RESEARCH ARTICLE

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Cancer Stem Cells as a Prognostic Biomarker and Therapeutic Target Using Curcumin/ Piperine Extract for Multiple Myeloma

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Abstract

Background: Multiple myeloma (MM) is a hematological bone marrow malignancy that can be treated but is usually fatal. Medication resistance is the major cause of relapses due to cancer stem cells (CSCs). As a result, this study aimed to identify multiple myeloma cancer stem cells (MMCSCs) in the bone marrow of twelve MM patients with pathological complete response (pCR) after chemotherapy and to investigate the potential effect of Curcumin/Piperine (C/P) extract as an anti-MMCSCs treatment in twenty newly diagnosed patients. **Methods:** This study included twenty bone marrow (BM) samples from newly diagnosed MM patients and twelve BM samples from pCR patients after a year of treatment. The MTT test was performed to assess the treatment's effective dosage. A flow cytometer was used to identify MMCSCs, cell cycle profile, extract's apoptotic activity, and proliferation marker in the selected samples. Also, a colony formation test and stemness protein were investigated. **Results:** In newly diagnosed MM patients, the C/P extract suppressed MMCSCs by 64.71% for CD138-/CD19- and 38.31% for CD38++. In MM patients' samples obtained after one year of treatment, the MMCSCs inhibition percentage reached 44.71% ($P < 0.008$) for CD138-/CD19- and 36.94% ($P < 0.221$) for CD38++. According to cell cycle analyses, the number of cells treated with C/P extract was significantly reduced in the S and G0/G1 phases (87.38%: 35.15%, and 4.83%: 2.17% respectively), with a rapid increase in the G2/M phases (1.1%: 2.2%.). MMCSCs apoptosis was identified using a flow cytometer and Annexin-V. Multiple myeloma stem cell (MMCSC) proliferation was inhibited. Clonogenicity was suppressed by 60%, and stemness protein expression was reduced by 70%. **Conclusion:** MMCSCs in the bone marrow of MM-pCR patients can be utilized as a prognostic tool to predict recurrent multiple myeloma incidence. Also, the therapeutic potential of C/P extract as a prospective anti-MM drug targeting MMCSCs.

Keywords: Multiple myeloma- cancer stem cells- multiple myeloma cancer stem cells- pathological complete response

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Introduction

Multiple myeloma (MM) is a hematological malignant tumor, accounting for 1% of all cancers and 10% of all hematological malignancies. Multiple myeloma (MM) is a treatable but incurable cancer. High expression of abnormal M protein in blood and urine will accumulate and result in organ dysfunction such as bone damage and kidney problems, then the patient becomes symptomatic (Khalife et al., 2021; Bekadja et al., 2022).

One of the most critical issues in this illness is identifying Multiple Myeloma Cancer Stem Cells (MMCSCs) and the cellular and molecular processes in these cells (Abdi et al., 2013). The core nuclei of these signaling networks, which are named by genes with the same name as stemness genes, are OCT4, Nanog, and SOX2 transcription factors. They play a crucial role in regulating gene expression and other genes in the signaling pathways (Kelly & Gatie, 2017). Those genes are naturally expressed in embryonic stem cells (Feyeux et al., 2012) and play a vital role in self-renewal, proliferation, differentiation, and maintaining the pluripotency capacity of these cells. Cancerous cells express stemness genes, particularly OCT4 and SOX2, and their expression is associated with cancer recurrence, treatment resistance, and poor prognosis (Gao et al., 2020; Szudy-Szczyrek et al., 2020).

Myeloma cells express surface indicators in different ways, and the expression of these markers varies between patients. Myeloma cancer stem cells can mark CD138-, CD38++, and CD19- on their surface. Flow cytometry revealed the expression of CD138- and CD38++ surface markers in all multiple myeloma patients (Brudno et al., 2018). In normal plasma cells, the surface marker CD19-

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is expressed, however in multiple myeloma, the CD19- marker is not present or is expressed in a reduced amount. CD19- expression on myeloma cells inhibits their growth and reduces their spread (Garfall et al., 2018).

G0/G1 phase represents the resting phase of the cell. S phase represents the synthesis phase, where DNA replication begins, and the G2/M phase represents the preparation phase for mitotic division. Cell cycle analysis is a prospective target for therapeutic medications used in cancer treatment because the regulatory systems sustaining this process are commonly disrupted in cancer cells. Cell cycle analysis is a DNA content determination technique that employs flow cytometry to distinguish between cells at different cell cycle stages (Vakili-Samiani et al., 2021).

Curcumin has a wide range of pharmacological activities due to its multiple molecular targets and can treat various diseases, including cancer. Breast, esophageal, bladder, hepatic, colorectal, kidney, skin, neurological, blood, and bone cancers are malignancies for which curcumin has therapeutic effects (Sharifi-Rad et al., 2020). Its limited solubility and poor bioavailability are limiting factors for therapeutic use. Studies have found that combining curcumin with piperine (black pepper's main component) increases curcumin bioavailability. Furthermore, piperine enhances plasma concentration and delays drug elimination (Kesarwani & Gupta, 2013).

