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Cytotoxicity and apoptotic activity of magnesium orotate nanoparticles towards human hepatoma cell line $HepG_2$

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The purpose of this study was to enhance the therapeutic effect of magnesium orotate (MO) via loading it to gum arabic nanocarrier. The magnesium orotate-loaded gum arabic nanoparticles (MOGANPs) were prepared by high pressure homogenizer. The freeze-dried powder of nanoparticles was characterized using X-ray Diffractometry (XRD) and Transmission Electron Microscopy (TEM). The obtained nanoparticles were spherical in shape with diameters ranged from 30 to 50 nm. The cytotoxicity of MOGANPs was determined against human hepatocellular carcinoma HepG2 cell line using 3-(4,5-dimethylthiazol-2-yl) 2,5-diphenyl tetrazolium bromide (MTT) assay which showed an enhanced efficacy of MO nano-carried with gum arabic when compared to pure compound alone. Moreover, the results revealed that MOGANPs induced apoptosis in the treated HepG2 cells and thus it is promising formulation, in addition, further anticancer studies are needed.

Keywords*:* Magnesium orotate; Gum; Liver cancer; Cytotoxicity.

1. INTRODUCTION

Recently, there has been renewed interest of research and applications of nanoparticles due to their capacity of reducing toxicity of chemotherapeutic agents [1-4].The main advantages of nanoparticles is the improvement of efficacy of drugs towards human cells and diagnostic of diseases such as the use of gadolinium nanoparticles for theranostic applications [5-12]. Due to the rapidly developing domain of nanomedicine , many bioactives loaded nanocarries systems are under investigation to reduce toxicity for cancer therapy [13-15]. Magnesium orotate is a complex

of magnesium and orotic acid. A recent study has reported the antitumor and anti-inflammatory effects of orotic acid. Researchers attempted to evaluate the impact of magnesium orotate on cancer therapy [16]. Gum arabic (GA) also known as *acacia gum*, enhanced the *in vivo* stability of nanoparticles [17].The MOGANPs nanoparticles were characterized using (XRD), (TEM), and cell cycle arrest was determined using cytometry analysis.

2. METHODS OF ANALYSIS

2.1. Characterization of MOGANPs nanoparticles

X-ray diffractogram patterns were recorded using CuK_α radiation, λ 1.5406 A° at 2θ =20°-60° and scan speed of 2° per minute. Transmission electron microscopy was carried out to visualise the morphology, size distribution, and determine the homogeneity of nanoparticles.

2.2. MTT Assay

Two hundred microliters at a concentration of 1×10^5 cells/mL of cancer cell line suspension was added to each well of a 96-well plate and incubation for 24 h. After that, the cells were treated with MO and MOGANPs at various concentration of 1.56-100µg/mL then incubation for 72h at 37 °C in 5% CO_2. Then, 20 µl of of 5mg/mL of MTT solution was added to each well. The plate was then incubated for 4 h at 37 °C in 5% CO_2 until formazan product was developed. The absorbance readings were recorded at 570 nm using ELISA reader.

2.3. Cell cycle analysis

The HepG2 cells were seeded at 1×10^6 cells/well for 24 h in a 6-well plate. The cells then were treated 12.5 and 25 µg/ml. After that, 250 µL of trypsin buffer solution was added to the tubes including harvested cells and incubation for 10 min at room temperature. 200µL of trypsin inhibitor and RNase buffer was further added to each tube and incubation for10 min. 200µL of PI stain solution was added then followed by incubation in the dark for 10 min. The PI fluorescence was measured under 620-640 nm excitation using flow cytometry analysis.

2.4. Statistical Analysis

The analysis of variance was carried out using the ANOVA technique ($p < 0.05$).

3. EXPERIMENTAL ANALYSIS

The preparation of MOGANPs nanoparticles was performed using freeze-drying method in 1: 1 molar ratio by dissolving 0.94g of MO in 50 mL of deionized water under mild agitation for 20 min. MO solution was added dropwise to GA solution with vigorous under stirring for 72 h. The dispersion was subjected to a high pressure homogeniser (Avestin Canada) at a pressure of 1000 bar for seven cycles followed by freeze-dried for 72 h.

4. RESULTS AND DISCUSSION

4.1. X-ray Diffraction

The patterns of MO, GA, Physical mixture, and MOGANPs nanoparticles are depicted in figure 1. A sharp diffraction peaks were revealed at several diffraction angles 25.94°, 27.96°, and 37.82°, illustrating the crystalline nature of MO. The MO peaks were totally vanished due to the complexation that happened between MO and GA (figure 1d).

