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Original Research Article

The reaction of curcumin-hydrazine and its effect on bone marrow mesenchymal stem cells

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ABSTRACT

In this work, the interaction between curcumin and hydrazine was studied in three different conditions: In the presence of ultrasonic waves, in the absence of ultrasonic waves, and while refluxing and the effects of the produced materials on the livability of bone marrow mesenchymal stem cells were investigated. Various methods including FT-IR, ^{1}H NMR and ^{13}C NMR were utilized to characterize the material. The results revealed that cell treatments with 40 µM dose of all three samples after 24 h were caused to the more biological ability of rat bone marrow mesenchymal stem cells (MSCs). This indicated that these compounds (curcumin-hydrazine) are effective in the livability of bone marrow mesenchymal stem cells (MSCs).

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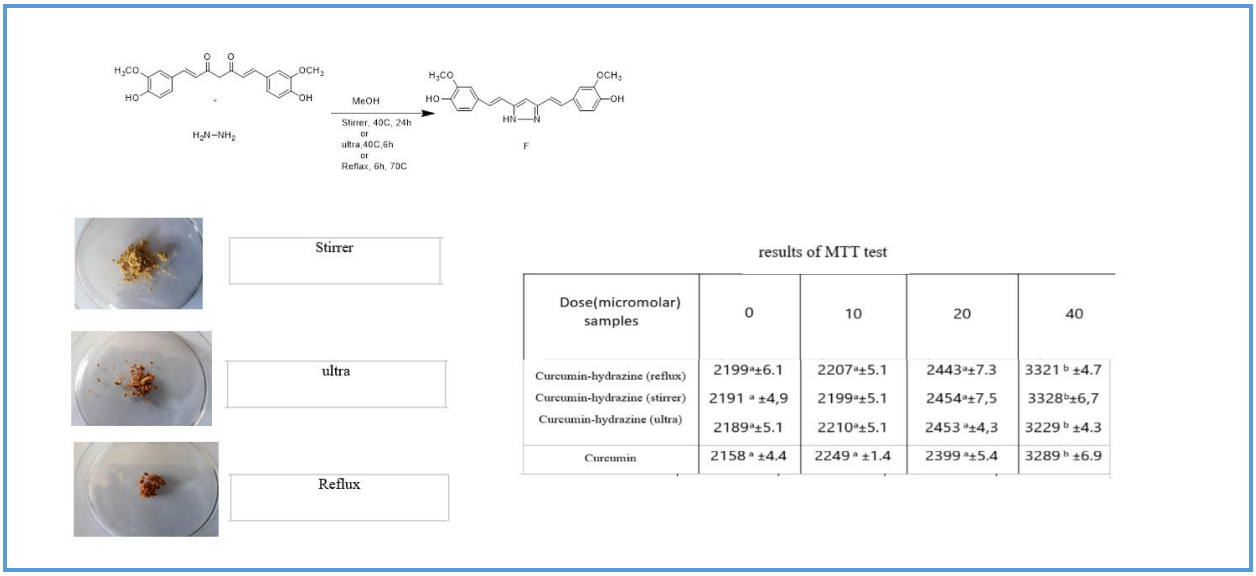
Graphical Abstract



Introduction

Cancer is one of the chronic and non-communicable complications which can occur in the form of various diseases. It is a genetic disease that has 277 different types [1]. Like any other chronic diseases, it has no personal, age or racial dependency and affects the community health. So that it is the second leading cause of death in developed countries after cardiovascular disease and the third one in the least developed countries. Cancer initiates when cells are losing their ability to divide and grow normally such that they invade and destroy healthy tissues. Consequently, a mass called "tumor" arises from the destruction of healthy cells and the aggregation of cancer ones [2].

Turmeric, also called Curcuma, is the underground stem of a plant of the ginger family and in various countries, it is widely used as a food seasoning [3]. Turmeric traditionally has been used in some countries like India, Iran and China for treating various complications such as gastric ulcer, jaundice, colds, sore throat, flatulency, skin diseases (e.g. Psoriasis), pulmonary diseases (e.g. asthma and allergies), liver diseases, rheumatism, and exploited for wound healing and blood purification [4–11].

Curcumin or dipherolyl methane ($C_6H_{12}O_4$) with the chemical formula of (6E, 1E)-1,7-bis-heptadiene-1,6-(4-hydroxy, 3-methoxyphenyl) 5,3-dione, is the most important biologically active compound of turmeric which makes up 2-8% of its weight [12, 13]. Curcumin is also a hydrophobic polyphenol derived from the turmeric rhizome. It has a well-favored yellow color that makes it possible to be used as a coloring agent in the food industry. Curcumin with a molecular weight of 368 daltons has a melting point of 177-177 °C. Although this powdery crystalline compound is insoluble in water, can be dissolved in some solvents such as ethanol, acetone and methanol. In fact, curcumin dissolves in water hardly. This compound is highly sensitive to pH change and is very poorly absorbed in the digestive tract [14–16].