Therefore, the current study intended to detect CSCS in MM patients' bone marrow samples and determine the C/P extract cytotoxic effect on MMCSCs.

Materials and Methods

Study design

The Egyptian National Cancer Institute (NCI), Cairo University, Egypt, provided thirty-two bone marrow samples. Twenty samples were from newly diagnosed MM patients and were divided into two groups: the control group and the treatment group (C/P). Twelve samples were from pathological complete response (pCR) patients after chemotherapy for one year. Patients with previous cancer or radiotherapy treatment, viral infections, and chronic diseases were excluded. Table 1 lists the clinical pathology parameters of MM patients. Curcumin and Piperine were purchased from Sigma-Aldrich (Sigma, St.Louis, MO, USA).

Primary samples preparation

The bone marrow samples from newly diagnosed and pCR patients (n=32) were added to lymphoprep (Ficoll/Hypaque) with a specific gravity (d) of 1.077 CE (Gibco, Cairo, Egypt) to separate the mononuclear layer using the density gradient centrifugation then cultured in complete media supplemented with 10% fetal and 1% L-glutamine bovine serum (Gibco, Cairo, Egypt) in a humidified incubator with 5% CO2, 100 U/ml penicillin, and 100g/ml streptomycin (Yehia et al., 2020).

Cell viability assays (MTT Cell viability assay)

The C/P extract cytotoxicity against myeloma cells was determined using the 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) colorimetric test (Sigma–Aldrich). Cells were plated in a 96-well culture plate at a density of 5×10^3 cells/well and then treated for 24 hours with five different dosages of the extract doubling triplet (12.5, 25, 50, 100, and 200 µg/ml). After treatment, each well was filled with 20 µL of MTT solution (5 mg/ml in PBS) and incubated for two hours. MTT formazan measurement was done using an ELISA reader (Tecan Group Ltd, Männedorf, Switzerland) at 595 nm after dissolving in 150 µl dimethyl sulfoxide (DMSO). The treated myeloma cells' relative vitality was calculated as a proportion of the untreated cells (Kuete et al., 2017).

Cancer stem cells identified by flow cytometric analysis

Flow cytometric analysis was used to identify the CSCs population in selected MM bone marrow samples. Fluorescently labeled monoclonal antibodies and related isotopic controls were tested in at least 200,000 cells. Ten microliters of specific conjugated antibody [anti-human CD138 isothiocyanate (CD138-FITC; Beckman Coulter Inc., Cairo, Egypt), anti-human CD38 isothiocyanate (CD38-FITC; Beckman Coulter Inc., Cairo, Egypt), and anti-human CD19 phycoerythrin (CD19-PE; Beckman Coulter Inc., Cairo, Egypt)] were added to 200,000 cells/100 µl and incubated for 15 to 20 minutes at a dark place after gentle vortex. The labeled cells were examined using a flow cytometer after washing with 1 ml of PBS, and then Diva 6.1.1 was used to evaluate all data.

Cell cycle analysis

Cell cycle analysis was performed on samples before and after C/P extract treatment (15 µg/ml) to investigate any alteration in cell cycle phases. One hundred and six cells were suspended in 0.5 mL PBS and fixed using 70% ethanol on ice. The ethanol-suspended cells were centrifuged. The pellet was centrifuged again, and 1 ml of propidium iodide (PI) staining solution was used to re-suspend the cells. The cells were then maintained in the dark for 30 minutes at room temperature (RT). The cell fluorescence was then monitored for cell cycle analysis (Beckman Coulter Inc., Cairo, Egypt). Cell Lab Quanta SC software was used to calculate the proportion of cells in the G0/G1, S, and G2/M stages of the cell cycle (Wu et al., 2017).

Cell apoptosis analysis

Propidium iodide and Annexin V-FITC (Beckman Coulter Inc., Cairo, Egypt) were used to assess the apoptotic activity induced by C/P extract on MMCSCs by flow cytometry. Before centrifugation, 106 cells were rinsed with PBS after 24 hours of culture in media supplemented with 15 g/ml C/P extract, resuspended in 1X binding buffer at freezing temperature, stained, and stored on ice for 15 minutes before incubation in the dark. Flow cytometry was used to examine the cells. The flow cytometric analyzer was used to calculate apoptotic cell proportion.

Colony-forming assay

The effect of the C/P extract (15 µg/ml) on self-renewal and CSCs differentiated capacity was assessed in-vitro using the colony-forming assay. A 6-well plate was used,

and each well contained two layers of agarose-RPMI medium. The bottom layer was solidified using 0.5% agarose. The other layer was composed of suspended cells (3.0 x104) in a 1.6 ml agarose- RPMI medium (10% FBS and 0.33% agarose). The plate was incubated for two weeks at 37º C in a humified environment of 5% CO2 and 95% air without fresh media. After that, colonies greater than 0.1 mm were counted and photographed (Horibata et al., 2015).

