment of 9,10-Dimethyl-1,2-Benzanthracene/thiophene-free benzene -induced leukemic mice with Graviola nano-composite in the presence of laser and/or ultrasound (pulsed/continuous US waves) and the combined therapeutic method is more efficient in the presence of Graviola nano-composite than using red laser or ultrasound alone (**Figure**: 3a-m).



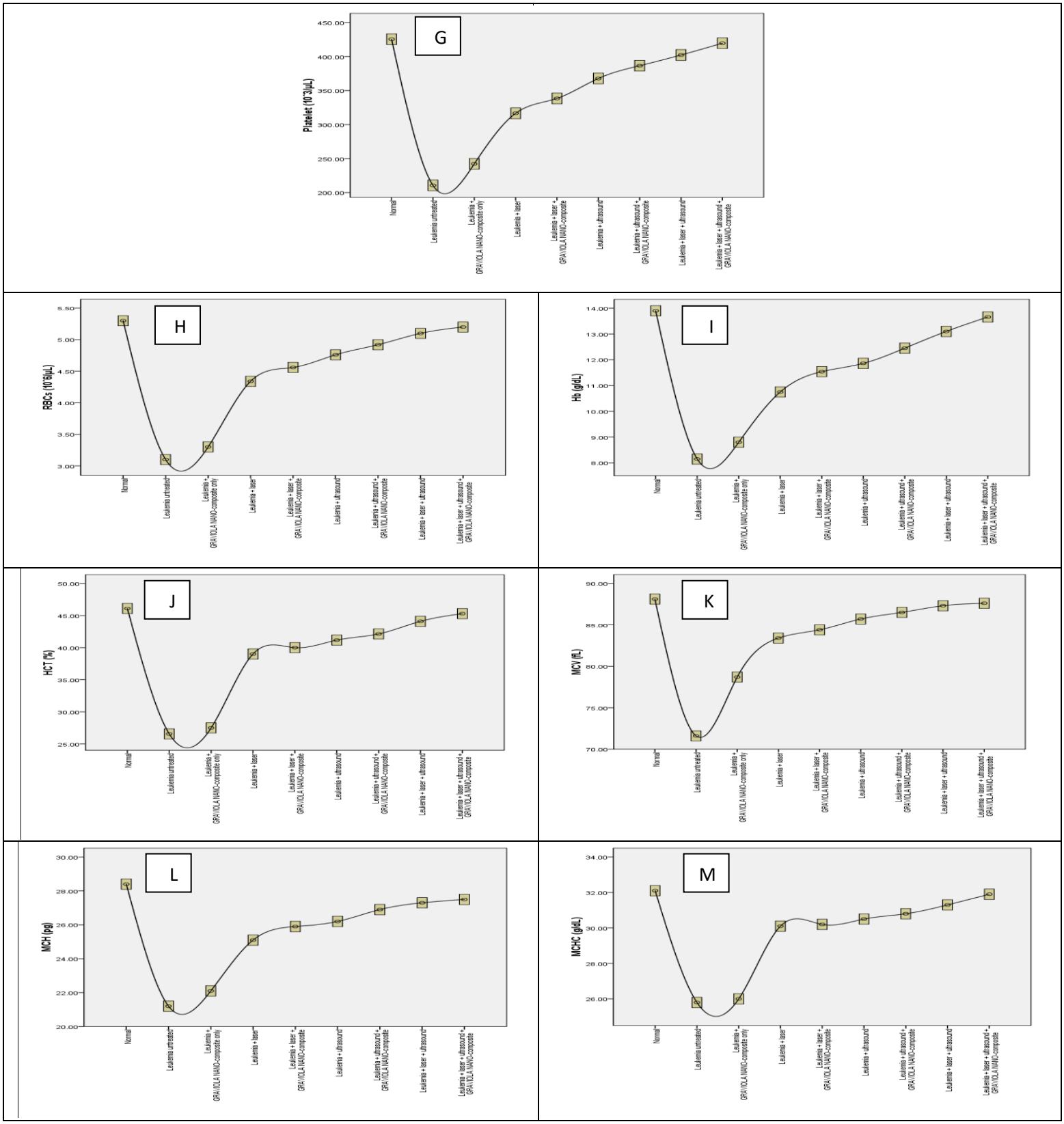


Figure (3): Complete blood count of all study groups: **A.** WBCs count (F=2545E3, p=0.001); differential leukocyte count **B.** neutrophils count (F=1910E3, p=0.001), **C.** lymphocytes count (F=2.428, p=0.001), **D.** monocytes count (F=33.027, p=0.001), **E.** eosinophils count (F=191.589, p=0.001), **F.** basophils count (F=8.096, p=0.001), **G.** platelet count (F=12.321, p=0.001), **H.** RBCs count (F=802.922, p=0.001); red blood cells indices as **I.** hemoglobin concentration (F=3.126, p=0.021), **J.** hematocrit ratio (F=61.023, p=0.001), **K.