

## RESEARCH ARTICLE

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# Synergistic Anticancer Activity of WO<sub>3</sub>/Graphene Oxide Nanocomposites: Structural, Optical, and Mechanistic Investigation

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## Abstract

**Objective:** To synthesize tungsten oxide (WO<sub>3</sub>) and a WO<sub>3</sub>/graphene oxide (GO) nanocomposite (1:0.5 molar ratio) and investigate their physicochemical properties and anticancer activity against human colon cancer (HT-29) cells. **Methods:** WO<sub>3</sub> and WO<sub>3</sub>/GO (1:0.5) nanocomposites were synthesized using an ex situ hydrothermal method and characterized by XRD, Raman spectroscopy, FTIR, UV-Vis spectroscopy, PL spectroscopy, zeta potential analysis, FESEM, and EDS. Anticancer activity was assessed using MTT cytotoxicity assays, AO/EtBr staining, apoptosis analysis, and ROS generation assays. **Results:** The incorporation of GO improved crystallinity, particle dispersion, and charge separation while reducing the optical band gap. The WO<sub>3</sub>/GO nanocomposite exhibited the strongest inhibition of HT-29 cell proliferation compared with pristine WO<sub>3</sub> and GO. Enhanced anticancer activity was associated with increased cellular internalization, membrane disruption, and ROS generation. AO/EtBr staining and flow cytometry confirmed significant apoptosis induction, while ROS assays demonstrated elevated intracellular oxidative stress that may contribute to mitochondrial dysfunction and apoptotic cell death. **Conclusion:** The WO<sub>3</sub>/GO nanocomposite (1:0.5) demonstrated enhanced ROS-mediated anticancer activity against HT-29 cells and represents a promising nanotherapeutic platform for colorectal cancer treatment.

**Keywords:** tungsten oxide (WO<sub>3</sub>), Graphene oxide (GO), hydrothermal method, Cytotoxicity, HT-29 cells, ROS

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## Introduction

Cancer is one of the leading causes of death. Conventional treatments for this disease include surgical tumor removal, hormone therapy, radiation therapy, and chemotherapy using a combination of drugs [1, 2]. As a result, researchers are constantly seeking non-surgical therapeutic strategies to combat cancer. Therefore, nanomaterials and their nanocomposites have received considerable attention for this purpose due to their unique properties and high efficiency for non-surgical treatment. Tungsten oxide (WO<sub>3</sub>) is an n-type semiconductor and belongs to the class of transition metal oxides. It is characterized by a low energy gap ranging from 2.6 to 3.0 electron volts [3, 4]. WO<sub>3</sub> can crystallize in several phases such as monoclinic, tetragonal, or cubic depending on the temperature. These structural transformations affect the main physicochemical properties of the material, including crystal symmetry, W-O bond arrangement, energy gap, photocatalytic activity, visible light response, and surface behavior, making it particularly important for medical and biological applications. The monoclinic phase (monoclinic I) is the most stable at room temperature [5, 6]. The structural, morphological, and electrical properties

of the material largely depend on the preparation method. However, pure WO<sub>3</sub> often suffers from a high rate of electron-hole (e-h) recombination and a limited oxygen storage capacity, reducing its photocatalytic efficiency [7]. To overcome these limitations, several strategies have been proposed, such as controlling the morphological shape and particle size, doping with metallic and non-metallic elements, loading with noble metals, or combining it with other semiconductors to form nanocomposites that enhance charge separation and improve performance under visible light irradiation. As for graphene, it is a two-dimensional carbon material composed of sp<sup>2</sup> hybridized carbon atoms arranged in a hexagonal lattice [8]. It has attracted tremendous attention due to its unique electrical, thermal, and mechanical properties [9]. Among its derivatives, graphene oxide (GO) is very promising in biomedical applications particularly in cancer treatment. GO can function either as a nanocarrier for drugs or as an active component in advanced drug delivery systems, thanks to its unique physical, chemical, and optical properties including a large surface area, tunable functional groups, excellent biocompatibility, and low toxicity [10, 11]. Additionally, GO possesses a strong photothermal conversion ability

[12], which enables the conversion of light into heat for the localized treatment of cancer cells. Published research indicates that the  $\text{WO}_3/\text{GO}$  composite exhibits superior antibacterial activity compared to  $\text{WO}_3$  and GO used separately [11]. This is attributed to the synergistic effect of  $\text{WO}_3$ 's activity under light and GO's ability to induce mechanical stress and damage to the bacterial cell membrane. Several studies have demonstrated that this composite inhibits the growth of common bacterial strains such as *E. coli*, *S. aureus*, and *P. aeruginosa*. This inhibition results from the increased production of reactive oxygen species (ROS) upon the combination of  $\text{WO}_3$  and GO, leading to oxidation of cell components and membrane distortion. Furthermore, the porous structure and increased surface area enhance the interaction between the composite and bacterial cells, while GO's properties improve light absorption and reduce the energy gap of  $\text{WO}_3$ , thus increasing its effectiveness under light. In general, the results of the previous studies suggest that  $\text{WO}_3/\text{GO}$  is a promising antimicrobial material due to its enhanced efficacy, stability, and potential for use in environmental and medical applications, with the need to assess cytotoxicity prior to biological application [13].  $\text{WO}_3/\text{GO}$  nanocomposites have demonstrated significantly higher photocatalytic performance than each material individually, which is attributed to increased visible light absorption and enhanced charge separation efficiency. Moreover, the photocatalytic processes in  $\text{WO}_3$  generate (e-h) pairs, when these materials are exposed to light, which result in formation Reactive oxygen species (ROS) on the external of nanoparticles. These species include hydroxyl radicals ( $\bullet\text{OH}$ ) and superoxide ions ( $\text{O}_2^-$ ), which induce oxidative stress in cancer cells, leading to cellular damage, apoptosis, and inhibition of cell proliferation.  $\text{WO}_3$  is known not only for its strong catalytic and photocatalytic activity but also for its biocompatibility and non-toxicity, making it suitable for use in medical and nanobiotechnology applications. It has been found that doping  $\text{WO}_3$  with noble metals such as silver (Ag), platinum (Pt), or gold (Au) enhances photocatalytic activity by capturing electrons and preventing recombination. However, the high cost of these metals has led to the search for more economical alternatives based on graphene and its derivatives. Recent studies have shown that  $\text{WO}_3/\text{GO}$  nanocomposites are capable of inhibiting the proliferation of cancer cells and inducing apoptosis in multiple human cancer cell lines [1, 14, 15]. These nanocomposites also possess photothermal conversion capability, allowing for selective destruction of cancer cells upon light exposure while minimizing negative effects on healthy cells. In this study, hydrothermal preparation method was employed to produce  $\text{WO}_3$  nanoparticles and  $\text{WO}_3/\text{GO}$  at molar ratio 1:0.5 of  $\text{WO}_3$ : GO, proving effective in controlling crystal growth, morphology, band gap and charge-carrier recombination. Moreover, HT-29 cells were chosen because they reliably represent human colon cancer, are easy to culture and replicate in vitro, and have clear pathways for programmed death (apoptosis), making them an excellent model for studying the effect of nanocomposites  $\text{WO}_3/\text{GO}$  and to assess whether these modifications enhance ROS generation and apoptosis in

HT-29 cancer cells.

## Materials and Methods

### Materials

Sodium tungstate  $\text{Na}_2\text{WO}_4 \cdot 2\text{H}_2\text{O}$  (purity 99.9%), sodium sulfates  $\text{Na}_2\text{SO}_4$  (purity 99.2%) and citric acids  $\text{C}_2\text{H}_8\text{O}_7 \cdot \text{H}_2\text{O}$  (99.9%) were purchased from Merck Company. Hydrochloric acid HCL (purity 38%) was purchased from Merck Company. Graphene oxide (GO, purity 98%, P. Size. 5-15 nm, D. 20 $\mu\text{m}$ ) used in this study were obtained from Platonic nanotech.