Effect of C/P extract on cell morphology

Leishman dye was used to stain the bone marrow samples. The morphological apoptotic alterations were assessed using an oil-immersed light microscope lens before and after treatment (Akhlaghi & Ahmadi-Hamedani, 2019).

Western blot analysis (Antibodies and chemicals)

Cell Signaling Technology Company provided antibodies for B-actin (BE5), OCT-4A (C30A3), SOX2 (D6D9), and NANOG (D73G4). PCI-32765, a BTK inhibitor, was obtained from Sigma Company.

On the ice, the cells were washed twice with PBS and lysed in the ReadyPrepTM protein extraction kit (Bio-Rad Inc) with 1 mM PMSF (Solarbio). The Bradford Protein Assay Kit was used to measure the protein supernatants (Bio basic Inc). On 10% SDS-PAGE gels, the same amount of protein was separated and transferred to PVDF membranes. The rest spots that were not connected were then blocked with 5% bovine serum albumin (BSA). The membranes were then treated overnight at 4° C with properly diluted primary antibodies B-actin (BE5), OCT-4A (C30A3), SOX2 (D6D9), and NANOG (D73G4) (1:1000). After that, the membranes were treated for 1 hour at room temperature with the secondary antibody rabbit anti-mouse IgG (Zhongshan Company, Beijing). Finally, using a CCD camera-based imager, the chemiluminescent signals were recorded. On the ChemiDoc MP imager, image analysis software was used to compare the band intensity of the target proteins to the control sample beta-actin (housekeeping protein) by protein normalization (Zhao et al., 2017).

Statistical analysis

Statistical Package for the Social Sciences was used for data analysis (version 24.0; IBM Corp., Armonk, NY, USA). Non-normally distributed data were reported as medians and interquartile ranges. Categorical data were reported as frequencies and percentages. P-values less than 0.05 were considered significant. The Student's t-test was used to compare the means of normally distributed variables between groups. The Mann–Whitney U–test compared non-normally distributed variables between groups. The distribution of categorical variables between groups was determined using the Chi-square test or Fisher's exact test.

Results

Cell viability assays (Cytotoxic effect of C/P extract on the myeloma cells)

When myeloma stem cells were treated with 12.5, 25, 50, 100, and 200 µg/ml of C/P extract, their 100% cell proliferation was significantly reduced in a dose-dependent manner to 52.2%, 43%, 35.2%, 31.3%, and 23.9 %, respectively. As a result, the IC50 value was 15 µg/ml, as indicated in Figure 1.

Cancer stem cells identified by flow cytometric analysis

CSCs population (CD138-, CD19-, and CD38++) was found in MM bone marrow samples. Furthermore, CD138-/CD19- expression was significantly decreased in the C/P group ($P < 0.008$), with a 64.71% inhibition percent. CD38++ was similarly reduced but insignificantly ($P < 0.221$) in the Curcumin/Piperine group with a 38.31% inhibition percent. In the pCR group, the same surface markers were found with a P-value of 0.549 and 44.71% inhibition percentage for CD138-/CD19-. The P-value for CD38++ of the control group versus the pCR group was 0.032, with a 36.94% inhibition percentage (Figure 2).

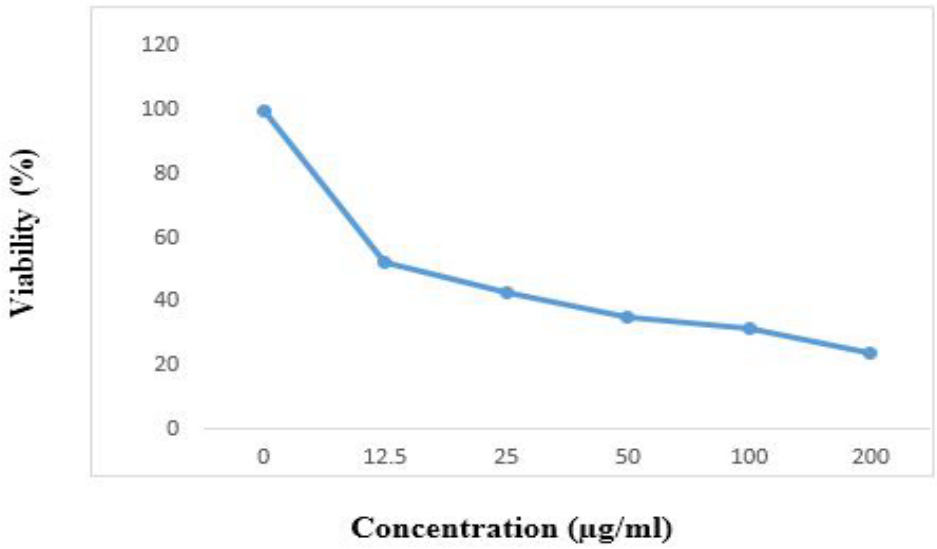

Figure 1. MTT Results to Detect the Viability of MM Cells after Treatment with C/P Extract.