** mean corpuscular volume (MCV) (F=1.368, p=0.0275), **L.** mean corpuscular hemoglobin (MCH)(F=0.743, p=0.0654), **M.** mean corpuscular hemoglobin concentration (MCHC)(F=0.968, p=0.0490).

Oxidative Stress and Antioxidant Status:

An increase in MDA; a lipid peroxidation marker was reported in the leukemia control group. In all radiation-exposed groups exposed to radiation and treated without Graviola nano-composite, a statistically significant elevation of MDA levels was observed.

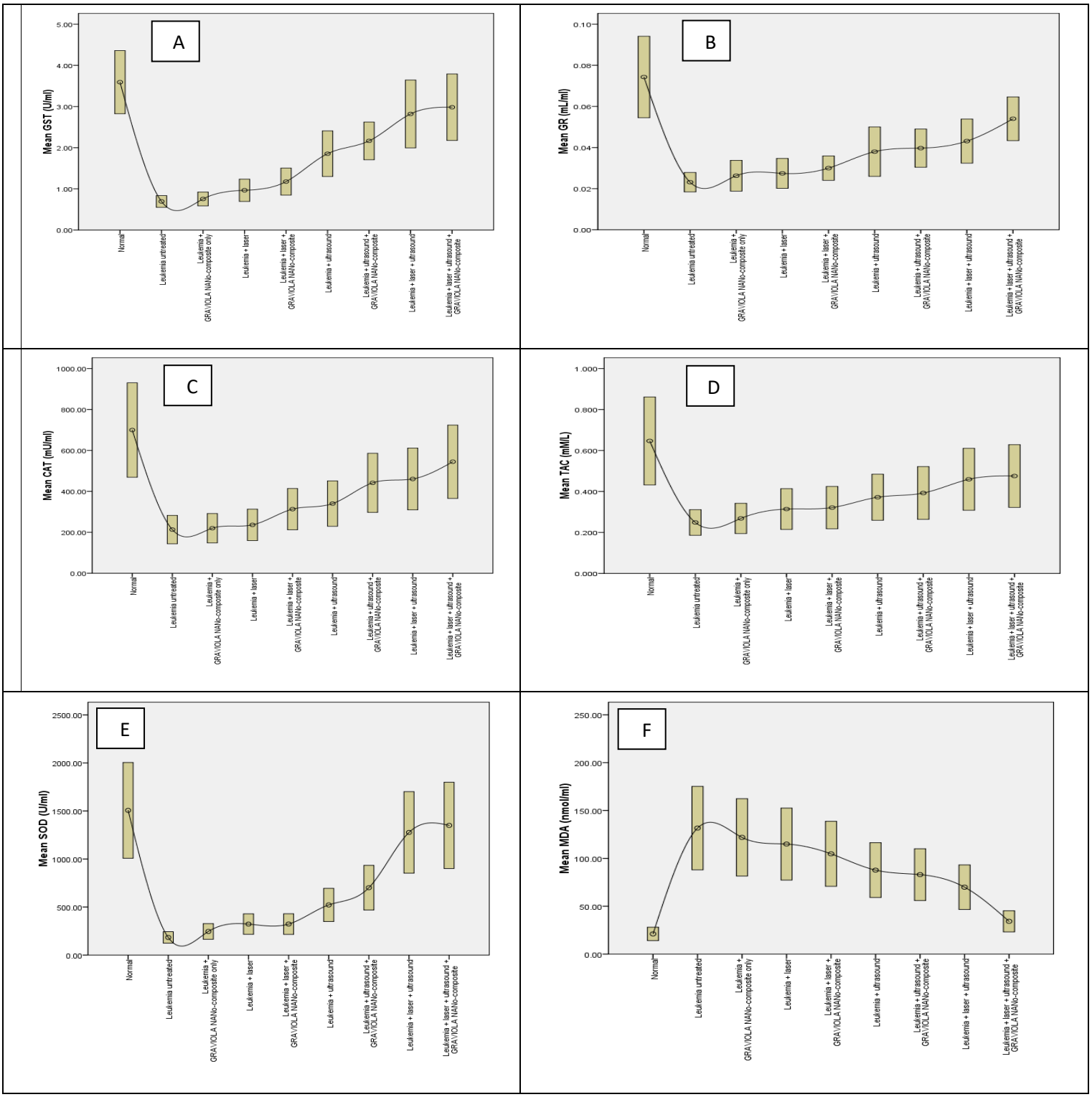
Animals in irradiated groups with red laser or US or both with Graviola nano-composite showed significantly lower levels of MDA, compared with the control group of cancer or with treated mice without activation of Graviola nano-composite, Figure (4.a.). At the same figure, leukemic mice showed decreased antioxidant activity (SOD, CAT, GR, GST, and TAC) compared with the normal control group.

