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Research Article

Analytical study and cytotoxicity test of azo dye in vitro

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Abstract

Analytical study of the azo dye (HA) showed that it can be affected by their solubility and the dielectric constant (D) of solvents. The MTT assay was used to test the effectiveness of HA against MCF-7 cell line, which indicated their sensible inhibition rates in contrast with the control (C). The assay also showed the great ability of the (HA) azo dye in destroying the MDA-MB231 cells and how it affected their viability%, which was better than their ability against the MCF-7 cell line. And this activity increased with the increase of their concentration. Statistical analysis gave $P<0.05$. Furthermore, the liver tissues of designated mice were normal in contrast with control (C). Therefore, after seeing its effectiveness as shown in the study, we recommend the specified HA to be a novel drug, that contains two medicines, (levothyroxine and paracetamol) in their structure. The new drug can give three effects at the same time, represented on thyroid medication, pain relive and anti-cancer effect.

Keywords Analytical study, MTT assay, cancer cell line, Rate of inhibition, Levothyroxine, Paracetamol.

Introduction

Cancer disease commonly treated via using chemotherapy as it common health problem (Johnson et al., 2015; Siegel et al., 2014). chemotherapy was related to different side effects. However, levothyroxine usually prescribes thyroid medication, that is used to treat hypothyroidism and goiter

(Wu et al., 2018). It has been effect the thyroid hormone, which are responsible for breast cancer cell propagation. While, the paracetamol is one of the most popular drug, that typically used as a pain-relieving and antipyretic drug everywhere in the world, that available without prescription (Jóźwiak-Bebenista & Nowak, 2014). Combination between paracetamol and L-thyroxin drugs used to prepare azo dye (HA) (Ali et al., 2019). The later has been diagnosed and tested against cancer, (MDA-MB231) cell line in vitro. In the current study analytical property of HA and its capacity to reduce the growth of MCF-7 cell line tested and then compared with MDA-MB231 cell line (Ali et al., 2019).

Laboratory method

The effect of solvents, (250-450 nm) using each of ethanol, methanol and DMSO, (1 x 10-4 M) studied through using of a spectrophotometer. However, the cancer cells were attained from the iRAQBiotech, Basrah, Iraq and then maintained (Al-Shammari et al., 2015).

The cytotoxicity Assay

The MTT cell viability assay of the MCF-7 cell line was done at 1 × 104 cells/well (Al-Shammari et al., 2019). After 24h or a confluent monolayer was attained, cells were treated with HA with final concentration (50,100, 250, 500 and 1000 μg/ml). The cell viability was measured after 72 hrs (Freshney, 2015).

The laboratory mice

The experimental mice, (aged 10-12 weeks and weight 21-23 g) (Al-Maliki, 2000) were divided into two groups, (three mice injected with 0.1 ml of HA (0.00124 mg / kg) and three attended as control group, (injected with 0.1 ml of normal saline). The groups were left for 15 days until dissected of two mice, which selected randomly from each group. The CBC blood test (Al-Maliki, 2000), was done for each mouse. the liver tissues of selected mouse injected with HA and from C were examined.

Results and Discussions

Analytical study approved on HA, (Fig. 1) and the solvent effect of HA was deliberated.

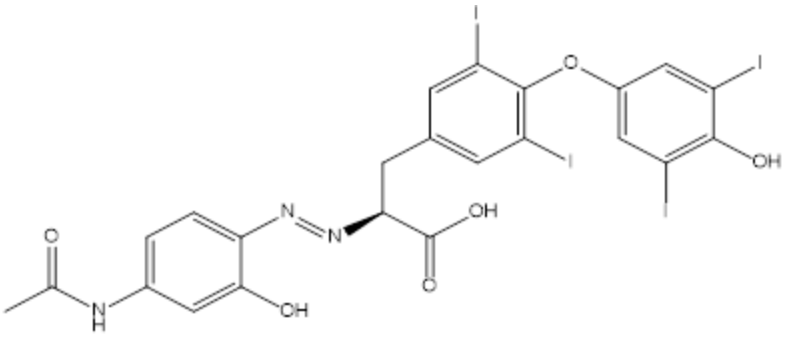

Fig. 1. The HA structure (Ali et al., 2019).

The solubility of HA in each of ethanol, methanol, and DMSO were represented in Fig. (2) below.