### Synthesis tungsten oxide ( $\text{WO}_3$ ) nanoparticles

In the first step, pure tungsten oxide ( $\text{WO}_3$ ) was formed via the hydrothermal method which provides controlled crystal growth. Typically 0.005 mol Sodium tungstate ( $\text{Na}_2\text{WO}_4 \cdot 2\text{H}_2\text{O}$ , 99.9%), 0.025 mol sodium sulfate ( $\text{Na}_2\text{SO}_4$ , 99.2%), and 0.006 mol citric acid ( $\text{C}_2\text{H}_8\text{O}_7 \cdot \text{H}_2\text{O}$ , 99.9%) were used to synthesize  $\text{WO}_3$  nanostructures. For this purpose, sodium tungstate, citric acid, and sodium sulfate were dissolved in 50 ml deionized water. 2M of HCL acid was gradually added to an aqueous solution when a transparent solution was formed and pH was approached to 1. The solution was kept stirred for 60 min and then transferred to a 100 ml Teflon-lined autoclave. After being sealed, the autoclave was maintained for 8 h at a fixed temperature of 200°C. The obtained precipitate was then filtered and washed several times thoroughly by deionized water and ethanol. The final product was dried in an oven with convection for 10 h at 80°C

### Preparation of $\text{WO}_3/\text{GO}$ nanocomposites

Depending on the same process of prepared  $\text{WO}_3$ , tungsten oxide ( $\text{WO}_3$ ) and graphene oxide (GO) were used at specific molar ratio of 1:0.5 by using the ex-suit hydrothermal method. 1 gram of  $\text{WO}_3$  was dispersed in 40 ml of deionized water under continuous magnetic stirring. Then, 0.5 grams of GO was gradually added to the suspension, and the mixture was stirred for an additional 60 mins to ensure consistent dispersion and homogeneity. The resulting solution was then transferred to a Teflon container and heated in an oven at a temperature of 200°C for 8 hours to complete the hydrothermal reaction. Later, the Teflon container was left to cool to room temperature. The resulting outcomes was dried in oven at temperature 80°C for 12 hr, after being rinsed several time with water and ethanol and resulting in the final powder of  $\text{WO}_3/\text{GO}$  nanocomposite.

### Defining characteristics

The crystalline composition of pure  $\text{WO}_3$ , GO and  $\text{WO}_3/\text{GO}$  nanocomposites at molar ratio 1:0.5 of  $\text{WO}_3$ :GO product, respectively was observed by XRD analysis (XRD diffraction 6000, shimadzu) with radiation ( $\lambda=1.542\text{\AA}$ ), the recorded data fell between the range of (10°-60°). To conduct photoluminescence (PL) Spectroscopy, a monochromatic wavelength of 350 nm was carried out using (Cary Eclipse Fluorescence Spectrometer, Agilent Co model, America) at room temperature to analyze the emission wavelengths of

samples. The optical properties were performed using UV-Visible spectroscopy (UV-1800 Spectrophotometer, Shimadzu). Raman spectra were measured at room temperature in range of (400-2800) nm using (Raman spectroscopy, Takram N1-541). The Field emission scanning electron microscope (FE-SEM, TESCAN, MIRA3) of pure WO<sub>3</sub>, GO and WO<sub>3</sub>/GO nanocomposites at molar ratio 1:0.5 samples were captured to study their morphology. Furthermore, the energy-dispersive X-ray spectroscopy (EDS) was conducted during FESEM measurements as a relatively simple but powerful technique for the elemental analysis of samples. The Zeta potential measurements were conducted to evaluate the surface charge and colloidal stability of the pure WO<sub>3</sub>, GO, and WO<sub>3</sub>/GO nanocomposites at molar ratio 1:0.5. These measurements were carried out using a Zeta sizer Nano ZS (Zetasizer Model, Malvern Panalytica, Malvern) at room temperature. The Zeta potential values provide insight into the surface interaction, dispersion stability, and potential biological behavior of the nanoparticles in suspension.

#### The MTT assay

Using the MTT assay, the cytotoxicity of pure WO<sub>3</sub>, GO and WO<sub>3</sub>/GO nanocomposites was examined. HT-29 cells were cultivated overnight and then seeded at a density of 1x10<sup>4</sup> cells per well on to 96-well plates. Following, the removal of the growth media and its replacement with 200 µL of fresh medium with different amounts of products at a concentration of 25 µg/mL for 24h. The cells were treated for 3 hours with MTT solution consisting 2 milligrams per milliliter (Invitrogen, Carlsbad, CA) after being with PBS. Each well was then filled with 100 µL of DMSO after the solution had been emptied away. The absorbance of each sample at wavelength of 492 nm was measured using a microplate reader. The formula used to calculate the percentage of cytotoxicity, or the rate of inhibiting of cell growth, is displayed:

$$\text{Cytotoxicity} = (A-B)/B$$

Where A exhibits the optical density of control and B exhibits the optical density of product [16, 17].

#### Acridine Orange/Ethidium Bromide stain (AO/EtBr)

After collecting, the HT-29 cells were then placed on 12-well plates. The cells were treated with WO<sub>3</sub>, GO and WO<sub>3</sub>/GO 1:0.5 nanocomposites for 24h after a 24-h incubation period. The cells were then stained for 2 min at 37°C by 10 µg/mL of AO/EtBr and examined under a fluorescence microscope.

#### Flow Cytometry test

A flow cytometry assay was used to quantify the generation of ROS in cells. Each well was seeded with 1x10<sup>6</sup> HT-29 cells. The cells were treated with WO<sub>3</sub>, GO and WO<sub>3</sub>/GO at 1:0.5 for 8 hours after being incubated overnight. The modified medium was then incubated for a further half hour in the dark with a ROS probe (DCFH-DA) at a concentration of 15 µM. A flow cytometer was used to quantify the cells fluorescence intensity.

Additionally, a Rhodamine probe was utilized in the flow cytometry test to detect mitochondrial dysfunction, and (JC-1) probe was used to measure mitochondrial membrane potential. The created methodology was used to measure the cells fluorescence intensity.

#### Apoptosis detection annexin V/PI assay

A flow cytometry test was used to examine cell apoptosis. The samples (WO<sub>3</sub>, GO and WO<sub>3</sub>/GO 1:0.5 nanocomposite) were introduced to HT-29 liver cancer cells at a dosage of 25 µg/mL. After a day, the cells were removed and gathered. After two cold PBS washes, the cells were stained with an Annexin V FITC and PI solution for 30 min. A flow cytometry experiment was then used to assess the labeled cells.

#### Statistical analysis

Three separate experiments produced the final data. Data are shown as mean ± SD. Graph Pad Prism (7) was used to perform the statistical analysis by applied of one-way ANOVA analysis of variance. LSD was used to evaluate the differences between means: p≤0.05 proved to be significant. P\*≤0.05, p\*\*≤0.01, p\*\*\*≤0.000139.