Meanwhile, there is a statistically significant elevation in enzymatic and non-enzymatic antioxidants in irradiated groups with either red laser and/or the US both in with and without Graviola nano-composite compared to control group of cancer or with treated mice group without activation of Graviola nano-composite.

Kidney and Liver Function Tests:

Kidney function tests, namely: creatinine and urea are estimated. It was found that s renal function tests increased in all leukemic animal groups. Meanwhile, Graviola nano-composite caused low levels of creatinine in serum and urea, which may be indicative of renal protection. This also underlines the Graviola nano-composite renal protective role. Transaminases, alkaline phosphatase, and gamma-glutamyl transferase were also examined **in Figure** (4.a.). Leukemic results in significant elevation of serum activity of transaminases, alkaline phosphatase, and gamma-glutamyl transferase in tumor-bearing groups. However, in leukemia-treated groups with Graviola nano-composite, a decrease in serum levels of transaminases, ALP, and GGT were observed, indicating the hepatic protection by Graviola nano-composite, this also underlines the Graviola nano-composite hepatic protective role.

p53, Bax, Bcl-2, Caspase (3,9), NFkB, TNF alpha, and VEGF gene expressions: Amplification of p53, Bax, Bcl-2, Caspase (3,9), NFkB, TNF alpha, and VEGF gene expressions in leukemic treated and untreated animals using qRT-PCR is illustrated **Figure (4.b)**. p53, Bax, Bcl-2, Caspase (3,9), NFkB, TNF alpha, and VEGF amplification with different levels of expression with different studied modality.


A
Mean GST (U/ml)
5.00
4.00
3.00
2.00
1.00
0.00
B
Mean GR (mL/ml)
0.10
0.08
0.06
0.04
0.02
0.00
C
Mean CAT (mU/ml)
1000.00
800.00
600.00
400.00
200.00
0.00
D
Mean TAC (mM/L)
1.000
0.800
0.600
0.400
0.200
0.000
E
Mean SOD (U/ml)
2500.00
2000.00
1500.00
1000.00
500.00
0.00
F
Mean MDA (nmol/ml)
250.00
200.00
150.00
100.00
50.00
0.00
Normal
Leukemia untreated
Leukemia + GRA\IOLA NANo-composite only
Leukemia + laser
Leukemia + laser + GRA\IOLA NANo-composite
Leukemia + ultrasound
Leukemia + ultrasound + GRA\IOLA NANo-composite
Leukemia + laser + ultrasound
Leukemia + laser + ultrasound + GRA\IOLA NANo-composite