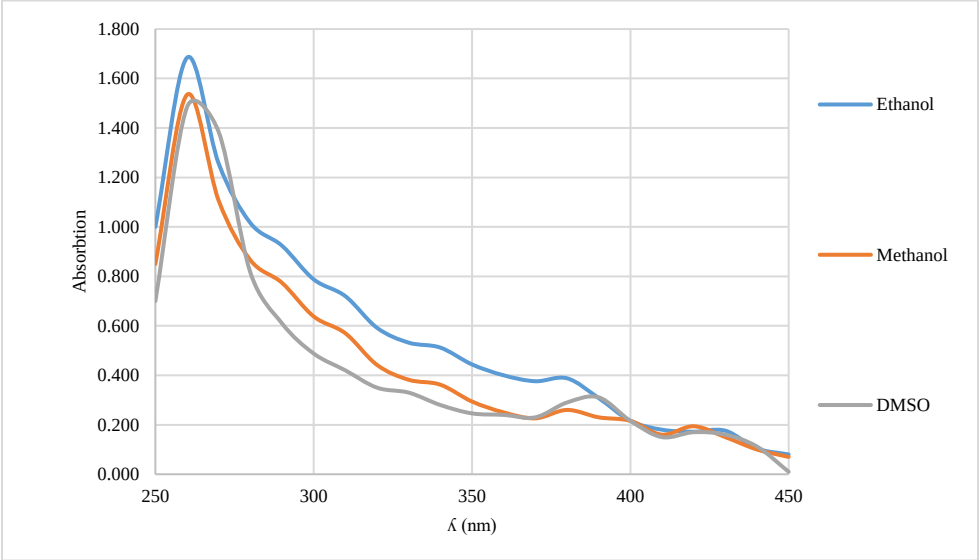

Fig. 2. Influence of three solvents in HA.

Fig. 2 above shows varied values of ʎmax, (Table 1) which related to π-π* and n -π* transitions. Revealed a slight red shift towards a higher wavelength using DMSO, owing to designate their affected through their solubility and the dielectric constant (D),

Table 1. HA`s UV-visible spectrum in three diverse solvents

Solvent	$ʎ_{max}$(nm)	$ε_{max}(\times 10^{-4})$
Ethanol	430	0.18
Methanol	420	0.19
DMSO	420	0.17

The results are specifying the effect of D of ethanol, methanol and DMSO, (24.55, 32.70 and

46.68 respectively) in HA. Therefore, the linear relationship of each three selected solvents were calculated using different selected functions, (Fig. 3).