## Results

#### Characterization of pure WO<sub>3</sub>, GO and WO<sub>3</sub>/GO nanocomposite

Figure 1, illustrates the XRD patterns of the pure WO<sub>3</sub>, GO and WO<sub>3</sub>/GO nanocomposite at specific molar ratio 1:0.5 WO<sub>3</sub>: GO prepared by hydrothermal method. For Graphene oxide (GO), an intense reflection related to GO had appeared at 2θ = 10.64° corresponded to (002) plane [18]. By applying Bragg's law, the calculated interlayer spacing of GO is 0.83 nm, providing an expansion than graphite analog depicted at 0.34nm. This is interpreted in terms of elongation of the c axis during graphite oxidation to GO via creation of oxygen containing functional groups. The XRD patterns of pure WO<sub>3</sub> exhibit stronger diffraction peaks at 2θ values of 23.1°, 23.6°, 24.3°, 26.5°, 33.5°, 34.1°

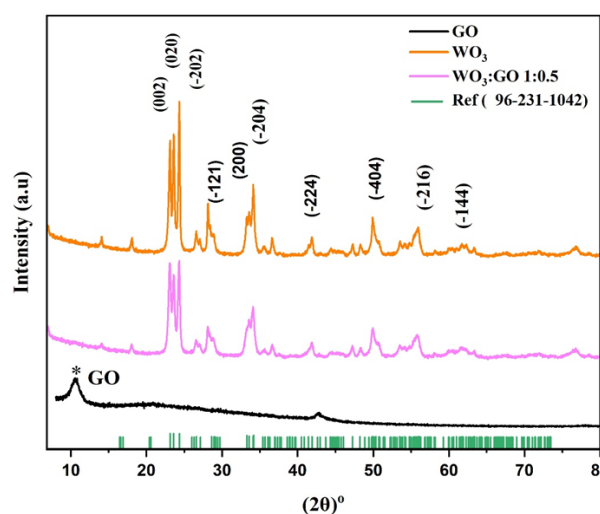


Figure 1. The XRD Patterns of GO, WO<sub>3</sub> and WO<sub>3</sub>/GO Nanocomposite at Specific Molar Ratio 1:0.5 prepared by Hydrothermal Method at 180°C and 8h

Table 1. The XRD Parameters of GO, WO<sub>3</sub> and WO<sub>3</sub>/GO Nanocomposite at Specific Molar Ratio 1:0.5 Prepared by Hydrothermal Method at 200°C and 8h

| Materials                                                       | 2θ   | Hkl    | d-spacing (Å) | FWHM (degree) | Crystalline size (nm) |
|-----------------------------------------------------------------|------|--------|---------------|---------------|-----------------------|
| WO <sub>3</sub>                                                 | 23.1 | (002)  | 3.76          | 0.22          | 27.5                  |
|                                                                 | 24.6 | (-202) | 3.65          | 0.207         | 39.2                  |
|                                                                 | 26.5 | (-121) | 3.35          | 0.331         | 24.6                  |
|                                                                 | 33.5 | (200)  | 2.66          | 0.235         | 35.3                  |
|                                                                 | 34.1 | (-204) | 2.62          | 0.316         | 26.3                  |
| WO <sub>3</sub> /GO nanocomposite at specific molar ratio 1:0.5 | 23   | (002)  | 3.85          | 0.226         | 36.6                  |
|                                                                 | 24.2 | (020)  | 3.66          | 0.298         | 27.2                  |
|                                                                 | 28.1 | (-202) | 3.16          | 0.49          | 16.7                  |
|                                                                 | 34   | (-121) | 2.62          | 0.453         | 18.3                  |
|                                                                 | 36.6 | (200)  | 2.45          | 0.429         | 19.5                  |

°, 41.9° and 49.9°. These peaks were related to the (002), (020), (-202), (-121), (200), (-204), (-224) and (-404) planes crystallographic, respectively. The diffraction peaks agreed with the structure of monoclinic WO<sub>3</sub> (JCPDS card No. 96-231-1042) [19]. The calculated lattice parameters obtained at the principal plane are  $a = 7.2$ ,  $b = 7.5$ ,  $c = 10.5$  Å. When compared to the XRD patterns of WO<sub>3</sub>, the state of the WO<sub>3</sub>/GO nanocomposite at molar ratio 1:0.5 showed no unique characteristics. The average crystallite size of WO<sub>3</sub>/GO nanocomposites, denoted as  $D = k\lambda/\beta \cos\theta$  [20], was calculated using the Scherrer formula, where  $k$  is the crystal shapes factor,  $\lambda$  is the wavelength of Cu-K radiation used [21], and  $\theta$  is Bragg angle as indicated in Table 1. The results showed that the crystal size of pure WO<sub>3</sub> is approximately 31.6 nanometers, whereas the grain size in the WO<sub>3</sub>/GO1:0.5 nanocomposite is reduced to about 22.8 nanometers. This decrease in grain size indicates the effect of introducing GO layers in inhibiting crystal growth during hydrothermal preparation, leading to a more uniform particle distribution and an increase in the effective surface area of the composite. It also reflects a strong interaction between WO<sub>3</sub> and GO and its impact on modifying the crystal lattice structure.

Moreover, the absence of GO peak confirmed the interaction between the GO and WO<sub>3</sub> during the nanocomposites formation cause changes in the crystal structure of both materials, potentially leading to shift in the diffraction peaks and disappearance of GO peak due to the strong bonding. The XRD pattern of nanocomposites showed only the characteristic peaks of WO<sub>3</sub>, suggesting that GO was effectively reduced and incorporated into nanocomposites without leaving a distinct signature in the XRD diffraction pattern [22]. There was no other significant peak observed in XRD pattern of these composite materials. Moreover, a shift in the values of  $2\theta$  of the WO<sub>3</sub>/GO at 1:0.5 nanocomposites was observed as  $2\theta$  as shown (23°.1-23°), (24°.6-24°.2), showed a slight shift towards lower  $2\theta$  angles compared to the pure WO<sub>3</sub> sample. Such a shift is indicative of tensile strain, which leads to an increase in the interplanar spacing (d-spacing) [23]. These findings suggest that the introduction of GO layers into the WO<sub>3</sub> matrix may have caused the crystalline network to expand, confirming a strong structural interaction between the two materials.

The same trends were observed for WO<sub>3</sub>/GO. The XRD patterns of the WO<sub>3</sub>/GO samples in Figure 1 did not show changes in crystal structures compared to the pure WO<sub>3</sub> [24]. We also notice that for some peaks, the value of shifted towards higher  $2\theta$  (26°.5 -28°.1), (33.5°-34°) and (34°.1-36°.6), as decrease in the value of (d spacing) as compared with pure WO<sub>3</sub>. Thus, it is the tensile strain reaches its maximum value which causes reduction of tensile strain or strain relaxation. A noticeable decrease was observed in the intensity of the X-ray diffraction (XRD) peaks of the WO<sub>3</sub>/GO sample compared to the pure WO<sub>3</sub> sample. This decrease can be attributed to the low crystallinity resulting from the incorporation of graphene oxide GO, which may cause defects in the crystalline lattice or stresses within it.

Figure 2, shows the Raman spectra of WO<sub>3</sub>, GO, and WO<sub>3</sub>/GO nanocomposite at specific molar ratio 1:0.5 in the wavenumber range of 400-2800 cm<sup>-1</sup>. In this context, Raman spectroscopy is considered a simple and non-destructive characterization technique, and it is highly sensitive to local structure and chemical composition. It is widely used to study phase transitions and disorder in metal oxides. Raman spectroscopy can also provide precise structural information and is considered a sensitive method for verifying crystallinity, local structure, and the

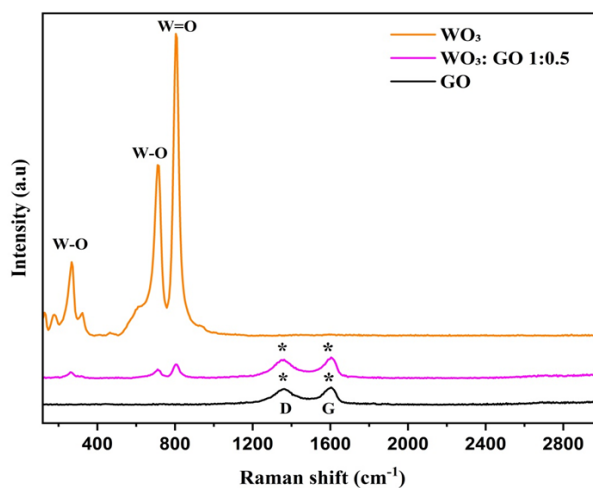


Figure 2, The Raman spectra of GO, WO<sub>3</sub> and WO<sub>3</sub>/GO Nanocomposite at Specific Molar Ratio 1:0.5 Prepared by Hydrothermal Method at 200°C and 8h