Curcumin has a great ability in trapping free radicals and can act as an inhibitor of cancer initiation. By inducing apoptosis (scheduled cell death), it inhibits the proliferation of cancer cells. Apoptosis occurs with the fragmentation

of DNA and stopping the cell cycle [17, 18]. So far, curcumin has been used separately or combinatorically in some clinical trials. Some clinical studies that use curcumin to treat cancer patients have found promising consequences [19, 20]. The most successful results were obtained in the treatment of inflammation and external lesions after surgery in patients suffering from pancreatic cancer [21–24]. Experiments are currently focused on the lung, ovary, breast, mouth, and tooth cancers [25–31]. In the recent years, research groups have conducted numerous studies to improve the biological sustainability of this compound and increase its effectiveness. Some investigations have also been conducted on the exploiting dendrimer and curcumin in medicine, pharmacy and cancer treatment [32–35].

Liang *et al*. [36, 37] reported that the presence of a 1,3 dicarbonyl site of curcumin leads to the instability and poor bioavailability of curcumin under the physiological structure and the deletion of the site could contribute to the enhancement in stability of curcumin [36, 37]. A modification at this site is not only simple to achieve, but also has indicated a superior activity consistently. It is one of the most attractive precursors for the incorporation of a heterocyclic group to synthesize stable derivatives. The presence of the ring not only provides stability to the molecule but is also known to display enhanced solubility which enables oral absorption and bioavailability [38].

Reddy *et al*. [39] elaborated that the incorporation of ring to curcumin leads to derivatives where the central 1,3-diketo-enol is masked or made rigid, which in turn improves the anti-tumor and the anti-proliferative activity [39]. Another analysis reported by Jankun *et al*. when it was pointed out that the ring cyclization of the central part of the compound and introduction of a heteroatom lead to the synthesis of analogues/derivatives with higher anti-angiogenic and anti-tumor activity [40]. In our initial research [41], we found that the modification at the diketo moiety of curcumin with poly (Propylene imine) dendrimer led to the preparation of novel derivative with superior soluble in aqueous solution. In order to investigate the diversity of curcumin warhead and the impact of different structures, some of compounds were designed.

In the present study, the curcumin-hydrazine interaction has been characterized and the effects of its product on the bone marrow mesenchymal stem cells have been studied.

Experimental

Materials and methods

Curcumin and hydrazine methanol were purchased from Merck, Germany. The BRUKER 400 (MHz) Nuclear Magnetic Resonance (NMR) device was used to identify the structure of the products, the number and types of carbon and hydrogen. PerkinElmer RX I model by KBr tablet was used to identify product functional groups. The purity of the products and the reaction progress were measured by thin-layer chromatography (TLC) and UV spectrophotometer. Silica gel or plate chromatography was used to separate and purify some products. Model sonication bath (Sonica) was used to dissolve the materials in the solvent and homogenize them. Model magnetic stirrer (Arec) was used to stir and heat the solutions. Sartorius TE313S digital scale weighing 0.001 decimal places was used to weigh the solids. Model centrifuge (Universal pars azma) was used to separate solid and liquid phases from suspension solutions. In this research, BRC-9 breast cancer cell line was purchased from the Pasteur Institute of Iran. Cells were cultured in RPMI (Roswell Park

Memorial Institute) medium (USA,Gipco) containing 10% Fetal bovine serum (FBS) (USA,ATCC), 2 mM L-glutamine solution and penicillin-streptomycin (Gibco, Germany). Cells was plated in 25 cm 2 flasks and incubated at 37ºC with atmosphere of 5% CO_2. Two days after culture initiation, the first medium replacement was performed and then medium was changed two times per week till the bottom of the flask was covered with the cells (till confluency). The cells were trypsinized (trypsin-EDTA, Gibco, Germany) and passed to another culture flask as the first passage and then the cultures were expanded through two additional subcultures which were used for further investigation.