Table 1. List of the Clinical Pathological Parameters of MM Patients

Clinical parameters	Patient number (n=32)
Age, median (range)	58 (35-75)
35-45 Years, No., (%)	3 (9.37%)
46-55 Years, No., (%)	7 (21.88%)
56-65 Years, No., (%)	16 (50%)
66-75 Years, No., (%)	6 (18.75%)
Gender	
Male, No., (%)	22 (68.75%)
Female, No., (%)	10 (31.25%)
WBC Count, Median (Range), × 10^9/L	7.86 (2.5-25)
	New 8.67 (2.5-25)
	Chemotherapy 4.8 (3.6-8.3)
Hemoglobin, Median (Range), g/dL	10.9 (7.1-15.6)
	New 10.37 (7.1-15.6)
	Chemotherapy 12.4 (10.6-16.1)
Platelet Count, Median (Range), × 10^9/L	237.1 (58-450)
	New 243.56 (80-450)
	Chemotherapy 214.43 (58-373)

C/P extract induces cell cycle arrest

Twenty-four hours following treating MMCSCs with C/P extract (15 µg/ml), a substantial drop in the G0/G1 phase was identified from 87.38% to 35.15% compared to the untreated cells (Figure 3). Compared to the untreated MMCSCs, the percentage of cells in the S-phase consistently decreased from 4.83% to 2.17% in the treated MMCSCs. In contrast to untreated MMCSCs, the proportion of MMCSCs in the G2/M phase after treatment with C/P extract increased considerably from 1.1% to 2.2%.

The apoptotic impact of C/P extract on MMCSCs

Flow cytometric analysis revealed that treating MMCSCs with C/P extract for 24 hours significantly increased apoptotic cells compared to untreated cells. Compared to the untreated MMCSCs, viable cell proportion in MM in the treated MMCSCs declined from 95.96% to 61.78%. Also, late apoptotic cells in the UR quadrant increased dramatically from 0.37% to 6.50%. Furthermore, early apoptotic cells in the LR quadrant increased significantly from 0.67% to 13.39%. At the same time, necrotic cell proportion in the first quadrant increased significantly from 3.01% before treatment to 18.32% after treatment (Figure 4-A and B).