Table 2. The Raman Bands of GO, WO<sub>3</sub> and WO<sub>3</sub>/GO Nanocomposite at Specific Molar Ratio 1:0.5 Prepared by Hydrothermal Method at 200°C and 8h

| Material                                         | Raman bands (cm <sup>-1</sup> ) | Assignment                             |
|--------------------------------------------------|---------------------------------|----------------------------------------|
| Pure WO <sub>3</sub>                             | 267                             | Due to the O-W-O deformation modes     |
|                                                  | 711                             | Stretching vibration of the o-w-o bond |
|                                                  | 804                             | Vibration of the w-o-w bonds           |
| WO <sub>3</sub> /GO nanocomposite at ratio 1:0.5 | 260                             | due to the O-W-O deformation modes     |
|                                                  | 704                             | stretching vibration of the O-W-O bond |
|                                                  | 810                             | vibration of the w-o-w bonds           |
|                                                  | 1356                            | D-band of GO*                          |
|                                                  | 1598                            | G-band of GO*                          |
| GO                                               | 1362                            | D-band of GO                           |
|                                                  | 1604                            | G-band of GO                           |

electronic properties of the sample, making it an important tool in the analysis of nanomaterials such as WO<sub>3</sub>, GO, and their compounds. The GO spectrum exhibits two intense bands as shown in Figure 2. The characteristic scatterings of graphene in the nanocomposites are observed at bands are related to D band and G band were about 1362 cm<sup>-1</sup>, 1604 cm<sup>-1</sup>, respectively [24, 25]. From the Figure 2, the former D-band represented the disordering sp<sup>2</sup>-hybridized C atoms of GO, while the G-band corresponded to the structural integrity of the sp<sup>2</sup>-hybridized C atoms of GO. The intensity ratio ID/IG of D and G bands is featuring the level of disorder in graphene [26]. However, the increase of disorder in graphene is characterized by two regimes displayed by ID/IG. The intensity ratio of the D band to the G band (ID/IG) is used to assess the density of defects in the structure of graphene oxide. In this work, the ratio intensity of GO was found to be (ID/IG=0.90), indicating a significant amount of structural defects within the graphene sheets. The Raman spectrum of WO<sub>3</sub> nanostructures prepared by hydrothermal method is shown in Figure 2. The most intense peak at 804 cm<sup>-1</sup> is corresponded to the stretching vibration of W-O-W bonds. The peak at 267 cm<sup>-1</sup> is most likely due to the O-W-O deformation modes. Besides, the peak at 711 cm<sup>-1</sup> represented a strong stretching vibration of the O-W-O bond, which is a major peak and a characteristic peak of tungsten oxide (WO<sub>3</sub>). The presence of peaks at 267 cm<sup>-1</sup>, 703 cm<sup>-1</sup> and 802 cm<sup>-1</sup> strengthen the possibility that the sample is highly crystalline. The recorded Raman spectra and findings XRD diffraction patterns are in agreement. WO<sub>3</sub>/GO nanocomposites at specific molar ratio of 1:0.5 WO<sub>3</sub>: GO, as shown in Figure 2. Two characteristic Raman peaks were observed at approximately 1362 cm<sup>-1</sup> and 1504 cm<sup>-1</sup>, corresponding to the D and G bands, respectively. As shown in Table 2, these peaks revealed the presence of the carbon network within the WO<sub>3</sub>/GO nanocomposites. Besides, the Raman spectra of WO<sub>3</sub>/GO nanocomposites at specific molar ratio of 1:0.5 WO<sub>3</sub>: GO show several distinctive changes compared to the spectra of pure tungsten oxide (WO<sub>3</sub>) and graphene oxide (GO) individually. Moreover, the Raman peaks around 261 cm<sup>-1</sup> are weakened and rather shifted to low frequencies in comparison to 267 cm<sup>-1</sup> of WO<sub>3</sub> pure, which supporting strong interaction between WO<sub>3</sub> and GO. A decrease in

the intensity of tungsten oxide (WO<sub>3</sub>) as shown in Raman peaks was identified after the addition of graphene oxide (GO), indicating an interaction between WO<sub>3</sub> and GO that alters the crystalline structure or affects the characteristic vibrational modes of WO<sub>3</sub> within the nanocomposites [27]. The intensity ratio ID/IG between the band-D and band-G in the nanocomposites can be used to determine the extent of graphene material defects after interaction between WO<sub>3</sub> and GO. This value was calculated to be (ID/IG=0.88) after addition GO to WO<sub>3</sub> at specific molar ratio of 1:0.5 WO<sub>3</sub>: GO nanocomposites. The increase in the intensity of D band reflects the emergence of new defects, possibly resulting from the interaction between WO<sub>3</sub> and GO or the conditions of hydrothermal preparation. At the same time, the increasing in the intensity of the G band suggests potential partial rearrangement or partial recovery of sp<sup>2</sup> domains. However, the slight decrease in the intensity ratio of ID/IG from 0.9 to 0.88 indicates that the number of sp<sup>2</sup> bonded carbon atoms is increased during their situ synthesis of GO/WO<sub>3</sub> nanocomposites. Besides, the findings reveal that there is a decreasing in the intensity ID/IG after the formation of the nanocomposite. The decrease in ID/IG ratio from 0.90 for GO to 0.88 for nanocomposites suggests that some defects or disorder in G sheets after interaction with WO<sub>3</sub>. This can be explained by the chemical bonding between GO and WO<sub>3</sub> where WO<sub>3</sub> could potentially interact with oxygen-containing groups on the surface of GO, leading to slight reduction in disorder in GO structure during the hydrothermal [22].

The optical properties of the synthesized pure WO<sub>3</sub>, GO, and the WO<sub>3</sub>/GO nanocomposite at molar ratio of 1:0.5 of WO<sub>3</sub>: GO by hydrothermal method were determined by UV-Vis spectroscopy in the range of (200-800) nm as shown in Figure 3 a,b,c,d. In state of prepared pure WO<sub>3</sub> exhibited a broad absorption range between 200-490 nm, indicating its ability to absorb UV and visible light. In contrast, the spectrum GO show a narrow absorption region in range between 200-300 nm with a characteristic peak at 230 nm attributed to  $\pi$ - $\pi^*$  electronic transitions of C=C bonds and a small peak near 300 nm resulting from n- $\pi^*$  transitions of C=O groups. The WO<sub>3</sub>/GO nanocomposite at molar ratio 1:0.5 of WO<sub>3</sub>: GO, displayed a broad absorption band in range between 200-420 nm as shown in Figure 3 a. In addition,