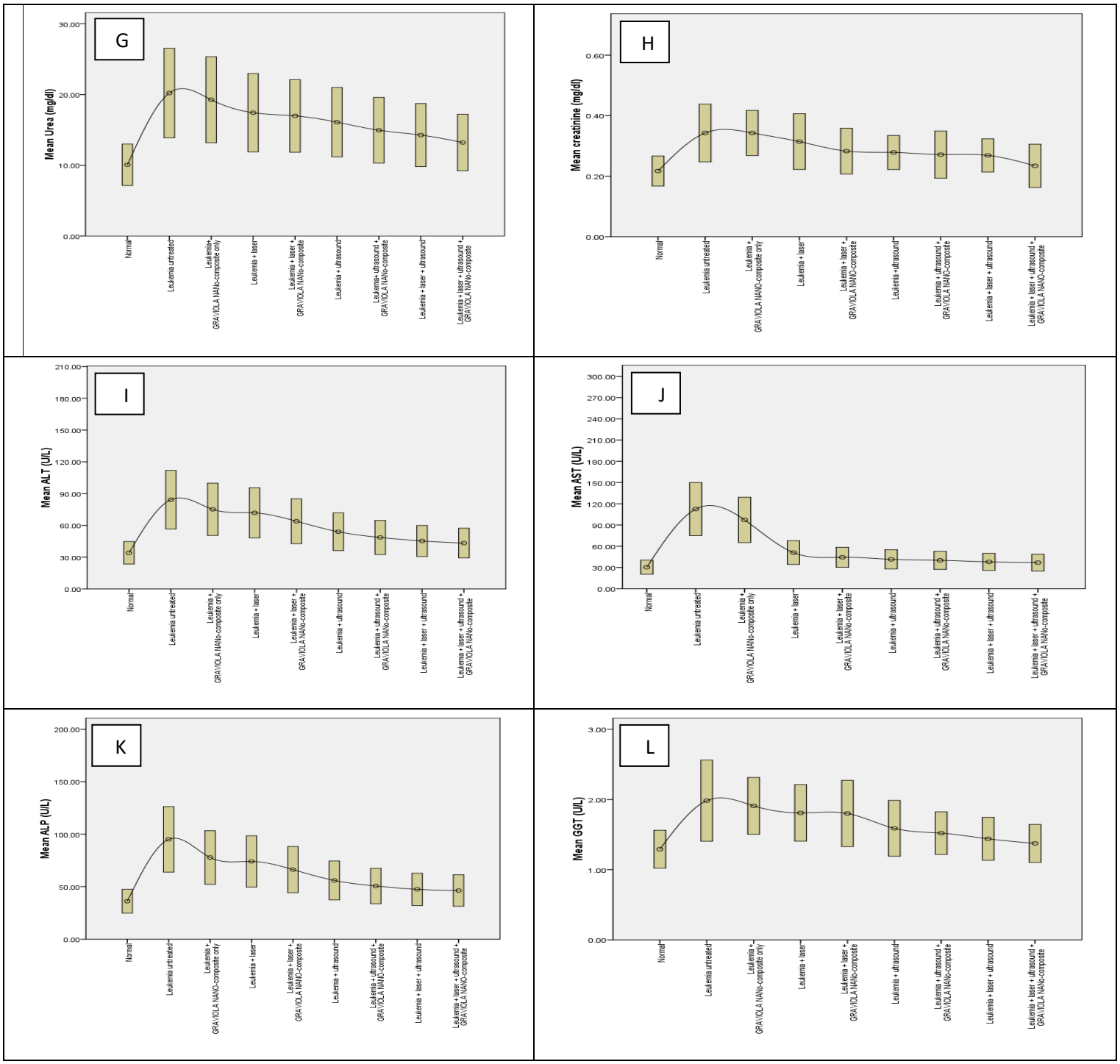


Figure (4.a): antioxidants activities, capacities, MDA, kidney and liver function tests of all study groups:**A.** GST (U/ml): 63.224 p<0.001*, **B.** GR (mU/ml): 6.211 p<0.001*, **C.** CAT (mU/ml): 1504.99 p<0.001*, **D.** TAC (mM/L): 585.337 p<0.001*, **E.** SOD (U/ml): 283.98 p<0.001*, **F.** MDA (nmol/ml): 1871.92 p<0.001*, **G.** Urea (mg/dl): 266.691 p<0.001*, **H.** Creatinine (mg/dl): 10.862 p<0.001*, **I.** ALT (U/l): 433.560 p<0.001*, **J.** AST (U/l): 1018.011 p<0.001*, **K.** ALP (U/l): 8386 p<0.001* and **L.** GGT (U/l): 4.862 p<0.001*).