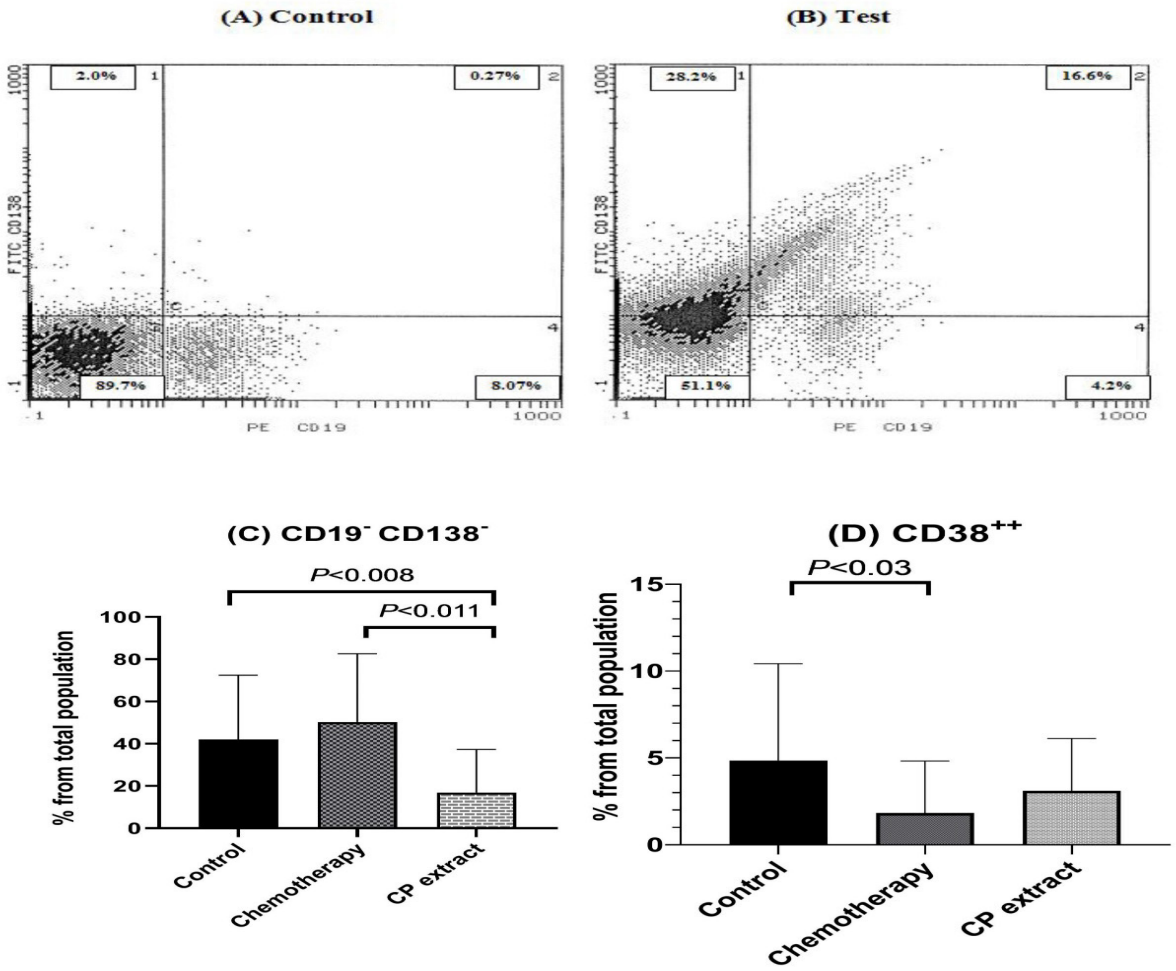

Figure 2. Identification of MMCSCs. (A, B) The cytotoxic effect of C/P extracts on the MMCSCs in comparison to control and chemotherapeutic groups using flow cytometry. Data were expressed as a median. (C) The ratio of CD19-/CD138- population was evaluated before and after treatment of C/P extract and chemotherapy. (D) The ratio of the CD38++ population was evaluated before and after treatment of C/P extract and chemotherapy

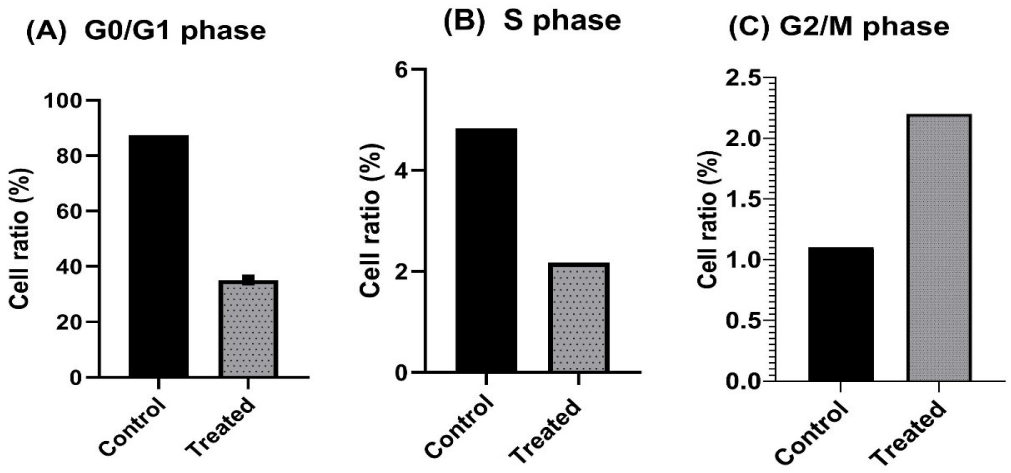

Figure 3. The Cell Cycle of MMCSCs Treated with C/P Extract Compared to Untreated Control.

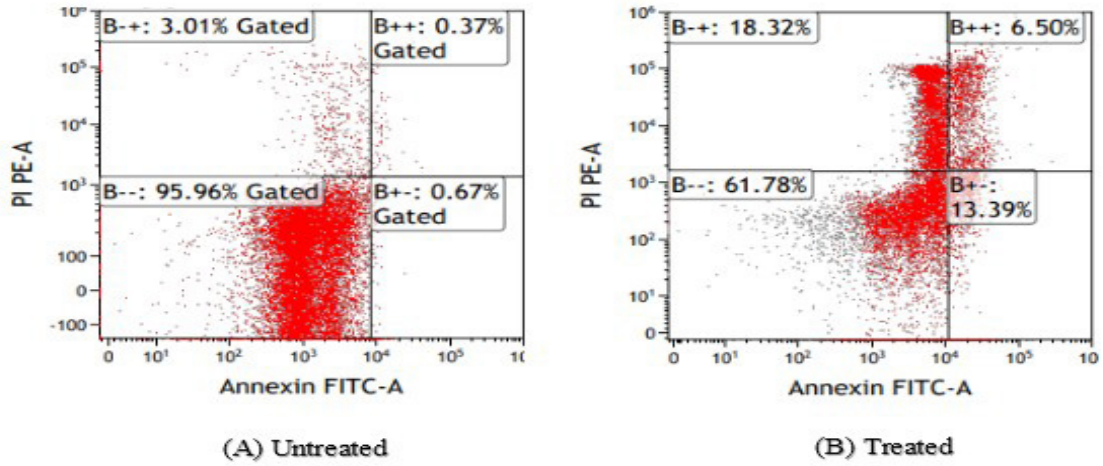

Figure 4. Apoptosis and Proliferation in MM Bone Marrow Samples Before and After C/P Extract Treatment (A) Annexin V and Propidium Iodide flow cytometric dot plot chart in untreated and treated MMCSCs. (B) Flow cytometric analysis was used to determine the percentage of apoptotic cells before and after treatment with the extract. When compared to untreated cells, data were reported as mean SEM (* P<0.05).