Curcumin-hydrazine synthesis in absence of ultrasonic waves

In this experiment, curcumin (0.5 mmol, 0.18 g) was agitated in 16 mL of methanol at a temperature of 60 °C for 1 h using an ultrasonic bath. After complete dissolution, hydrazine (1 mmol, 0.0136 mL) was added to the reaction mixture. The solution was then stirred for 24 h at 40 °C. After completion of the reaction, the solution was dried at room temperature, and the product purity was determined by thin-layer chromatography (TLC) technique using acetone/hexane mixture (60:40). FT-IR, ^{1}H NMR and ^{13}C NMR techniques were also used for product characterization.

Curcumin-hydrazine synthesis in presence of ultrasonic waves

In this experiment, curcumin (0.5 mmol, 0.18 g) was agitated in 16 mL of methanol at a temperature of 60 °C for 1 h using an ultrasonic bath. After complete dissolution, hydrazine (1 mmol, 0.0136 mL) was added to the reaction mixture. The solution was then subjected to ultrasonic bath vibration for 6 h at 40 °C. After completion of the reaction, the solution was dried at room temperature, and the product purity was determined by thin-layer chromatography (TLC) technique using acetone/hexane mixture (60:40). FT-IR, ^{1}H NMR and ^{13}C NMR techniques were also used for product characterization.

Curcumin–hydrazine synthesis while back distillation (reflux)

In this experiment, curcumin (0.5 mmol, 0.18 g) was agitated in 16 mL of methanol at a temperature of 60 °C for 1 h using an ultrasonic bath. After complete dissolution, hydrazine (1 mmol, 0.0136 mL) was added to the reaction mixture. The solution was then refluxed for 6 h at 70 °C. After completion of the reaction, the solution was dried at room temperature, and the product purity was determined by thin-layer chromatography (TLC) technique using acetone/hexane mixture (60:40). FT-IR, ^{1}H NMR and ^{13}C NMR techniques were also used for product characterization.

Isolation and culture of rat bone marrow mesenchymal stem cells

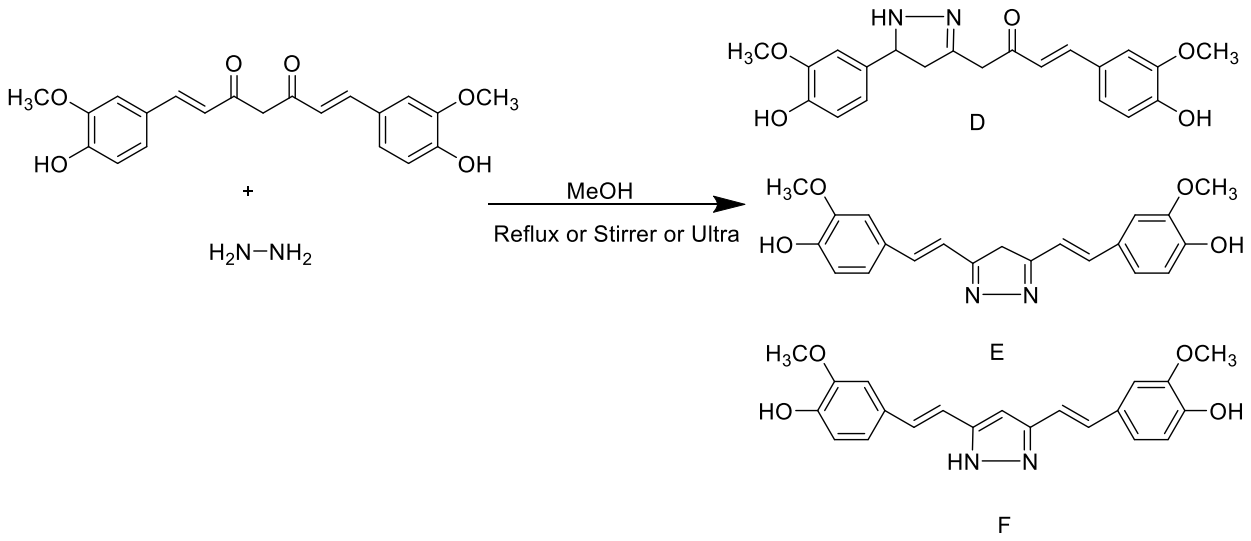
In the present study, wistar rats (6-8 weeks old) were purchased from Razi institute (Tehran, Iran) and kept in the animal house under standard condition of light and food and were treated according to the institutional guidelines for animals' care. The animals were sacrificed by excessive chloroform inhalation. After that, their tibia and femur bones were removed and cleaned from the adherent soft tissue. Then the two ends of the bones were cut off and bone marrow was flashed out using 2 mL DMEM (Dulbecco's Modified Eagles Medium, Gibco, Germany) supplemented with 10% FBS (Fetal Bovine Serum, Gibco, Germany) and penicillin-streptomycin (Gibco, Germany). Bone marrow content was centrifuged at 1200 rpm

for 5 min and resuspended in DMEM containing 15% FBS and then plated incubated in 25 cm^2 flasks at 37 °C with atmosphere of 5% CO_2. Two days after culture, medium was changed two times per week till confluency. The cells were trypsinized (trypsin-EDTA, Gibco, Germany) and passed to another culture flask as the first passage. In order to purification of the MSCs, passage of cells was repeated three times which were used for further investigation.