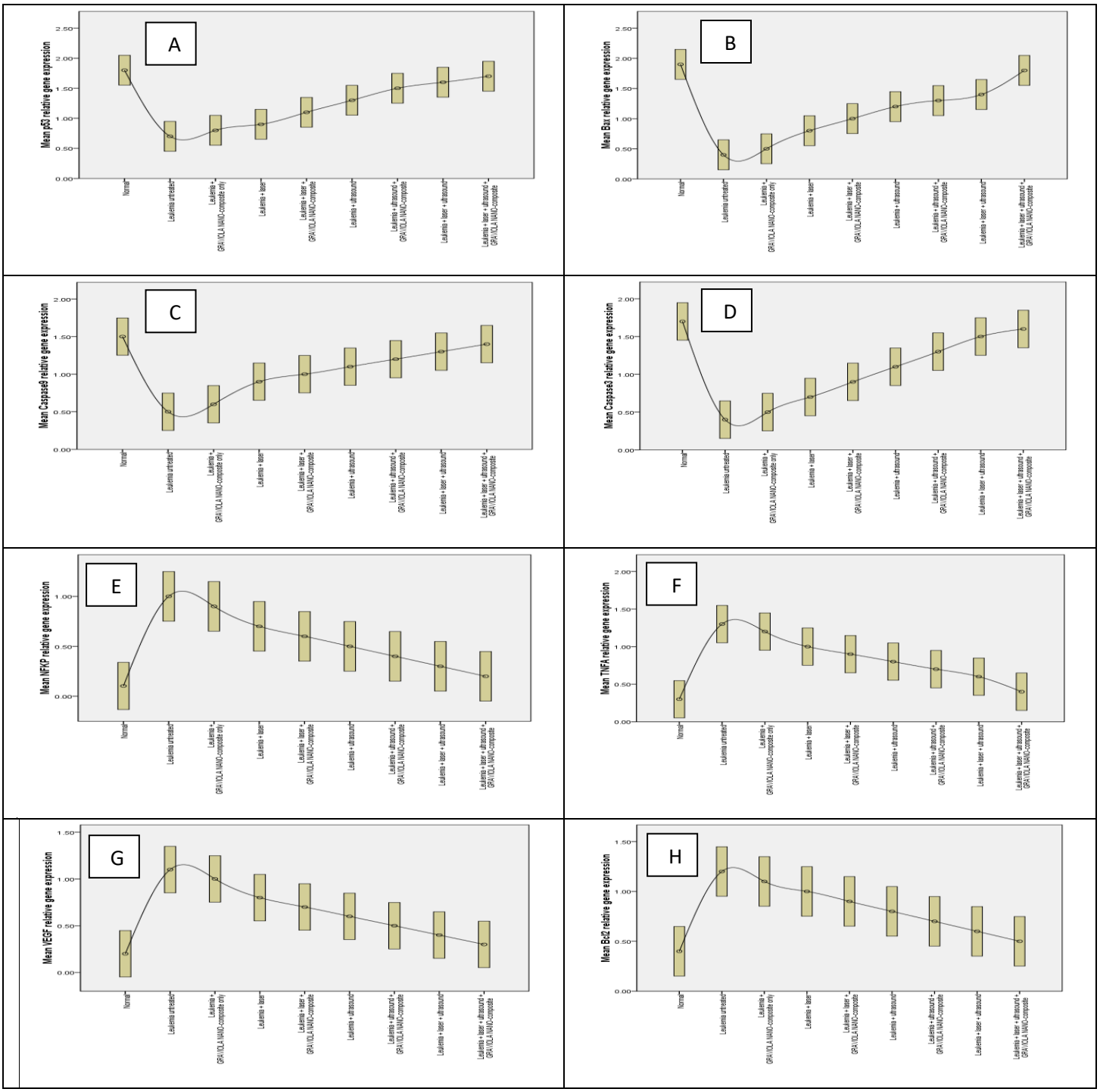


Figure (4.b): p53, Bax, Caspase (9,3), NFkB, TNFalpha, VEGF, Bcl-2 qRT-PCR relative gene expression of all study groups: **A.** p53: 50.250 p<0.001*, **B.** Bax: 82.583 p<0.001*, **C.** Caspase 9: 38.333 p<0.001*, **D.** Caspase 3: 69.583 p<0.001*, **E.** NFkB: 28.331 p<0.001*, **F.** TNFalpha: 34.500 p<0.001*, **G.** VEGF: 28.333 p<0.001*, **H.** Bcl-2: 22.500 p<0.001*.

Discussion

In the present manuscript, Graviola nano-composite was demonstrated as a sensitizer for both red laser and ultrasound and to determine whether photodynamic therapy and sonodynamic therapy alone or together can be managed safely, providing an increasingly toxic response to carcinogenic cells locally also offer a promising way for cancer eradication.