Effect of C/P extract on Clonogenicity

The MMCSC colony-forming potential was reduced after treatment with C/P extract. The number of colonies and their size decreased in the treated cells compared to the untreated cells, as shown in Figure 5.

The morphological apoptotic changes induced by C/P extract

Compared to untreated MMCSCs, the effect of C/P extract on the morphology of all investigated bone marrow films demonstrated apoptotic alterations, which were

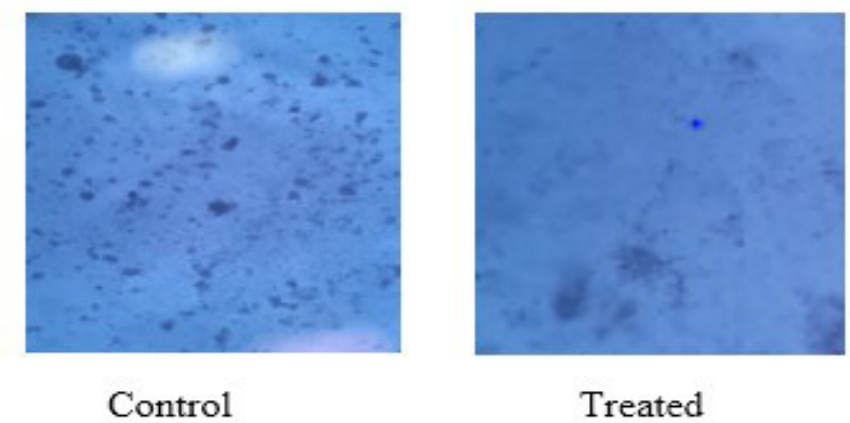

Figure 5. Under an Inverted Microscope, the Size and Number of the Colonies Reduced Following Treatment with the Extract.

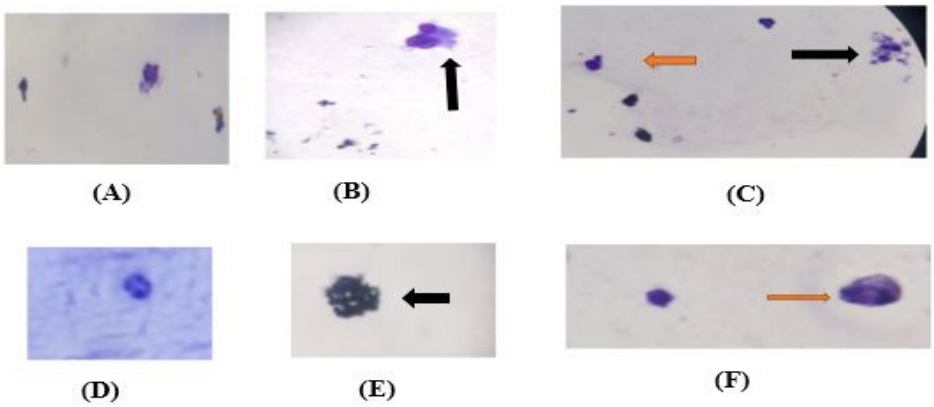

Figure 6. Morphological Apoptotic Changes in MM were induced by C/P extract and visualized at 1000X magnification. (A) Apoptotic body development in a plasma cell after treatment in a bone marrow smear aspirate. (B) A big binucleated leukemic blast cell with typical apoptotic bodies is shown with black arrows. (c) The black arrow indicates the discharge of apoptotic corpses from a damaged cell, whereas the red arrow indicates the creation of separate cytoplasmic or psedopods. (D) Apoptotic bodies are formed in giant plasma cells. (E) Apoptotic bodies develop in a plasma cell as shown by the arrow. (F) A blast cell's nuclear chromatin condenses along with the nuclear envelope, as shown by the red arrow.

recognized under a light microscope and included released apoptotic bodies from a shattered cell, dispersed apoptotic bodies, apoptotic bodies production in MMCSCs, and nuclear chromatin condensation along with the nuclear envelope, as seen in Figure 6A-F.

Western blot assay of stemness proteins

Western blotting findings showed that the C/P extract significantly reduced stemness in MMCSCs compared to untreated cells, as evidenced by OCT-4A, Nanog, and SOX2 ($P < 0.31$, $P < 0.002$, and $P < 0.037$, respectively), as indicated in Figure 7.

