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Study of chrysin induced apoptosis of HepG2 cells through mitochondrial pathway

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ABSTRACT

Discussion of chrysin inhibiting effect on HepG2 cells and its mechanism.Determination of IC_{50} of chrysin on HepG2 cells by MTT method; The cell morphology was observed by transmission electron microscope; Detect cell apoptosis of chrysin on HepG2 cells after 48h rate of flow cytometry; Effect of flow cytometry of chrysin on mitochondrial membrane potential of HepG2 cells; Effect of fluorescence microscopy of chrysin on HepG2 cell mitochondrial membrane channel rotating hole; Effects of chrysin on MPTP kit for detecting the protein content and Caspase-3,9 content in HepG2 cells. Chrysin inhibited the growth of HepG2 cells, its IC_{50} value is 47.62umol/L; Chrysin can effectively induce the apoptosis of HepG2 tumor cells, concentration increased apoptosis rate increased; chrysin appears to decrease of mitochondrial membrane potential in HepG2 cells; Chrysin makes the mitochondrial of HepG2 cell membrane channel open; chrysin improves the content of Caspase- 3,9 in HepG2 cells. Chrysin could induce apoptosis of HepG2 cells.

Keywords: Chrysin; Mitochondrial membrane potential; Caspase-3, 9; Apoptosis

1. INTRODUCTION

Chrysin[1-3] (Chrysin, CR) chemical name is 5, 7- two hydroxy flavone, light yellow prismatic crystal (crystallization from methanol), molecular weight 254, melting point 285 ℃, dissolved in sodium hydroxide solution, slightly soluble in ether, ethanol and chloroform, insoluble in water. Chrysin has effects of vasodilatation, anti-inflammatory and antioxidant [4], can induce muscle relaxation and damage the motor ability[5]. Chrysin could significantly inhibit the growth of human thyroid cancer cells, chrysin and its derivatives can inhibit human lung cancer cell line, murine lymphocytic leukemia cell line, human acute myeloid leukemia cell line, human gastric carcinoma cell line, human colon cancer cell proliferation and the growth of cells and tumor cells, and in a dose and time dependent. Study on the inhibitory effect of chrysin on these tumors provides space to imagine [6-7] reasonable for its potential role as an effect on liver cancer cells. Now researchers think it's the most common and abundant virus in the human stomach [8]. This experiment using cell culture in vitro tumorigenicity experiment method, to the way from the mitochondrial mediated apoptosis of poplar, effects of anti hepatocellular carcinoma cell line HepG2, and provide a theoretical basis for clinical treatment of hepatocarcinoma.

2. EXPERIMENTAL

2.1. Materials

2.1.1. Drugs

Chrysin (purity: >98%) :purchased from Sigma company.

2.1.2. Cells

HepG2: from the College of pharmacy of Harbin University of Commerce

2.1.3. Reagent and instrument

RPMI1640,Trypsin,MTT,DMSO,KH_2PO_4,PI, Rhodamine123, TritonX-100, glutaraldehyde, glacial acetic acid, potassium dichromate, Caspase-3, 9 activity assay kit. CO_2 incubator, JJT-900/1300 super clean bench, Olympus IX70 inverted microscope, Adventurer 1/10000 electronic balance, SUNRISE microplate, CLOUTER EPICS-XL flow cytometry, optical microscope, C-4040ZOOM camera, high speed refrigerated centrifuge.

2.2. Methods

2.2.1. Chrysin inhibits the growth of HepG2 cells

Logarithmic growth phase cells at 1,000 cells each well were seeded in 96-well plates, the next day each well add 100μL Chrysin, located seven dose groups provided 6 parallel holes, culture 48h. Add 100μL per well containing 0.5mg/mL MTT solution, Discard the supernatant added to each well 200μL DMSO. Microplate detection wavelength 490nm optical density measured under the conditions (OD).