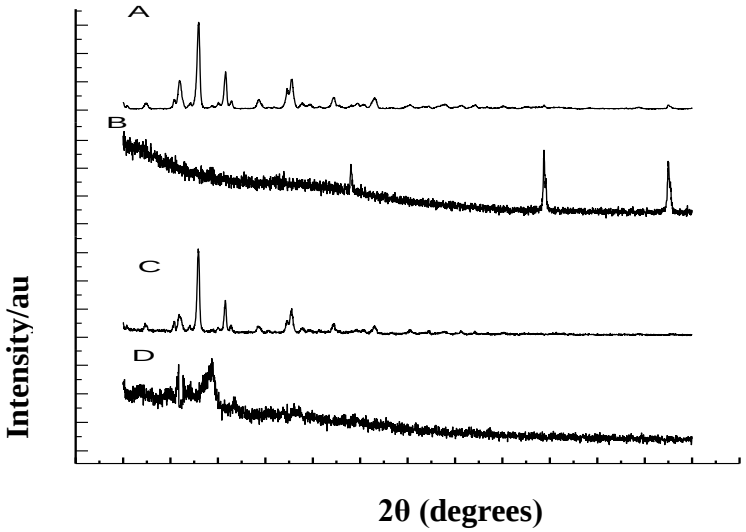

Fig.1 X-ray diffractogram: (A) MO, (B) GA, (C) physical mixture,(D) MOGANPs

4.2. Transmission Electron Microscopy (TEM)

Nanoparticle prepared presents approximately spherical elliptical shape as individual particles with narrow size distribution ranging from 21 to 45nm.

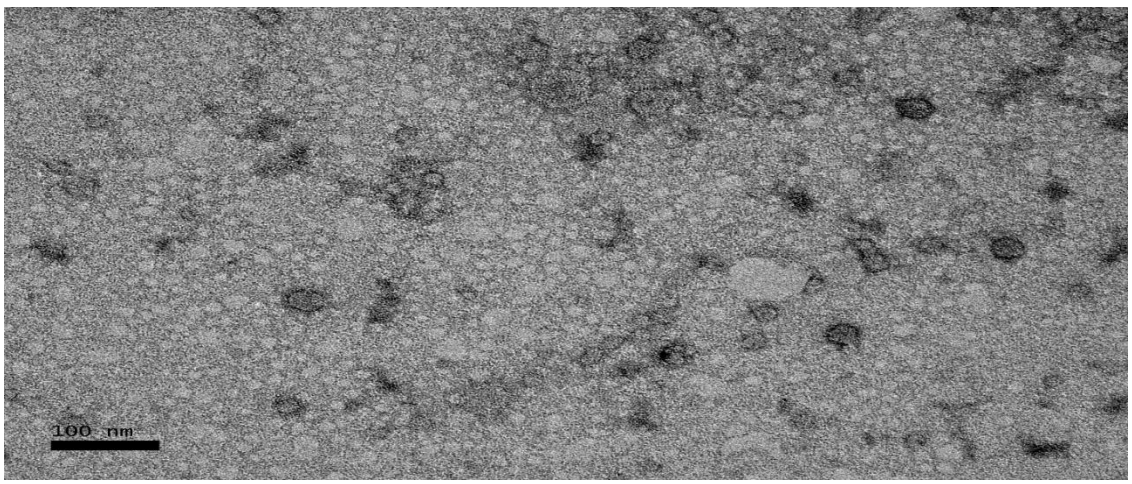

Fig 2. Transmission electron microscopy of MOGANPs nanoparticles

4.3. Cytotoxicity assay

The anticancer activity of MOGANPs was slightly higher than the free MO due to the galactose group of GA polymer [18] (p value < 0.05).