Discussion

Multiple myeloma is a hematological bone marrow malignancy defined by M protein and plasma cell accumulation in hematopoietic organs, particularly the bone marrow. Multiple myeloma cancer stem cells are the main cause of therapy resistance and recurrence. As a

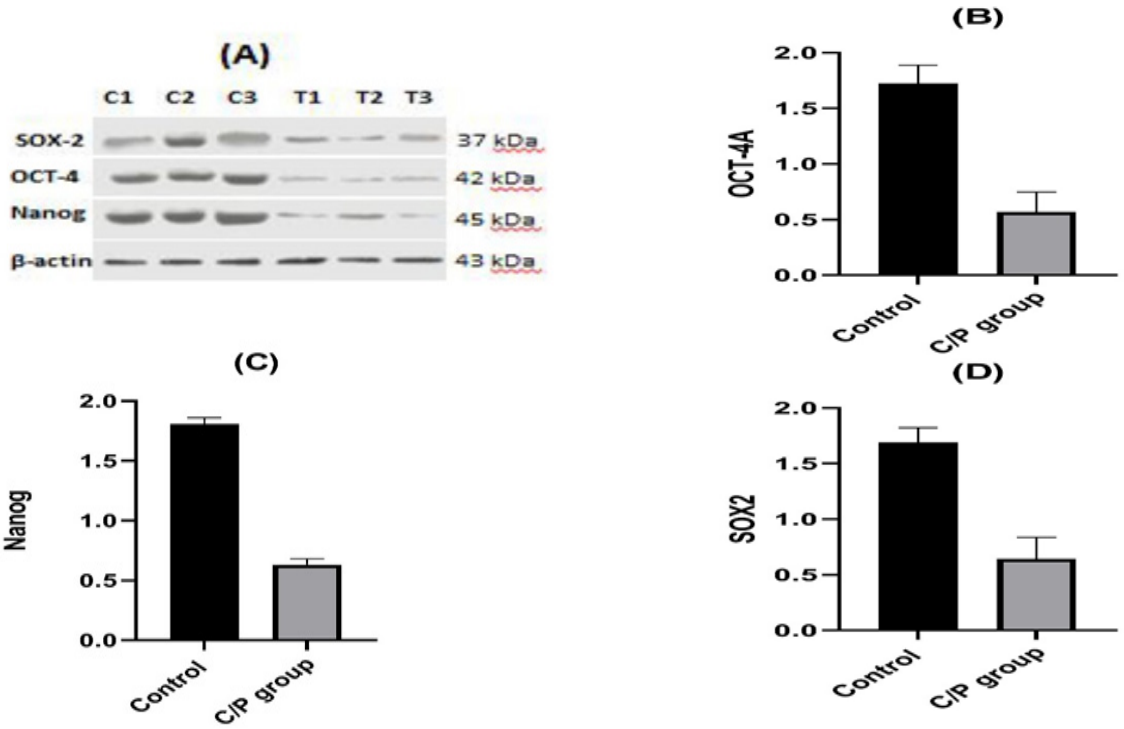

Figure 7. The Western Blotting Results for Stemness Protein in the Control Group, and C.P. Extract Group. (A) The membrane of a western blot for the stemness proteins (OCT-4A, NANOG, and SOX2) (C1, C2, C3: control for sample no. 1, control for sample no.2, control for sample no.3) (T1, T2, T3: test for sample no.1, test for sample no.1, test for sample no.1). (B-D) illustrate the effect of C/P extract on the levels of different proteins when compared with the control group.

result, anti-MMCSCs drugs as novel therapeutic remedies were required to improve patients' prognoses and decrease myeloma-related mortality (Krejcik et al., 2021).

At first, we detected MMCSCs in the bone marrow of patients treated with chemotherapy for more than one year and found that the number and aggressiveness of CSCs were very high. This population is resistant to chemotherapy, explaining why MM is incurable. Therefore, investigating a new therapy with high efficiency in this population is very important. We started to identify this population in newly diagnosed patients, and the CD19-CD138-CD38++ cell population was previously identified (Calton et al., 2018; Zhao et al., 2018). CD138- cells from human MM cell lines and primary patient samples had higher clonogenic capacity than CD138+ plasma cells, according to Matsui et al. [(2004). Furthermore, these CD138- cells could develop into CD138+ plasma cells and phenotypically resemble post germinal center B cells, and rituximab, an anti-CD20 monoclonal antibody, inhibited their clonogenic proliferation. These results imply that CD138- B cells are similar to MMCSCs.

According to Matsui et al. (2016), CD138- B cells are also resistant to clinical anti-MM medications (dexamethasone, lenalidomide, bortezomib, and 4-hydroxycyclophosphamide) and have a high drug efflux capacity and intracellular drug detoxifying activities. In the 16 samples investigated by Singh et al (2017), CD138-CD19-CD38++ plasma cells could produce MM colonies in vitro, whereas CD19+ B cells never did. CD138-CD19-CD38++ plasma cells recovered from three of nine patients engrafted in the SCID-rab model also produced MM, although CD19+ B cells did not. As expected, our findings revealed the expression of the CSCs population CD19-CD138-CD38++ in Egyptian MM patients.