The data presented here show that Treatment with Graviola nano-composite without activation has a slight effect on the two human leukemia cell lines in vitro and in vivo models of 9,10-Dimethyl-1,2-Benzanthracene mice treated with laser and pulse or continuous US waves without Graviola nano-composite has little effect on the two human leukemia cell lines and in vivo models of 9,10-Dimethyl-1,2-Benzanthracene mice. The presence of Graviola nano-composite increases both the laser and US effectiveness. Moreover, pulsed/continuous US wave is more efficient than laser with Graviola nano-composite. The combined therapeutic method is more efficient on the two human leukemia cell lines and in vivo models of 9,10-Dimethyl-1,2-Benzanthracene mice than using IRL or US alone.

The data presented here showed the successful leukemia-induction using (50µg 9,10-Dimethyl-1,2-Benzanthracene/0.1ml thiophene-free benzene/mouse twice weekly for 8 weeks) associated with marked anemia and leukocytosis compared with the normal control group. Further, the restoration of deteriorated hematological parameter results in all treated groups compared with the leukemia group were observed and the potential treatment efficacy of Graviola nano-composite against 9,10-Dimethyl-1,2-Benzanthracene-induced leukemia. Obtained results elucidated that puls continuous US is more efficient than the laser in the presence of Graviola nano-composite and the combined therapeutic method is the most efficient in presence of Graviola nano-composite on 9,10-Dimethyl-1,2-Benzanthracene/thiophene-free benzene-induced leukemia mice.

The evaluation of MDA is used as an oxidative stress marker, indicating the role that lipid peroxide plays in cancer development. Free radical oxidizes polyunsaturated lipids, resulting in MDA, one of the reactive electrophile species, causing cells toxic stress [22,23]. The probable cause of MDA elevated level in cancer could be a deteriorated oxidant-antioxidant balance system that results in an accumulation of cancerous tissues with lipid peroxides followed by secretion into the bloodstream [24]. Highly cytotoxic MDA is the polyunsaturated lipids oxidation final product.

MDA is reported to inhibit protective enzymes, thus, it can have a tumorigenic impact. In this work, MDA elevation in the untreated leukemia control group was reported. MDA levels increase in leukemic animal groups when compared to the normal untreated group. Oxidative stress Inhibition by Graviola nano-composite is mainly due to trapping of ROS causing peroxidation [19-20,25].

Animal groups administered Graviola nano-composite as a treatment showed a significantly MDA lower level compared with non-nano-treated Graviola nano-composite groups. These are achieved by anti-lipid peroxidation characteristic of Graviola nano-composite through searching for and scavenging free radical generated. Prevention of cellular destruction by ROS results from the defense anti-oxidant system.

The defensive antioxidant system can reveal ROS, which is of great significant value in oxidative stress initiation and MDA formation, thus acting as a defensive part in cancer. Defense system function via non-enzymatic components (mainly GSH) and enzymatic (including GPx, SOD, CAT, and GST). SOD is the defense mechanism primary step involving the oxidant-antioxidant system resisting oxidative stress, catalyzing superoxide anion (O_2) breaks down into H_2O_2 and O_2. H_2O_2 can be deleted and converted into harmless byproducts by Gpx and catalase, thus allowing protection against ROS [26-28].

GPX is highly effective in free radicals

neutralization reacting in ROS stress response to also peroxides and hydroxide detoxification that lead to GSH oxidation. Also, GST catalyzes the association of GSH thiol functional groups with electrolytic xeobiotics, eliminating or converting the xenobiotic-GSH conjugate. In this interaction, glutathione is converted to oxidized form GSSG and reassembled with NADPH consumption by GR. The non-enzymatic antioxidant inside a cell is GSH. GSH is involved in lots of cellular pathways, including internal and external compound detoxification, and conserves cells against the adverse effects of oxidation efficiently by scavenging, removing free radicals and/or H_2O_2, and lipid peroxidation suppressing [29-32].

In this work, studied leukemia groups evoked low antioxidant activities. Current data is in agreement with reported results [18,33-39]. It was reported that this subsequent reduction of antioxidants because of low expression of these defense systems during cancer. Meanwhile, there was a significant enzymatic antioxidant and non-enzymatic increase in custody in leukemic animals subjected to Graviola nano-composite, red laser, and/or the US. [33]

This increase because of the competence of Graviola nano-composite to hinder the synthesis of reactive free radicals, promote the activity of the endogenous defense antioxidant system beyond its ROS trapping moiety and enhance the lowering of leukemia oxidative stress [36]. The elevation of antioxidant enzyme activity in Graviola nano-composite treated mice indicates its protective effect [34,39-40].