Result and Discussion

The structure of curcumin-hydrazine reaction product obtained under different experimental conditions was characterized using infrared (IR) and nuclear magnetic resonance (NMR) spectroscopy methods. Expected probable products are shown in Scheme 1. Based on the spectral data discussed below, structure will be accepted.

The product of curcumin-hydrazine interaction by the molar ratio of 1:1 in the absence of the ultrasonic waves is a beige-colored dry powder with a melting point of 67 °C (Figure 1 shows an image of the product). In the FT-IR spectrum, specific absorption bands can be seen in wave numbers of 2916.96 cm^{-1}, 1597.64 cm^{-1}, (1513.82 and 1450.92) cm^{-1}, (1351.55, 1450.00 and 1124.24) cm^{-1} that belong to tensile vibrations of CH, aromatic and aliphatic rings, C=N of imine and heterocyclic CN, respectively (ESI, Figure S2).



Scheme 1. Probable product

Figure 1. Images of curcumin-hydrazine reaction products under different reaction conditions

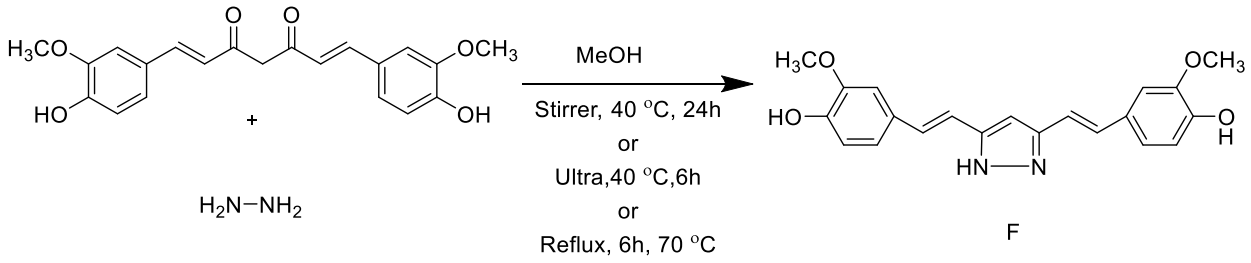
The proton NMR spectrum was obtained in DMSO and the solvent hydrogens appeared at the peaks of 1.06 to 2.50 ppm (ESI, Figure S3). The maximum point of absorption bands originated from phenolic hydroxy, hydrogens of aromatic rings, CHs, hydrogens of methoxy groups, CH_2 have appeared at 6.734 ppm, 6.606 ppm, (3.468, 3.925) ppm and 3.374 ppm, respectively. The carbon NMR spectrum was obtained in DMSO solvent, too, and the absorption peaks of the solvent carbons have appeared in the range of 39.33 to 40.58 ppm (ESI, Figure S4). The absorption peak of methoxy CH_3 has appeared at 55.93 ppm. The peak originated from vinyl and methyl aromatic ring carbons; CHs has appeared at 110 ppm. The peaks of the fourth type carbons of imine (C=N) have appeared in the absorption band of 147 ppm. The disappearance of the carbonyl peak indicates that the carbonyls have reacted with NH_2 of hydrazine and thus the reaction product has an F structure.

The dried product of curcumin-hydrazine interaction with a molar ratio of 1:1 in the presence of ultrasonic waves is a light orange powder which has a melting point of 84 °C. In the IR spectrum of this product, several absorption bands have appeared in the wavenumbers of 3197.38 cm^{-1}, 2917.83 cm^{-1} and 1601.48 cm^{-1}, which belong to the tensile vibrations of phenolic OH, aromatic and aliphatic CH and C = N of imine (ESI, Figure S5) respectively. Also, the bands appeared in the wavenumbers of 1456.45 cm^{-1}, 1433.61 cm^{-1} can be attributed to C = C aromatic rings. The product structure has been shown in Scheme 2. ^1H NMR spectrum was obtained in DMSO solvent, and the absorption peak of the solvent hydrogens has appeared in the range of 1.23 to 2.88 ppm (ESI, Figure S6). The highest point of absorbed bands appeared at 6.734 ppm, 6.584 ppm and 3.601 ppm are related to phenolic OH, aromatic ring H and CH_3 of the methoxy core group, respectively. ^{13}C NMR spectrum was also obtained in DMSO solvent (ESI, Figure S7). The absorption peak of the carbons of fourth type imine appeared in the band of 147.86 ppm. Also, the absorption peaks of aromatic ring carbons and vinylic carbons C=CH were found in the range of 115.69 ppm and 119.30 ppm. The methoxy OCH_3 band also has appeared at 56.02 ppm. Obviously, the NMR spectral data and carbonyl removal confirm the main structure of the product, as seen in Figure S7.