2.2.2. Effect of Chrysin on HepG2 cell morphology

Logarithmic growth phase of the cells,final concentration dosing was 40mg•L^{-1}, 50mg•L^{-1}, 60mg•L^{-1},collect the cells after 48h,fixed with glutaraldehyde for 1h, precipitated cells embedded in the agar with 1% osmic acid fixed after 2h, after washed with 35%, 50%, 75% , 90% gradient series of dehydrated; soaked in 100% acetone and embedding medium (1:1) mixture of 37 ℃ 3h, ultrathin sections were stained with lead citrate, electron microscopy, photographed.

2.2.3. Flow cytometry detect chrysin on HepG2 cells inhibition rate and apoptosis rate

Logarithmic growth phase cells were seeded in 6-well plates, dosing chrysin final concentration of 40mg•L^{-1}, 50mg•L-1, 60mg•L^{-1}, cultured 48h, cells were collected by centrifugation. Resuspend cells, add 1ml of 70% ice-cold ethanol, fixed at 4℃ refrigerator overnight; centrifuged, discard the supernatant fluid, add 1ml PI dye, avoid light (20-25℃) staining 30min; flow cytometry, excitation wavelength 488nm, emission wavelength of 630nm.

2.2.4. Effect of chrysin on HepG2 cell membrane potential

Logarithmic growth phase cells were seeded in six-well plates, add the high, medium and low doses of chrysin and set the control group, cultured 72h; collect the cells, fixed at 4℃ refrigerator overnight, centrifuged, the supernatant was discarded, each tube were added 1ml Rhodamine 123 dye, dark staining 30min; flow cytometry, excitation wavelength 488nm, emission wavelength of 630nm.

2.2.5. Effect of chrysin on mitochondrial HepG2 cell membrane channel rotating hole

Cells were treated with drugs after 48h, GENMED cleaning, add GENMED staining solution, 37℃ cell cultures incubated for 20 minutes, remove GENMED dye working solution. Add GENMED cleaning solution to the cell culture wells, using an inverted fluorescence microscope with an excitation wavelength 488nm, distribute wavelength 505nm.

2.2.6. Chrysin on HepG2 cells, Caspase-3, Caspase-9 content

Logarithmic growth phase cells were seeded in six-well plates, medicate the final concentration was 120, 180, 240, 300μmol•L^{-1} ; drugs sucked out after 72h culture in strict accordance with the kit instructions to operate, Measured Caspase-3, Caspase-9 enzyme activity.

2.2.7. Data processing

The results were expressed as mean± standard deviation (± s) that the use of statistical software SPSS15.0 analysis indicators among groups were compared using ANOVA, with P <0.05 as statistically significant difference, P <0.01 for the difference was considered statistically significant.

3. RESULTS AND DISCUSSION

3.1. Chrysin inhibits the growth of HepG2 cells

As can be seen from table 1 chrysin effector cells at different concentration for 48 h, the growth of cell was inhibited, showed significant dependence between the effects of each treatment group had significant difference compared (P<0.01). IC_{50} value is 47.62umol / L.

Tab.1 Inhibitory rate of CR on growth of HepG2 measured by MTT assay ($\bar{x}$ ±s, n=6)

CR	48h	
(μmol·L^{-1})	OD	IR%
Control	0.538±0.0379	—
CR-5	0.472±0.0764**	8.530
CR-10	0. 467±0.0702**	9.50
CR-20	0.462±0.0666**	10.47
CR-30	0.446±0.0458**	13.57
CR-40	0.320±0.0194*	37.98
CR-50	0.256±0.0265**	50.39
CR-60	0.178±0.0153**	65.50
5FU-150	0.204±0.0121**	60.47

Note:*P<0.05, **P<0.01 vs control

3.2. Effect of chrysin on HepG2 cell morphology

With the control group (Figure 1-a), compared by low concentration of 40 uM chrysin treated cells (Figure 1-b) smaller size, increased electron density, membrane occur within a small space chamber; the concentration of 50 uM in chrysin treated cells (Figure 1-c) of cell volume than the former result is an increase in the phenomenon of swelling of the cytoplasm

marginalization curved membrane; after 60 uM to the treated cells (Figure 1-d) formation of nuclear fragmentation, cell surface membrane spine disease pathological changes, resulting in apoptotic bodies, foaming even more serious, the membrane body completely changed, appear crushed shape.