According to Giordano and Tommonaro (Giordano & Tommonaro, 2019), curcumin has anti-cancer properties by targeting several cell signalings pathways such as growth factors, cytokines, transcription factors, and genes that modulate cellular proliferation and death. On the other hand, curcumin is not immune to side effects such as nausea, diarrhea, headaches, and yellow stools. Furthermore, it has low bioavailability due to low absorption, quick metabolism, and systemic clearance, limiting its efficiency in treating disorders. Piperine overcomes this constraint (Sharifi-Rad et al., 2020). As a result, the current study aims to assess the therapeutic potential of C/P extract by focusing on the plasma and MMCSCs subpopulations identified and expressed in MM patients (Pan et al., 2018).

The findings of the MTT experiment confirmed C/P extract safety and its cytotoxic impact. These findings might be attributed to the fact that Curcuma longa is one of the most promising groups of bioactive natural chemicals, particularly in treating various cancers.

It is noteworthy that the C/P extract was efficient against the population of CSCs. In the MMCSC compartment, there was a greater expression of retinoic acid receptor alpha 2 (RAR2), which enhanced drug resistance. Further study revealed that in MMCSCs expressing RAR2, the expression of anti-apoptotic Bcl-2 family members increased, endowing these cells with greater drug resistance (Phi et al., 2018; Vinogradov & Wei, 2012). The BTK expression was previously upregulated in MMCSCs, and BTK overexpression caused treatment resistance, which was largely mediated by induction of the anti-apoptotic gene BCL-2 (Suskil et al., 2021). The C/P extract apoptotic action was further validated by its morphological effects. As indicated, there was a considerable reduction in the number and size of colonies in the treated MMCSCs.

The C/P extract effect on cell cycle arrest was validated in this study by substantial reductions in the G0/G1 and S phases and an increase in the G2/M phase. Recent studies reported that curcumin causes cell cycle arrest at the G2/M phase in gastric cancer cells by lowering cyclin D1 and raising cyclin B1 in a dose-dependent manner. Also, it can inhibit colorectal cancer proliferation by interrupting the cell cycle and speeding up apoptosis. This occurs via altering thymidylate synthase and the transcription factor E2F-1 that regulates it. Cell cycle inhibition was induced by the downregulation of NF-B and other survival pathways. Also, curcumin inhibited the enzyme CDK2, resulting in the G1 cell cycle (Pricci et al., 2020; Rajitha et al., 2016).

Normal stem cells and CSCs express stem cell markers. The octamer-binding transcription factor 4 (OCT4), one of the stem cell factors required for embryogenesis and pluripotency, is gaining popularity. OCT4 is overexpressed in cancer stem cells (CSCs). However, most CSC research found a favorable link between OCT4 expression and chemoresistance and an inverse link between OCT4 and clinical outcome (Zhang et al., 2020).

Tumor cells expressing SOX2 can initiate and promote tumor development, according to Boumahdi et al. (2014) , and are closely linked to the cancer stem cell phenotype in numerous cancer types. According to Zhao et al. (2017), BM-MSCs, as a new key factor, can suppress MM stemness by reducing the expression of key stemness proteins such as OCT4, SOX2, and NANOG by targeting BTK, with PCI-32765 can reduce MMCSCs and cure myeloma. In line with this, our results showed a reduction of those stemness genes at the protein level, indicating that the C/P extract is effective as a therapeutic agent.

In conclusion, MMCSCs were identified using a flow cytometer, which revealed significantly decreased CD138-/CD19- expression in the C/P extract-treated group and reduced CD38++ but without significance. According to cell cycle analyses, the number of cells treated with C/P extract was significantly reduced in the S and G0/G1 phases, with a rapid increase in the G2/M phases. MMCSCs apoptosis was identified using a flow cytometer and Annexin-V. Multiple myeloma stem cell (MMCSC) proliferation was inhibited. Clonogenicity was suppressed, and stemness protein expression was reduced. Therefore, MMCSCs can be used as a diagnostic marker and therapeutic target to enhance the treatment of MM patients.

Author Contribution Statement

Ibrahim M. Abdel-Salam, Mohga S. Abdalla conceived and designed experiments. All authors contributed to the

analysis and/or interpretation of data equally. Sara A. Mekkawy, Mohamed M. Omran drafted the manuscript. Sara A. Mekkawy, Mohamed M. Omran revised it critically for important intellectual content. All authors have read the manuscript and approved the submission.

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Scientific Body approvment

This research is part of an approved student MSC thesis (Sara A. Mekkawy).

Informed consent

Informed consent was obtained from all individual participants included in the study.

Ethics approval and consent to participate

The study was approved by the Institutional Review Board (IRB) of the National Cancer Institute, Cairo University, Egypt (IRB approval No. 2220-402-031). Cancer stem cells as a prognostic biomarker and therapeutic target using Curcumin/ Piperine extract for multiple myeloma. The study protocol was approved by the Institutional Review Committee and conformed to the ethical guidelines of the 1975 Helsinki Declaration.

Availability of data and materials

The authors declare that all generated and analyzed data are included in the article.

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