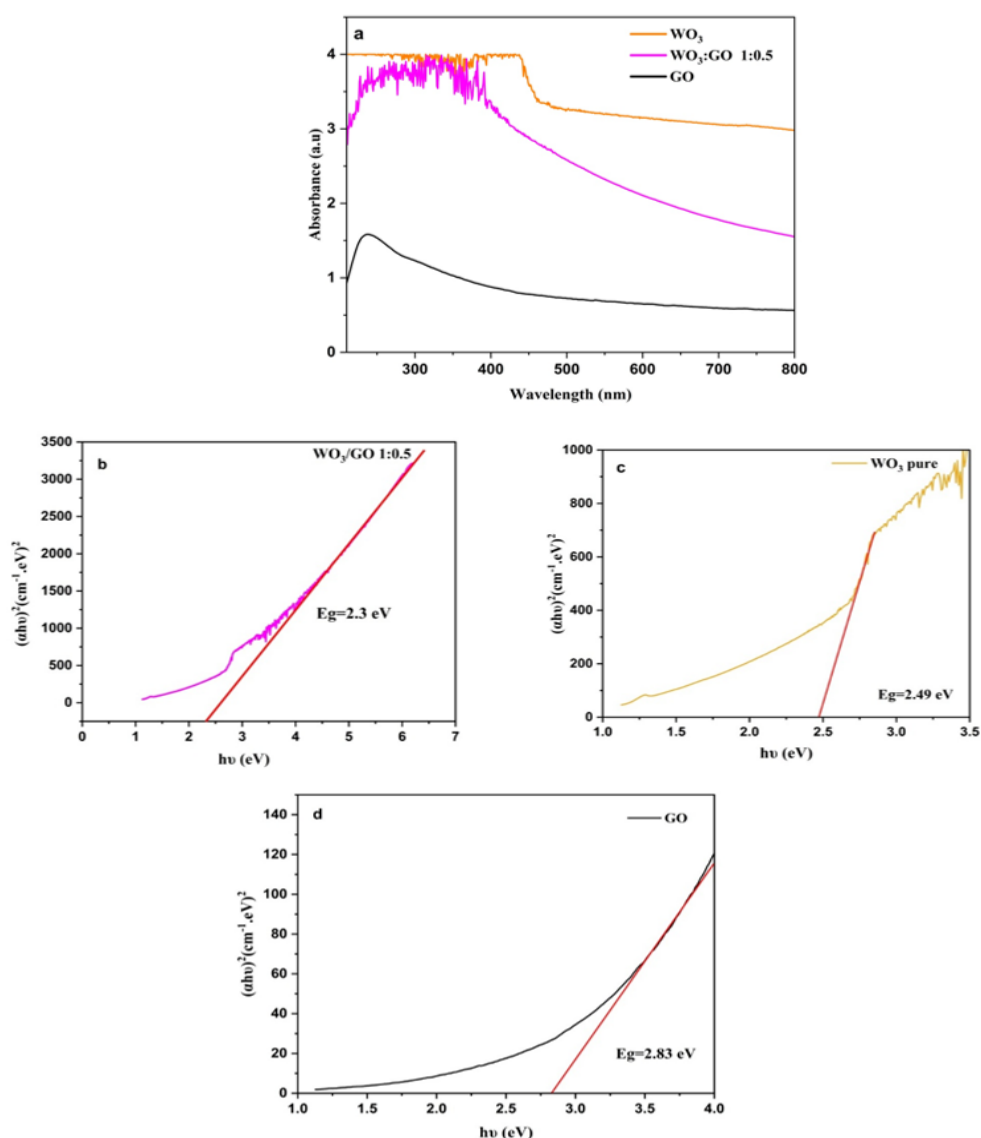


Figure 3. a) the optical absorption of  $\text{WO}_3$ , GO and  $\text{WO}_3/\text{GO}$  nanocomposites at specific molar ratio 1:0.5 of  $\text{WO}_3$ : GO and the calculated band gap energy of b)  $\text{WO}_3$ , c) GO, d)  $\text{WO}_3/\text{GO}$  using hydrothermal method

the absorption edges were at 450 nm, 410 nm and 255 nm for the pure  $\text{WO}_3$ ,  $\text{WO}_3/\text{GO}$  nanocomposite and GO, respectively. It was clear that the absorption edge shifted to shorter wavelength for the  $\text{WO}_3/\text{GO}$  nanocomposites after introduction GO content and result in a slight blue shift compared to pure  $\text{WO}_3$ , indicating a reduced bandgap energy and improved light absorption. This enhancement is attributed to the formation of W-O-C bonds and increased charge transfer efficiency between  $\text{WO}_3$  and GO, which reduces electron-hole recombination as improved by FTIR and PL analysis. Moreover, Figure 3 b,c,d, illustrated the optical band gap energy of pure  $\text{WO}_3$ , GO and  $\text{WO}_3/\text{GO}$  at molar ratio 1:0.5 by using Tauc plot. The calculated energy band gap of  $\text{WO}_3/\text{GO}$  nanocomposites was found to be 2.3 eV in Figure 3b, which is significantly lower than the bandgap of pure  $\text{WO}_3$  2.49 eV in Figure 3c and energy gap of GO was found to be 2.8 eV in Figure 3d. This notable reduction of the bandgap is attributed to the strong interfacial interaction between  $\text{WO}_3$  nanoparticles and GO sheets, leading to the formation of W-O-C bonds, as confirmed by FTIR analysis. Additionally, the

introduction of intermediate levels within the gap (such as oxygen vacancies) enhances charge transfer pathways. As shown in Table 3, the reduction in band gap improves visible light absorption and decreases (e-h) recombination, likely contributing to the observed enhancement in photocatalytic activity and anticancer performance (ROS generation and photothermal effect) of the  $\text{WO}_3/\text{GO}$  at molar ratio 1:0.5.

Photoluminescence (PL) spectra analysis is considered a key aspect in evaluating the optical properties of tungsten oxide  $\text{WO}_3$ , GO and  $\text{WO}_3/\text{GO}$  nanocomposite

Table 3. the Optical Absorption of  $\text{WO}_3$ , GO and  $\text{WO}_3/\text{GO}$  at Specific Molar Ratio 1:0.5 of  $\text{WO}_3$ : GO and the calculated band gap energy using hydrothermal method

| Materials                             | Wavelength (nm) | Energy band gap (eV) |
|---------------------------------------|-----------------|----------------------|
| Pure $\text{WO}_3$                    | 450nm           | 2.49                 |
| GO                                    | 255 nm          | 2.83                 |
| $\text{WO}_3/\text{GO}$ nanocomposite | 410             | 2.3                  |

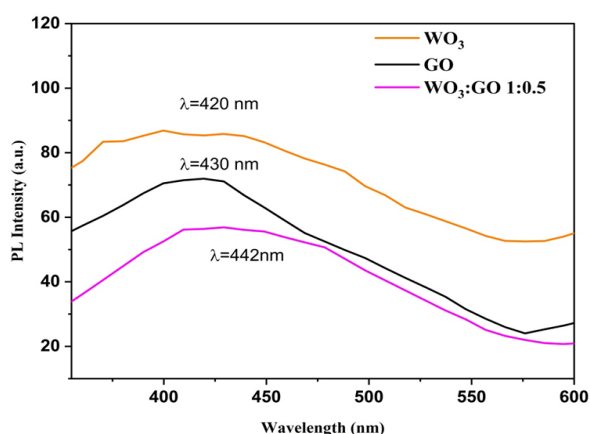


Figure 4. The PL Spectrum of WO<sub>3</sub>, GO, and WO<sub>3</sub>/GO Nanocomposite at Specific Molar Ratio 1:0.5 Prepared by Hydrothermal Method at 200°C and 8h.

at specific molar ratio 1:0.5 WO<sub>3</sub>: GO as shown in Figure 4. PL analysis provides valuable information about the recombination of charge carriers and the efficiency of photocatalysis in the visible region [10, 18]. As shown in Figure 4, the PL spectra of the samples exhibit clear peaks when excited with light of 350 nm. The pure WO<sub>3</sub> sample demonstrated a high PL emission intensity ( $I = 80$  at  $\lambda = 420$  nm), indicating a high rate of electron-hole pair recombination, which limits the efficiency of photocatalysis. In contrast, the WO<sub>3</sub>/GO nanoscale composite, prepared at a molar ratio of 1:0.5, showed a significant decrease in PL intensity ( $I = 35$  at  $\lambda = 442$  nanometers). This substantial decrease indicates a low rate of recombination of photon-generated electron-hole pairs. This reduction is attributed to the role of graphene oxide in capturing and transferring emitted electrons, preventing their recombination with holes. Furthermore, the red shift in the emission peak from 420 nm to 442 nm and the decrease in the energy gap observed in the UV-Vis absorption spectra confirm the modification of the electronic structure of tungsten oxide due to graphene oxide. This enhances the material's response under visible light and supports better charge carrier separation.