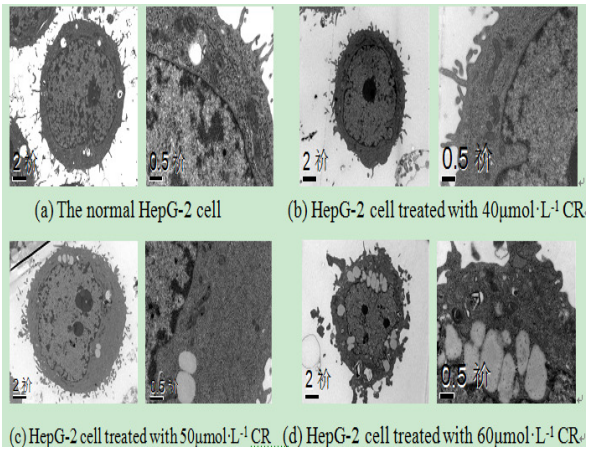
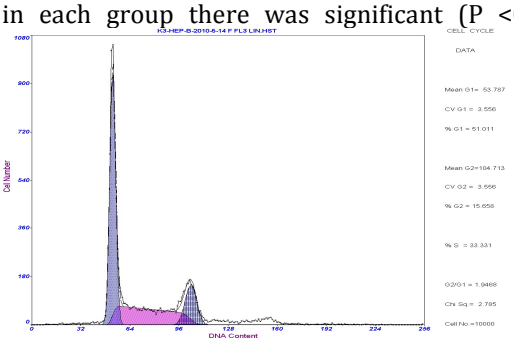


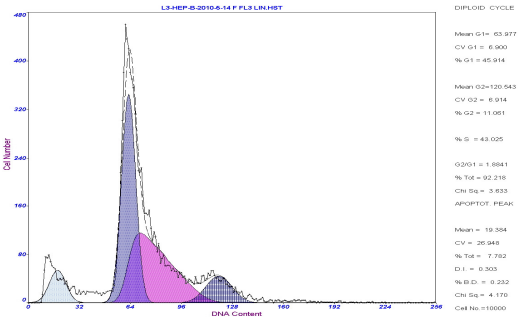
Figure – 1: Transmission analysis the morphology of apoptosis in HepG2

3.3. Flow cytometry of chrysin on HepG2 cell inhibition rate and apoptosis rate

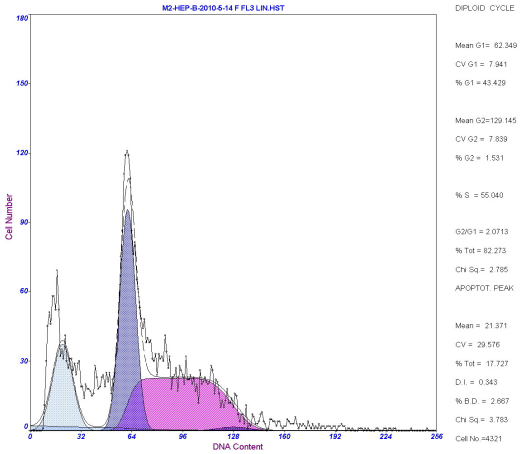
The following can be seen from the drawings, more cells were arrested in S phase. Table understand salicin on cell cycle to a certain extent, and can block tumor cells in M phase. Meanwhile 60, 50,40 uM of chrysin on HepG2 cell apoptosis rates were 41.09%, 17.73%, 7.762%, significantly higher than the positive drug, cell apoptosis rates were dose dependent differences in each group there was significant (P <0.01).