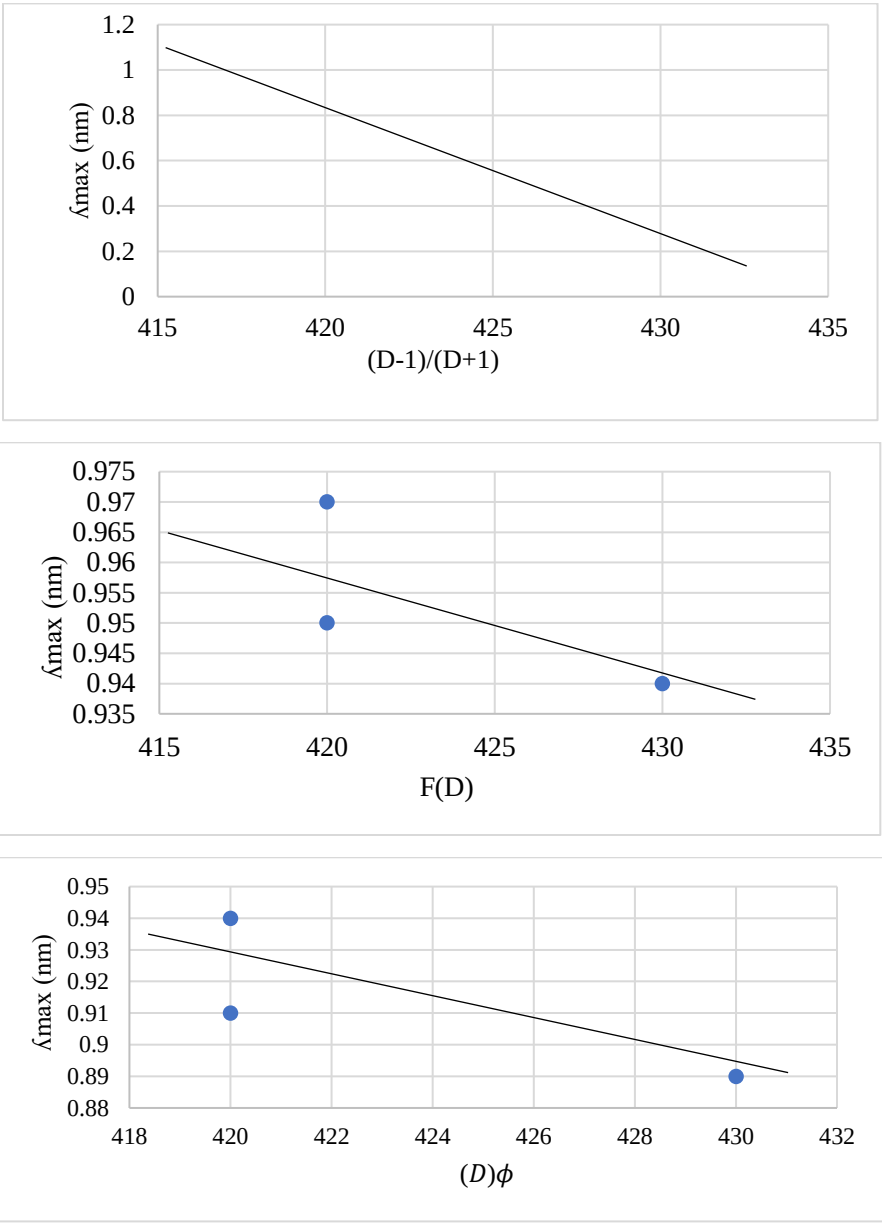

Fig. 3. Relation between Some functions and λ_{max}(nm) of the designated solvents.

The outcomes from figs. above can have indicated, that there is no deviation from the linear relationship, which is due to give details can explain the effect of D in control the shift of the absorption beaks.

Determination of the cytotoxicity test shows that mtt assay method is that HA has effects on the MCF-7 cell line of breast cancer, and this was followed by calculation of inhibition rates% of

infected cells using different concentrations of HA, (50, 250, 500, 750 and 1000) as seen Fig. 4.

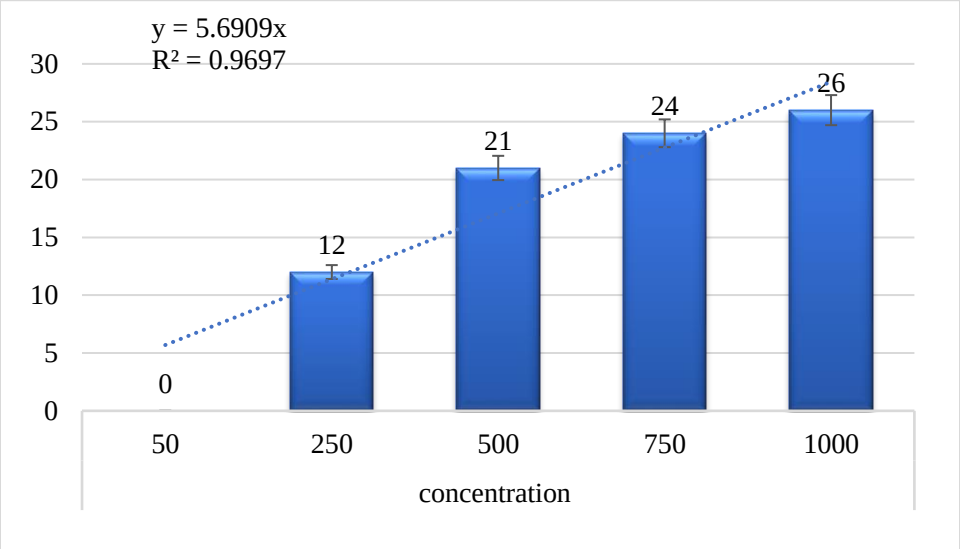

Fig. 4. Rate of inhibition % of (1) using selected concentrations.

Morphologically, (Comşa et al., 2015; Loibl et al., 2021) the viability assay was made to each of MCF-7 and MDA-MB-231 cell lines in order to compare the effect of different concentrations of HA in each, (Fig. 5).