The morphology and microstructure of WO<sub>3</sub>, GO and WO<sub>3</sub>/GO nanocomposites at molar ratio 1:0.5 of WO<sub>3</sub>:GO were examined using field emission scanning electron microscopy (FESEM), as shown in Figure 5 a,b,c. The GO sheet in Figure 5a, demonstrated the formation of thin, smooth sheets devoid of defects and surface disfigurement. Additionally, it indicates that the plate has certain surface folds that result from the plate twisting or from one plate component piling on top of another to create this structure. Showed layered wavy structures stacked on top of one another created the GO structures [28]. Figure 5 b, exhibited quasi-cubic particles of pure WO<sub>3</sub> with smooth surfaces and relatively sharp edges, with size distribution ranging from 39.5-80.89 nm with monoclinic WO<sub>3</sub> phase. Besides, this structure reflects a high degree of regularity in crystal growth and indicates good control over preparation conditions. This morphology is governed by anisotropic crystal growth along the direction of monoclinic structure. The theoretical method explains the

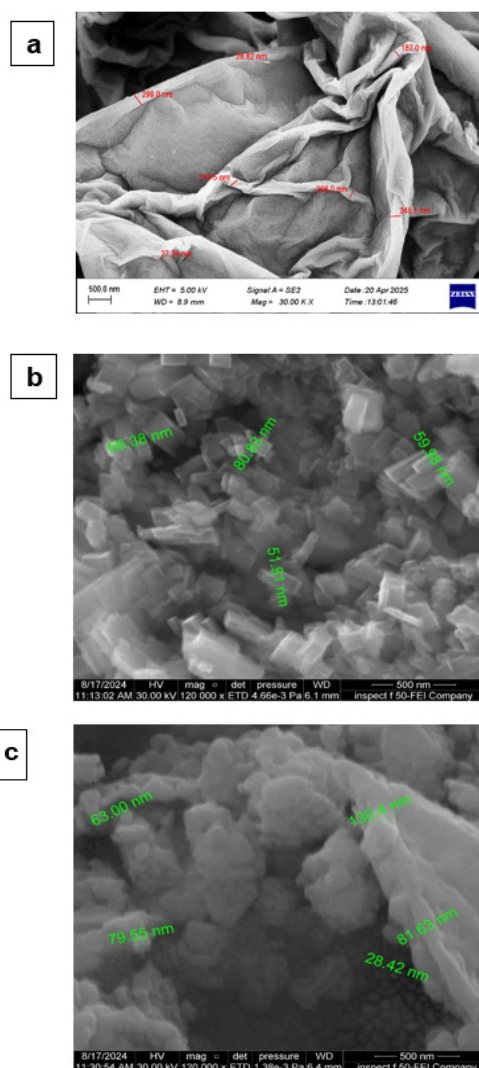


Figure 5. The FESEM Images of a) GO b) WO<sub>3</sub>, and c) WO<sub>3</sub>/GO nanocomposite at specific molar ratio 1:0.5 prepared by hydrothermal method at 200°C and 8h.

morphology through surface energy, controlled nucleation kinetics and thermodynamic equilibrium during synthesis. The resulting microstructure offers enhanced surface area, improved crystallinity and directional properties that are valuable for various applications. In contrast, the FESEM image displays the morphology of WO<sub>3</sub>/GO nanocomposite at molar ratio 1:0.5 prepared by the hydrothermal method at 200 °C for 8 h. The morphology reveals spherical particles, extensive agglomeration and rough surface texture with ranging in size from 28.42-81.63 nm as illustrated in Figure 5 c. This behavior is attributed to the low graphene oxide GO content in this ratio, which did not allow for full coverage of the WO<sub>3</sub> particles, leading to WO<sub>3</sub> agglomeration and the formation of irregular particles with varied sizes. Besides, the GO integration basically alters the nucleation and growth kinetics, leading to enhanced surface area, improving synergistic functionality that makes the nanocomposites superior for photocatalysis applications. As a result, WO<sub>3</sub> particles appear distributed on GO sheets within a layered structure with a larger surface area and higher surface roughness. This interfacial interaction between

Table 4. Wavenumber Position and Attribution of the Transmittance Peak Observed by FTIR Analysis of Pure WO<sub>3</sub>, GO and WO<sub>3</sub>/GO 1:0.5 Nanocomposite Prepared by Hydrothermal Method at 180°C and 8

| Material                           | Peak position (cm <sup>-1</sup> ) | Attribution                                                               |
|------------------------------------|-----------------------------------|---------------------------------------------------------------------------|
| Pure WO <sub>3</sub>               | 819                               | O–W–O stretching vibrations                                               |
|                                    | 760                               | O–W–O stretching vibrations                                               |
|                                    | 1535                              | O–H bending vibrations                                                    |
|                                    | 3444                              | O–H stretching vibrations                                                 |
|                                    | 3753                              | O–H stretching vibrations                                                 |
|                                    | 3417                              | O–H stretching vibrations                                                 |
|                                    | 1646                              | H–O–H bending vibration                                                   |
|                                    | 2358                              | CO <sub>2</sub> adsorption                                                |
|                                    | 3417                              | W–OH stretching vibrations                                                |
| WO <sub>3</sub> /GO nanocomposites | 817, 758                          | O–W–O stretching vibrations                                               |
|                                    | 1228                              | C–O–C stretching                                                          |
|                                    | 1058                              | C–O vibrational mode                                                      |
|                                    | 1597                              | C=C structure                                                             |
|                                    | 1741, 1627                        | C=O stretching vibration of the carboxyl group.                           |
|                                    | 3417, 3747                        | O–H stretching vibrations groups and water molecules                      |
|                                    | 2353                              | O=C=O vibration                                                           |
| GO                                 | 3429                              | O–H stretching vibrations                                                 |
|                                    | 2364                              | CO <sub>2</sub> stretching vibrations                                     |
|                                    | 1685                              | (C=O), carbonyl group                                                     |
|                                    | 1583                              | the primary graphitic domain resulting from sp <sup>2</sup> hybridization |
|                                    | 1386                              | C–OH group                                                                |
|                                    | 1215                              | C–O–C stretching                                                          |
|                                    | 1062                              | C–O vibrational mode                                                      |
|                                    | 819                               | C–H bonds                                                                 |
|                                    | 670, 594                          | C–H bending vibrations                                                    |

WO<sub>3</sub> and GO is expected to contribute to improved charge separation efficiency and reduced recombination, which positively impacts the photocatalytic activity and biological properties of the compound compared to pure WO<sub>3</sub>. Moreover, the energy-dispersive X-ray spectroscopy (EDS) was performed to determine the elemental composition of WO<sub>3</sub> and WO<sub>3</sub>/GO nanocomposite at a molar ratio of 1:0.5 respectively as shown in Supplementary Figure 1 a, b. The EDS spectrum of WO<sub>3</sub> showed weight percentages about 15% of O and 84.5%, of W, respectively as shown in Supplementary Figure 1 a. The outcomes demonstrate the high purity of the sample and the agreement of the weight percentage of oxygen with the expected chemical composition of tungsten oxide, where each tungsten atom is bonded to three oxygen atoms. The high weight percentage of tungsten is due to the high atomic mass of this element. On the other hand, the WO<sub>3</sub>/GO nanocomposite in Supplementary Figure 1b reveals the presence of carbon C, at weight percentages 17.2%, 21.9% oxygen (O), and tungsten (W) about 60.9%, respectively. These results indicate the successful deposition of tungsten oxide WO<sub>3</sub> on graphene oxide GO.