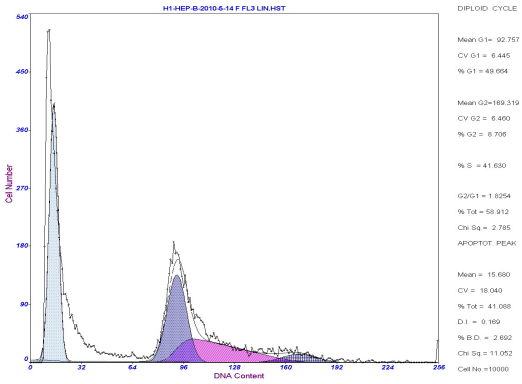
(a)RPMI-1640



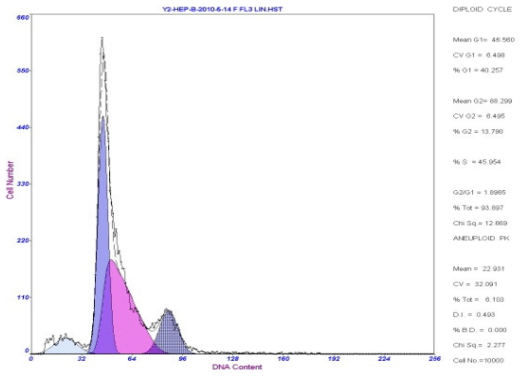
(b)CR-40μM



(C) CR-50μM



(d) CR-60μM

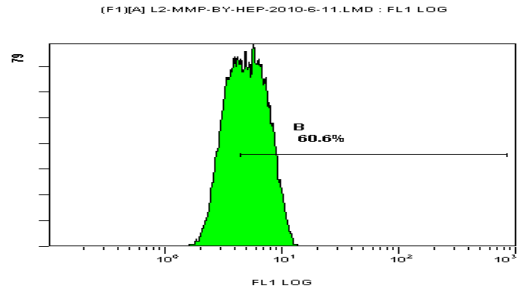


(e) 5-Fu-150μM

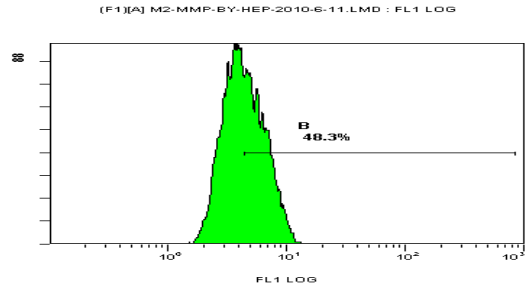
Figure – 2: Effect of CR on cell cycle distribution and apoptosis rate of HepG2

3.4. Effect of chrysin on HepG2 cell membrane potential

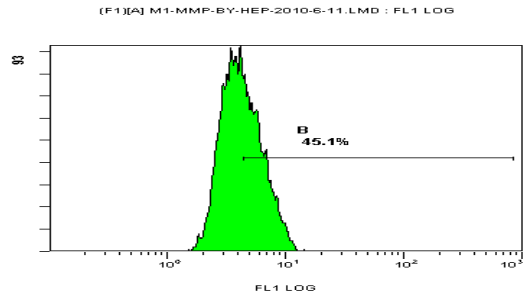
The effect of different concentration of chrysin on HepG2 cells after 48h, HepG2 cells in the inner mitochondrial membrane potential were 38.6 ± 2.6,45.1 ± 4.3,48.3 ± 3.6 . Blank group intracellular mitochondrial membrane potential was 60.6 ± 4.3, cell activity is better. CR can led to HepG2 cells the inner mitochondrial membrane potential decreased, compared with control group differences were statistically significant (P <0.01), shown in figure 3.