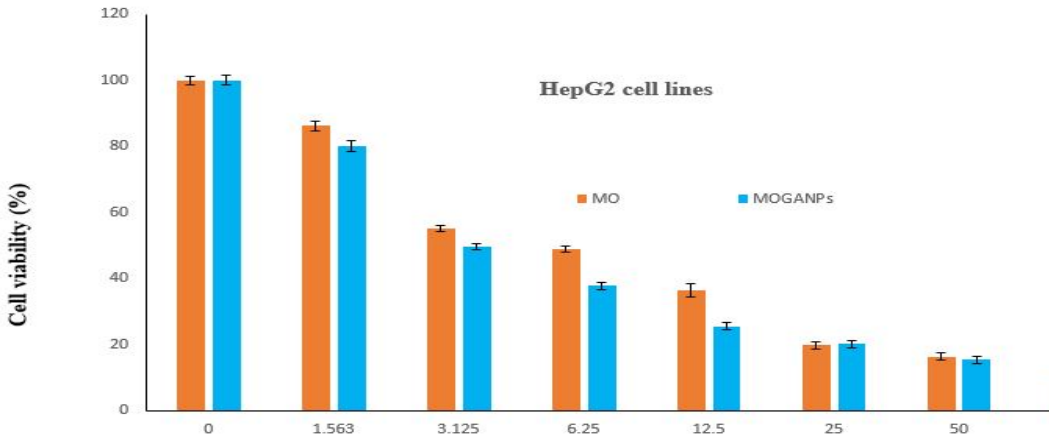

Fig.3 Cytotoxicity activity of MO, MOGANPs exposed to HepG2 cell lines

Table 1 IC_{50} (µg/mL) values of MO and MOGANPs nanoparticles on HepG2

Cell lines	MO	MOGANPs
HepG2	24.20 ±0.1	12.08±0.2

4.4. Induction of cell cycle analysis by MO and MONPs nanoparticles

MOGANPs nanoparticles at 12.5 µg/mL increased both G_0/G_1 (7%) and S arrest (19%) in addition to reduce the total number of cells in G_2/M phase which had 28 % decrease when compared to control. Moreover, MOGANPs nanoparticles induced apoptosis which had 13.9-fold increase at 12.5 µg/mL, and 28-fold increase at 25.0 µg/mL, when compared to control. For 24-hour treatment, MOGANPs nanoparticles at 25.0 µg/mL induce G_0/G_1 arrest which had 13% increase, and 53% and 26% decrease at S and G_2/M phases, respectively when compared to control (Figure 4). Also, pure MO dissolved in DMSO induced apoptosis which had 1410 % increase at 12.5 µg/mL, and 1926 % increase at 25.0 µg/mL when compared to control.

For 24-hour treatment, MO induced G_0/G_1 arrest which had 8 % decrease at 12.5 µg/mL, and 3% decrease at 25.0 µg/mL when compared to control. MO showed no significant effect ($p \geq 0.05$) on the cell cycle at S-phase in HepG2 cancer cells after treatment with both 12.5 and 25.0 µg/mL when compared to control. Besides, MO reduced cell numbers at G_2/M which had 30 % decrease at 12.5 µg/mL, and 67 % decrease at 25.0 µg/mL, when compared to control (Figure

4).

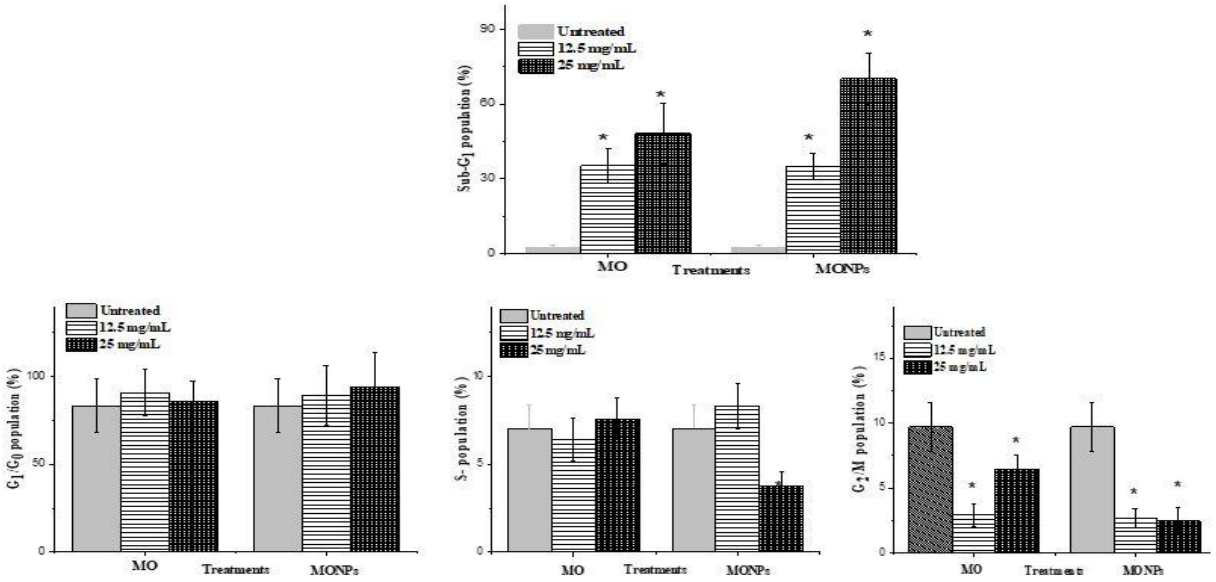

Fig 4 Effect of MOGANPs nanoparticles and MO (IC_{50} values) on cell cycle distribution of HepG2 liver cancer cells determined by flow cytometry

5. CONCLUSION

The results confirmed the incorporation of MO into GA cavity. This study has found that MOGANPs nanoparticles can be considered as a potential candidate compound for the evaluation of prevention and cancer treatment. Thus, MO, either pure or MONPs, had effect on cell cycle of HepG2 cancer cell. However, the main cause of their antiproliferaive activity on HepG2 cancer cell was apoptosis as it got the largest change when compared to other phases.

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