Supplementary Figure 2, illustrated the Zeta potential obtained for the tungsten oxide nanoparticles WO<sub>3</sub> NPs prepared by hydrothermal method is about -35 mV, and the conductivity value of 0.067mS/cm. Previous studies

suggested that a higher value (~30 mV) of zeta potential (either positive or negative) was associated with the greater stability of aqueous suspension. In addition, the positive surface charge of NPs and NCs in deionized water (DI) and culture media favors their interaction with negatively charged biomolecules cells. These results reveal high surface negative, good stability which indicates the high dispersion of the material and the lack of agglomerates, which is an essential property in medical applications and many other applications. This strong negativity is attributed to the presence of abundant surface hydroxyl groups (-OH) forming during the hydrothermal process, as well as the small particle size, which increases surface charge density. In contrast, the nanocomposite with a ratio of 1:0.5 of WO<sub>3</sub>: GO showed a zeta potential value is about -36 mV. We notice a slight change and an increase in value in the zeta potential was observed in nanocomposites compared to the pure WO<sub>3</sub> sample, indicating that the compound retained its high negative surface charge. This is attributed to the fact that the GO layers did not occupy or neutralize the surface charge sites of tungsten oxide. However, the electrical conductivity increased to 0.126 mS/cm, likely due to the partial integration of the conductive GO sheets within the WO<sub>3</sub> matrix, which enhanced charge transport across the composite.

The FTIR spectrum of WO<sub>3</sub>, GO and WO<sub>3</sub>/GO

nanocomposite at a molar ratio of 1:0.5 are demonstrated using Fourier-transform infrared (FTIR) spectroscopy in the spectral range of 500 to 4000 cm<sup>-1</sup> as shown in Supplementary Figure 1 a,b,c, respectively. In Supplementary Figure 3 a, GO spectrum includes characteristic vibration bands of graphene oxide, which comprise carbonyl (C=O), aromatic (C=C), carboxyl (-COOH), epoxy (C-O-C), and hydroxyl (O-H) groups, as described in Table 4. A broad and intense absorption peak is observed at 3429 cm<sup>-1</sup>, attributed to the stretching vibrations of the hydroxyl (O-H) groups, due to the presence of absorbed water molecules and hydroxyl groups on the surface of the graphene oxide. The absorption peaks at 2364 cm<sup>-1</sup> represent the asymmetric and symmetric stretching vibrations of the CO<sub>2</sub> bond. The peak at 1685 cm<sup>-1</sup> is attributed to the carbonyl group (C=O), while the main peak at 1583 cm<sup>-1</sup> is associated with the primary graphitic domain resulting from sp<sup>2</sup> hybridization (C=C) [29, 30]. The peak at 1386 cm<sup>-1</sup> arises from C-OH group. The peak at 1215 cm<sup>-1</sup> denotes C-O-C stretching and the peak at 1062 cm<sup>-1</sup> corresponds to the vibrational mode of the C-O group [31]. The absorption peak at 819 cm<sup>-1</sup> corresponds to the deformation of aromatic C-H bonds, whereas the bumps at 670 cm<sup>-1</sup>, 594 cm<sup>-1</sup>, arise from C-H bending vibrations. The chemical bonds of tungsten oxide WO<sub>3</sub> sample were identified using FTIR spectroscopy as displayed in Supplementary Figure 3 b. As shown, a strong and broad band between 819 cm<sup>-1</sup> and 760 cm<sup>-1</sup>, attributed to O-W-O stretching vibrations, which are characteristic of the monoclinic phase. The peak at 1535 cm<sup>-1</sup> is attributed to bending vibrations of O-H groups associated with adsorbed water or crystalline water. Similarly, the Bands at 3444 and 3753 cm<sup>-1</sup> are attributed to stretching vibrations of -OH groups and water molecules [32]. Besides, the peak at 2366 cm<sup>-1</sup> is due to the CO<sub>2</sub> adsorption for the WO<sub>3</sub> prepared. Supplementary Figure 3c, illustrates the FTIR spectra of WO<sub>3</sub>/GO at a ratio of 1:0.5 nanocomposites. A strong and broad band between 817 and 758 cm<sup>-1</sup>, attributed to O-W-O stretching vibrations, The peak at 1228 cm<sup>-1</sup> denotes C-O-C stretching and the peak at 1058 cm<sup>-1</sup> corresponded to the vibrational mode of the C-O group in GO. The peak at 1597 cm<sup>-1</sup> is assigned to the C=C structure, while the peak around 1627 and 1741 cm<sup>-1</sup> are attributed to the C=O stretching vibration of the carboxyl group. Bands at 3417 and 3747 cm<sup>-1</sup> are attributed to stretching vibrations of -OH groups and water molecules. The characteristic peak at 2362 cm<sup>-1</sup> corresponds to the O=C=O group vibration. Based on the findings, it can be concluded that GO nanosheets strongly interact with WO<sub>3</sub> molecules through oxygen-containing functional groups [33]. These results reveal that the peak at 1685 cm<sup>-1</sup> (GO) has shifted to 1627 cm<sup>-1</sup> 3/GO) after the formation of the nanocomposite, and this may be due to the formation of W-O-C bonds between WO<sub>3</sub> and GO.

Based on FTIR spectrum, the shift is considered evidence of the chemical interaction between WO<sub>3</sub> and GO and the formation of a chemically bonded stable composite. These changes indicate the formation of WO<sub>3</sub>/GO nanocomposites. The interactions between residual carboxyl groups of graphene and WO<sub>3</sub> are also confirmed

by the obvious asymmetric -COO stretching bonds around 1597 cm<sup>-1</sup> [34].

#### *The Cytotoxicity of Prepared Nanoparticles*

By using cancer cells type HT-29 as a model for studying colorectal cancer, the resulted nanoparticles like GO, WO<sub>3</sub> and WO<sub>3</sub>/GO nanocomposite prepared at specific molar ratio 1:0.5 are treated with these cells to investigate the determine how nanoparticles interact with human cells at the molecular and cellular levels to inhibit growth inhibition and proliferation. As shown in Supplementary Figure 3, the cytotoxicity influence of GO and pure WO<sub>3</sub> against HT-29 cells at lower concentrations (12 µg/ml and 25 µg/ml), reveal low cytotoxicity, suggesting that these concentrations are not high enough to cause substantial damage. As the higher concentrations (50 µg/ml and 100 µg/ml), the cytotoxicity increases significantly, especially for WO<sub>3</sub>/GO nanocomposite, indicating that at higher doses, the nanocomposites is significantly disrupt the cells integrity due to the combined effects of membrane damage from GO and oxidative stress from WO<sub>3</sub>. These findings reveal the highest cytotoxicity of nanocomposites, due to the synergistic effects between GO membrane-disrupting properties and WO<sub>3</sub> oxidative stress generation leading to increased ROS production and activation of apoptotic pathways. As a result of this study suggested that GO, WO<sub>3</sub> and WO<sub>3</sub>/GO nanocomposite at higher doses caused cell death. Moreover, the results reveal inside the cells, the WO<sub>3</sub> component can generate ROS, leading to oxidative stress. GO may also exacerbate this process by creating physical interactions with the cell membrane, allowing more ROS to penetrate the cells and causing further damage. Previous studies demonstrated the ability GO and WO<sub>3</sub> to destroy cancer cells. G. Jeevitha et al., was studied the viability of lung cancer (A-549) cell line, by evaluation the cytotoxicity of WO<sub>3</sub>-GO nanocomposite. They found that increasing the concentrations of WO<sub>3</sub> leads to increase in cell death. Moreover, in state of WO<sub>3</sub>-GO nanocomposites, the cell death rate was higher even at minimum concentrations in comparison to pure WO<sub>3</sub> and improved that nanocpmposite has better anticancer activity. Besides, Ngigi, Eric M. et al., demonstrated that Ag-doped PEGylated WO<sub>3</sub> showed better cytotoxicity and anticancer activates towards breast cancer cells (MCF-7). This could be attributed to the apoptosis effects of Ag NPs in the WO<sub>3</sub> and with an increasing in dosage of Ag-doped PEGylated WO<sub>3</sub> [29].