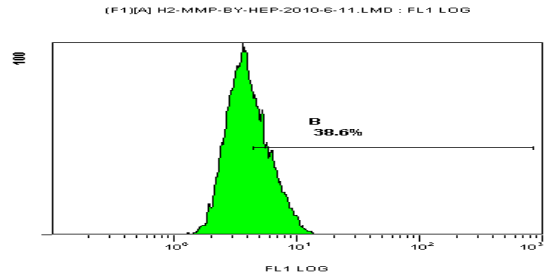
(a) with RPMI-1640



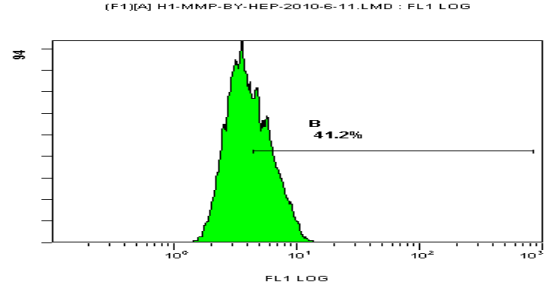
(b) with CR-40μM



(C) with CR-50μM



(d) with CR-60μM

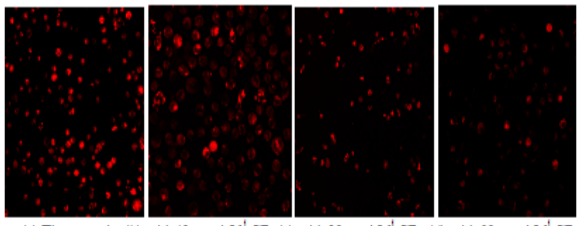


(e) with 5-Fu 150μM

Figure – 3: Effect on the mitochondrion Δψm of HepG2 cell of CR

3.5. Effect of chrysin on HepG2 cell mitochondrial membrane channel rotating hole

Chrysin role in HepG2 cells 48h, laser scanning confocal microscope as shown in Fig4:CR can lead to HepG2 mitochondrial membrane channel shift empty (channel protein) open.



(a) The normal (b) with 40 μmol·L^{-1} CR (c) with 50μmol·L^{-1} CR (d) with 60 μmol·L^{-1} CR

Figure – 4: Effect on the mitochondrion MPTP of HepG2 cell of CR

3.6. Effect of CR on the content of the Caspase-3,Caspase-9 in HepG2

As can be seen from Table2, CR can increased HepG2 cells Caspase-3, Caspase-9 levels. It also proved that CR can obvious induced HepG2 tumor cell apoptosis.

Table – 2: Effect of CR on the content of the Caspase-3, Caspase-9 in HepG2 ($\bar{x}$ ±s,n=4)

Concentration (μmol·L^{-1})	Caspase - 3	Caspase - 9
Control	0.063±0.003	0.076±0.004
CR-40	0.071±0.004*	0.119±0.004**
CR-50	0.096±0.003**	0.128±0.004**
CR-60	0.115±0.005**	0.214±0.005**

4. CONCLUSION

Chrysin inhibits HepG2 cell proliferation and its mechanism of action related to apoptosis induced by start the mitochondrial pathway. Chrysin is probably by cutting the Bcl-2 gene expression, increased Bax gene expression, which induce the mitochondrial permeability transition hole open channel (PT), promote Cyt-c to the release of the cytoplasm in the mitochondria. Release of Cyt-c could further and, ATP/dATP Apaf-1 in combination with the formation of apoptotic body, prompting Apaf-1 CARD domain exposed outside the structure, and the CARD with the same structure of the domain procaspase-9 unifies, cause the self-activation of procaspase-9, effectively cutting and activation of downstream caspase-3, finishing chrysin mitochondria induced by HepG2 cell apoptosis process[9-12]. But whether chrysin can trigger mitochondrial ways other than those induced by HepG2 cell apoptosis still needs

further research. In rare congenital disease, in cancer and in infections, genomic insights are already transforming diagnosis and treatment[13].

The experimental results showed that with chrysin concentration and time increases, the tumor cells Caspase - 9 were significantly increased, and in a dose-dependent relationship, understand that salicin can improve the content of Caspase-9 to induce tumor cell wither die.

5. REFERENCES

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