The dried product of curcumin-hydrazine reaction with a molar ratio of 1:1 under reflux conditions is a brick red powder with a melting point of 87 °C. A photo of the product is demonstrated in Scheme 2. In the IR spectrum of this product, the absorption band of the phenolic OH has appeared in the wavenumber of 3322.12 cm^{-1} and the peaks seen in the wavenumbers of 2958.58 cm-1 and 2929.15 cm^{-1} were attributed to the tensile vibrations of aromatic and aliphatic CHs (ESI, Figure S8). The absorption band of imine C=N has also appeared at the peak of 1731.91 cm^{-1}. Aromatic ring carbons C=C were also found in the absorption bands of 1519.86 cm^{-1} and 1457.36 cm^{-1}. Based on IR spectral data, it may be concluded that the structure of this product is similar to the product structure of curcumin-hydrazine reaction in the presence and absence of ultrasonic waves.

Next, the clinical implications of curcumin-hydrazine interaction will be reviewed. The results of statistical analysis showed that at treatment dose of 0 (control group), 10 and 20 µM of curcumin and hydrazine-curcumin after 24 h, no significant change was observed in cell number, but after treatment with 40 µM dose of both curcumin and hydrazine-curcumin, the number of cells increased significantly. Also, considering the proliferation of mesenchymal stem cells (MSCs), there was no significant difference between curcumin and hydrazine-

curcumin at any utilized doses. Therefore, it could be said that the application of 40 μM dose of curcumin and hydrazine-curcumin after 24 h increases the biological ability (livability) of rat bone marrow mesenchymal stem cells (MSCs).
A comparison of the number of living cells after treatment with different doses of curcumin-hydrazine and curcumin after 24 h was performed using MTT assay and values are reported as means ± sd (Table 1). Mean values obtained from compounds with different letter codes have significant differences (one-way ANOVA, $p < 0.05$).



Scheme 2. View of curcumin-hydrazine interactio

Table 1. The results of MTT test

Samples	Dose(micromolar)			
	40	20	10	0
Curcumin-hydrazine (reflux)	٢١٩٩ a±٦,١	٢٢٠٧ a±٥,١	٢٤٤٣ a±٧,٣	٣٣١ b ±٤,٧
Curcumin-hydrazine (stirrer)	٢١٩١ a±٤,٩	٢١٩٩ a±٥,١	٢٤٥٤ a±٧,٥	٣٣٢٨ b ±٦,٧
Curcumin-hydrazine (ultra)	٢١٨٩ a±٥,١	٢٢١٠ a±٥,١	٢٤٥٣ a±٤,٣	٣٢٢٩ b ±٤,٣
Curcumin	2158 a ±4.4	٢٢٤٩ a ±١,٤	٢٣٩٩ a ±٥,٤	٣٢٨٩ b ±٦,٩

Conclusions

This study provides a systematic insight into the different methods of synthesis adopted to achieve heterocyclic compounds. The effects of some parameters on the reaction between curcumin and hydrazine were studied with the design of the experiment. The presence and absence of ultrasonic method was applied for the synthesis of heterocyclic compounds. The revealed products were prepared with reflux condition as well. The results showed that the all three methods have a significant influence on the purity of the product and yield of the process. This work also can provide applicable data in the process design and development for the synthesis of heterocyclic derivatives of Curcumin. The received outcomes represented that the F structure exhibited the best product performance under all methods among other products. It is because of the presence of more extensive conjugation, which may be said that ring formation plays more key role in this process. Studies also showed that the cell treatments with 40μM dose of all three samples after 24 h were caused to the more biological ability of rat bone marrow mesenchymal stem cells (MSCs). This means that these compounds (curcumin-hydrazine) are effective in the livability of bone marrow mesenchymal stem cells (MSCs).

Disclosure Statement

No potential conflict of interest was reported by the authors.

Orcid

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Supporting Information

Additional supporting information related to this article can be found, in the online version, at DOI: 10.26655/AJNANOMAT.2021.3.7

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