#### *WO<sub>3</sub>/GO nanocomposite induces apoptosis in HT-29 cells*

In order to study the effect of GO, WO<sub>3</sub> and WO<sub>3</sub>/GO nanocomposite on treated cells, DNA damage was used as standard for evaluation apoptotic cells. In this study, AO-EtBr staining was employed to investigate the apoptotic characteristics of the nuclear changes as shown in Supplementary Figure 4 a,b,c,d. After being stained with AO-ETtBr, the untreated cells appear uniformly green color indicating high viability and intact cell membrane, meaning no apoptosis in Supplementary Figure 4 a. whereas apoptotic cells in state of GO- treated HT-29 cells show a noticeable increase in orange\ red color compared to control as shown in Supplementary

Figure 4 b, indicating GO induces moderate cytotoxicity and apoptotic changes. Besides, the ROS in GO result in damage cellular membrane and DNA, causing oxidative stress. In Supplementary Figure 4 c, the  $\text{WO}_3$  shows higher cytotoxicity than GO with strong red/ orange color, due to their ability to produce ROS under visible light leading to oxidative stress mitochondrial dysfunction. In state of HT-29 cells treated with  $\text{WO}_3/\text{GO}$  nanocomposites at specific molar ratio 1:0.5, the most intense become red color, with maximum apoptotic cell death as compared to control cells that had not been treated as shown in Supplementary Figure 4 d. Besides, the nanocomposites exhibit synergistic effects that result in enhance cellular uptake, ROS generation, and DNA fragmentation leading to increasing apoptosis in HT-29 cancer cells.

Supplementary Figure 5 a,b,c,d, shows flow cytometry (Annexin V-FITC/PI) analysis of apoptosis in HT-29 colon cancer cells after treatment with GO,  $\text{WO}_3$  and  $\text{WO}_3/\text{GO}$  nanocomposite at specific molar ratio 1:0.5 compared to the untreated control. According to the flow cytometry data, the Annexin V-FITC/PI was used to determine the cells in quadrant Q3 that were going through apoptosis. In Supplementary Figure 5 a, most cells are in viable ( $\text{Q4}=94.4\%$ ) and minimal apoptosis in Q3 is about ( $\text{Q3}=0.15$ ) confirming normal cell morphology and non-apoptotic. In state of treated HT-29 cells with GO and  $\text{WO}_3$ , respectively for 24 h at an  $\text{IC}_{50}$  concentration are presented in Supplementary Figure 5 b, c. For GO, the percentage of apoptotic cells increase to ( $\text{Q3}=81.8\%$ ) and was decreased in variable cells. This indicates that GO trigger apoptosis. While the  $\text{WO}_3$  treated HT-29 cells, the percentage of apoptotic cells increase 84.6 % demonstrating higher cytotoxicity than GO. When the cells treated with  $\text{WO}_3/\text{GO}$  nanocomposite, the results displayed highest apoptosis percentage is about ( $\text{Q3}=93\%$ ) as shown in Supplementary Figure 5 d, confirming a synergetic effect between GO and  $\text{WO}_3$ . Besides, the possible mechanism of this state is represented by the  $\text{WO}_3/\text{GO}$  nanocomposite act as oxidative stimuli, which in turn supports DNA damage, resulting in massive early apoptosis with minimal necrosis. The findings reveal that viability of HT-29 cells was potentially decreased. Besides, in previous work, Yousefi et al. [35] showed that the synergic experiment of  $\text{ZnO}/\text{CNT}@\text{Fe}_3\text{O}_4$  nanocomposites enhanced the cytotoxicity effects of imatinib on K562 cells b indicig a ROS-dependent apoptotic cells death apparently by uprgulation of FOXO3a and SIRT1 in CML-derived K562 cells [35, 36].

#### *$\text{WO}_3/\text{GO}$ nanocomposite induces ROS generation*

Supplementary Figure 6 a,b,c,d represents intercellular reactive oxygen species (ROS) generation in HT-29 colon cancer cells after treated with GO,  $\text{WO}_3$  and  $\text{WO}_3/\text{GO}$  nanocomposites at specific molar ratio 1:0.5, analyzed by DCFDA 2,7-dichlorofluorescein diacetate ) fluorescence assay. In state of control untreated HT-29 cells, the fluorescence intensity is minimal; indicating normal ROS levels consistent with healthy cellular metabolism and no oxidative stress is detected as shown in Supplementary Figure 6 a. A significant shift toward higher DCF fluorescence intensity is observed in Supplementary

Figure 6 b and c after treated with GO and  $\text{WO}_3$  compared to control cells. The increasing of ROS levels was seen in HT-29 cells that had been treated with GO and  $\text{WO}_3$ . This enhanced ROS production can lead to oxidative damage to proteins, lipids and DNA, initiating apoptotic. When the liver cancer cells were treated with  $\text{WO}_3/\text{GO}$  nanocomposites, the outcomes revealed the levels of ROS was enhanced as shown in Supplementary Figure 6d. The fluorescence intensity resulting from ROS was found to be higher after treated with  $\text{WO}_3/\text{GO}$  nanocomposites as compared to control untreated cells. These findings confirm the large surface area of GO that improve cellular uptake of  $\text{WO}_3/\text{GO}$  nanocomposites and strong catalytic ROS-generating activity of  $\text{WO}_3$ . Moreover, this combination leads to intracellular oxidative stress, which is consistant with the high apoptosis percentage seen in Annexin V assay.

## Discussion

In conclusion, in this study,  $\text{WO}_3$  and  $\text{WO}_3/\text{GO}$  nanocomposites at molar ratio 1:0.5 of  $\text{WO}_3$ : GO were effectively formed using a hydrothermal process. The characterization results reveal that  $\text{WO}_3$  retained its monoclinic phase and the introduction of small amount of GO had little effect on the morphology, structure, optical and composition of tungsten oxide as shown in the FESEM that reveal spherical particles, extensive agglomeration with ranging in size from 28.42-81.63 nm. In UV-Vis analysis, the notable reduction of the band gap is attributed to the strong interfacial interaction between  $\text{WO}_3$  nanoparticles and GO sheets, leading to the formation of W-O-C bonds, as confirmed by PL, FTIR and Raman analysis. Zeta potential exhibit a slight increase from 35 eV to 36 eV after introduction GO to  $\text{WO}_3$ , indicating that the nanocomposites retained its high negative surface charge. This is attributed to the fact that the GO layers did not occupy or neutralize the surface charge sites of tungsten oxide. Overall, these findings suggest that  $\text{WO}_3/\text{GO}$  nanocomposites at specific molar ratio 1:0.5 of  $\text{WO}_3$ : GO exhibit superior anticancer potential through the induction of apoptosis, primarily facilitated by ROS-dependent pathways. The synergistic interaction between the GO and  $\text{WO}_3$  nanoparticles enhances the therapeutic efficacy while maintaining good dispersion and cellular interaction properties. Besides, the fact that  $\text{WO}_3/\text{GO}$  nanocomposite at specific molar ratio 1:0.5 of  $\text{WO}_3$ : GO shows higher toxicity than either GO or  $\text{WO}_3$ , reveals that the combination of GO and  $\text{WO}_3$  nanoparticles enhance their biological activity, potentially through mechanism such as increased cellular uptake and ROS.

## Author Contribution Statement

(N.K.O), (D.S.A): Investigation, (N.K.O): Validation, (D.S.A), (A.M.A) (N.K.O) : Data curation, (M.S.J), (D.S.A):Writing-original draft. (N.K.O): Methodology, (N.K.O), (A.M.A),(M.S.J): Formal analysis, (A.M.A),(D.S.A),(M.S.J):Resources, (N.K.O) (D.S.A): Writing – review & editing, (D.S.A) , (M.S.J): writing reviews and editing. All authors reviewed the manuscript.

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## Data availability

All data were represented in this published article.

## Conflicts of Interest

The authors declare no conflict of interest.

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