In this work, antioxidant activities are inversely related to MDA. The elevated level of MDA can be elucidated by an imbalance in the oxidant-antioxidant defense system and ROS lipid peroxide accumulation in the tumor. In addition, it was reported that decrease in TAC with a higher MDA level in the cancer group when compared to the normal untreated group [25,34,39].

Creatinine and urea, removed from the bloodstream by the kidneys, are metabolic byproducts to prohibit their accumulation. Elevations of all these indices indicate loss of kidney function [40]. The results of this study indicate that leukemia induction in all mice groups has caused kidney function loss compared to normal mice, which is inconsistent with other studies [16-17,39-40]. Vital indicators of kidney function, urea, and creatinine, have been estimated in our work. In the present work, Graviola nano-composite improves kidney function tests, as an indicator of affording protection to the kidney and evokes the Graviola nano-composite protective role [16,41]. The liver is the main organ concerned with the biological transformation of xenobiotic substances. Levels of bilirubin and liver transaminases, ALP, and transferase enzymes consider a reliable marker of liver toxicity [42].

Increased transaminases due to leakage from liver-destructed cells (liver injury). Alkaline phosphatase and transferase enzyme are linked to the cellular membrane. Elevations of ALP and transferase enzyme activities manifest conditions of liver injury [42-46]. Vital indications of liver function, liver transaminases, the ALP, and transferase were taken in our work.

In this work, implanted mice groups, with leukemia significantly increased the activity of serum transaminases, ALP, and transferase. Transaminases are primarily localized in hepatic cell cytoplasm and mitochondria [16-17,47]. In this work, Graviola nano-composite therapy was treated against elevated levels of serum AST, ALT, ALP, and GGT, an indicator of liver protection by Graviola nano-composite.

In current work, the molecular study of p53, Bax, Bcl-2, Caspase (3,9), NFkB, TNF alpha, and VEGF gene expressions as markers for leukemia treatment and inhibition of angiogenesis; manifested a significant positive agreement between p53, caspase 3,9, Bax, NFkB and TNF alpha gene expressions and treatment modalities in the presence of Graviola nano-composite, on the other hand, a negative agreement between leukemia, development, and p53, caspase 3,9, Bax, NFkB and TNF alpha expressions in untreated leukemia group. p53, caspase 3,9, Bax, NFkB and TNF alpha gene

expressions significantly elevated in sono-photodynamic therapy with (Graviola nano-composite) exposed mice groups than exposed to PDT-modality or SDT-modality only with (Graviola nano-composite) alone followed by laser-modality or US-modality only without (Graviola nano-composite) alone and expression was lowest among the leukemia untreated group.

Meanwhile, a negative significant agreement was between Bcl-2 and VEGF expressions and treatment modalities in the presence of sensitizer while a positive agreement was between leukemia development and expressions of Bcl-2 and VEGF in the untreated leukemia group. Bcl-2 and VEGF gene expressions significantly lower in mice groups treated with sono-photodynamic therapy with (Graviola nano-composite)] than those treated with PDT- modality or SDT-modality only with (Graviola nano-composite) alone followed by laser-modality or US-modality only without (Graviola nano-composite) alone and expression was highest among leukemia untreated group.

The current study indicated that p53, Bax, Bcl-2, Caspase (3,9), NFkB, TNF alpha, and VEGF gene expressions using RT-PCR a good predictor of cancer treatment and inhibition of angiogenesis and was inconsistent with other studies done by other authors [50-56] In the end, the application of photo- and sono- activated phytochemical nanoparticle (Graviola nano-composite) as in vivo anti-tumor therapy open up a new way of research in cancer treatment.

Finally, it can be elucidating the potential of Graviola nano-composite for sono-photodynamic leukemia treatment in vitro and in vivo. Also, the application of nanoparticles enables the great potential for effective drug delivery, allows targeted treatment, improves and amplifies the response to sono-phototherapy.

Conclusion

The current work results displayed that the application of the sono-photodynamic method, which uses ultrasound and laser exposure in the presence of Graviola nano-composite to treat leukemia in vitro and in the Vivo experimental model a promising trend for the treatment of cancer.

Recommendation

Current work offered new trends for the treatment of cancer that require furthermore investigations. More experimental study protocols need with more investigations to be introduced to conduct this promising modern method to humans safely and to monitor other biophysical and biochemical indices change.

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