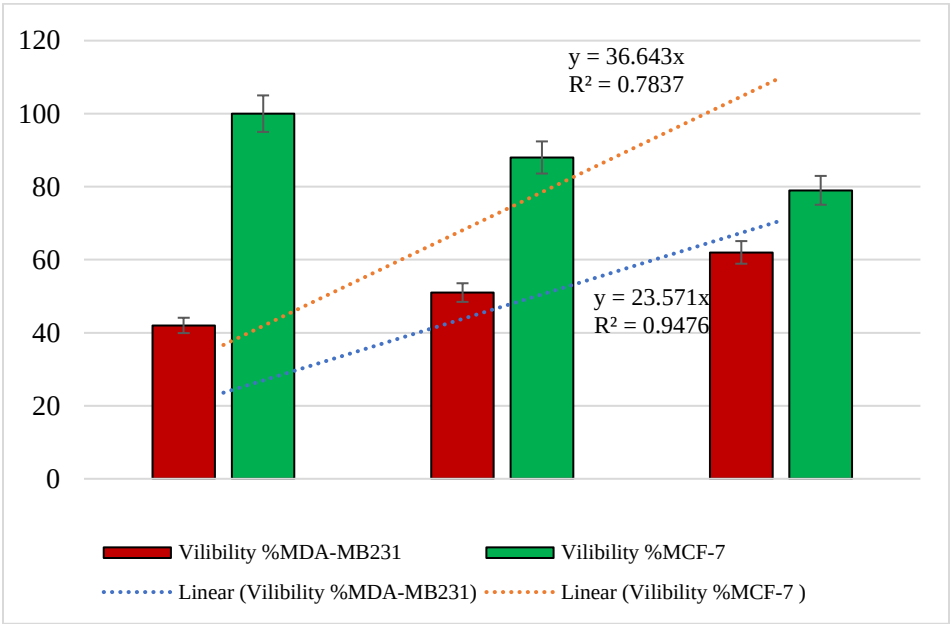

Fig. 5. The cell viability of HA in two different breast cancer cell lines.

The result reveals, that the effect of HA in MDA-MB231 cell line better than in MCF-7 cell line. This effect situated as a direct proportion to the concentration. The CBC blood tests, of mice injected with HA were established in contrast with the C. So, the statistical analysis using SPSS version 20 showed $P<0.05$. Furthermore, the liver tissues of designated mouse from the group, that injected with HA were studied in contrast with the liver tissues of the selected mouse from group (C), (Fig. 6 (a & b) respectively).

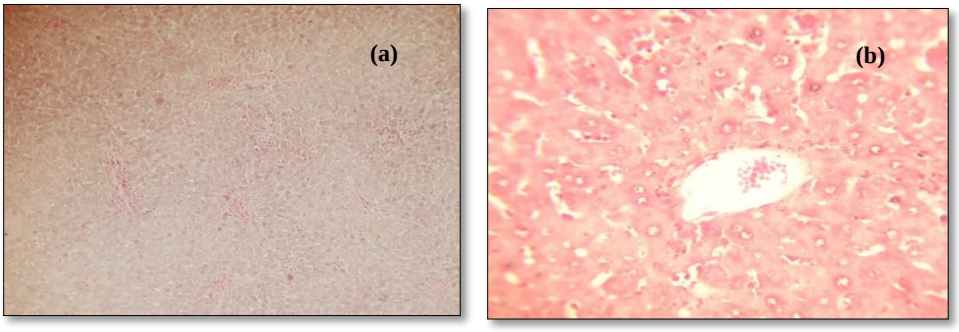

Fig. 6. Effect of HA in the liver tissues of designated mouse, (a) in contrast with the liver tissues of mouse from C (b).

Fig. 6 shows that the liver tissues of the designated mouse, (a) and the selected mouse from C (b) were normal. Our decision, that the HA can distinguished between MDA-MB231 and MCF-7 cell lines in vitro with high biological activity as anti-cancer drug. This activity was effected by rise of the concentration. Moreover, the HA did not affect the liver tissues and contain thyroid medication and pain relive in their structure.

Conclusion

The HA expanded virtuous colour, harmless effect, effected by polar solvents and obligated good ability in destroying MDA-MB231 cells and affect their viability%. The HA effected the MDA-MB231 cell line better than the MCF-7 cell line. Our conclusion, that the HA can differentiate between MDA-MB231 and MCF-7 cell lines and its activity increased as a direct proportion to its concentration. Moreover, HA did not affect the liver tissues due to suggested HA as new drug represented three effects in one, (thyroid medication, pain relive and anti-cancer drug).

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