行政院國家科學委員會專題研究計畫　成果報告

牛樟芝菌絲體發酵過濾液對人類肝癌細胞株細胞週期之影響

計畫類別：個別型計畫
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計畫主持人：沈立言

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行政院國家科學委員會專題研究計畫期末成果報告

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Effect of the filtrate of fermented mycelia of *Antrodia camphorata* on the cell cycle of human liver tumor cell line

計畫編號：NSC91-2316-13-002-010

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主持人：沈立言　　國立台灣大學食品科技研究所

共同主持人：蔣丙煌

一、中文摘要

癌症近年來為國人十大死亡原因之首，其中肝癌更是男性癌症罹患率之首位。近來，以天然食物來達到預防癌症之功效已成為世界性熱門的研究課題。本研究分別以食工所研發之牛樟芝菌絲體發酵過濾液 (filtrate of fermented mycelia of *Antrodia camphorata*, FFMA) 及自行發酵之牛樟芝菌絲體萃取液 (extract of fermented mycelia of *Antrodia camphorata*, EFMA) 探討對肝癌細胞株之影響。在細胞生存力方面，實驗結果顯示以食工所 FFMA 處理 Hep G2 及 Hep 3B 肝癌細胞，其 IC_{50} 分別為 447 及 244μg/ml；當以自行發酵之 EFMA 處理 Hep G2 及 Hep 3B 肝癌細胞時，IC_{50} 分別為 24.6 及 48.1μg/ml。而在細胞形態方面，以 500μg/ml 食工所 FFMA 及自行發酵 EFMA 處理 Hep G2 與 Hep 3B 肝癌細胞，發現其細胞形態與控制組有明顯差異，處理組之肝癌細胞皆有皺縮情況發生，且增加細胞懸浮現象。在細胞週期的影響方面，利用 500μg/ml 食工所 FFMA 處理，Hep G2 有被滯留在 G0/G1 期的現象，而 Hep 3B 在 300 及 500μg/ml 食工所 FFMA 濃度處理下，壞死細胞增加，有部分細胞被滯留在 S 期；而以 50μg/ml 自行發酵 EFMA 處理肝癌細胞時，則使 Hep G2 被滯留於 G2/M 期，而 Hep 3B 被滯留於 S 期。

關鍵詞：牛樟芝、菌絲體、過濾液、萃取液、人類肝癌細胞株、細胞週期

Abstract

Cancer is the number one of the ten leading death causes in Taiwan in recent years. Among the leading causes of cancer death, liver cancer is the first one for male. Cancer chemoprevention by natural edible products is a hot topic for researchers worldwide recently. In this study, the effects of filtrate of fermented mycelia of *Antrodia camphorata* (FFMA) from Food Industry Research and Development Institute (FIRDI) and extract of fermented mycelia of *Antrodia camphorata* (EFMA) from our lab on the human liver tumor cell lines, i.e. Hep G2 and Hep 3B, were investigated. In terms of cell viability, the IC_{50} of Hep G2 and Hep 3B treated with FFMA from FIRDI was 447 and 244μg/ml, respectively. The IC_{50} of Hep G2 and Hep 3B cells treated with EFMA from our lab was 24.6 and 48.1μg/ml, respectively. In addition, the cell morphologies of Hep G2 and Hep 3B cells treated with 500 μg/ml FFMA from FIRDI and EFMA from our lab showed the cell shrinkage and cell suspension in medium, with significant difference as compared to the control group. The cell cycle of Hep G2 was arrested in G0/G1 phase by the treatment with 500μg/ml FFMA from FIRDI. While with the treatments of 300 and 500μg/ml FFMA from FIRDI, the number of necrotic cells of Hep 3B was increased, and some of Hep 3B cells were arrested in S phase. However, the cell cycles of Hep G2 and Hep 3B were arrested in G2/M and S phase, respectively, when those liver tumor cells were treated with 50μg/ml EFMA from our lab.

Key words: *Antrodia camphorata*, mycelium, filtrate, extract, human liver tumor cell line, cell cycle

二、緣由與目的

牛樟芝（*Antrodia camphorata*; niu-chang-chih）又稱樟芝或牛樟菇，為本省特有種真菌，僅生長於台灣特有之牛樟樹（*Cinnamomum kanehirai* Hay）。目前民間認為牛樟芝具有解毒、抗癌及止癢作用，為一本土性非常具有經濟效益的保健食品材料。牛樟芝生長於老齡牛樟樹，由其中空樹幹內

面生長出真菌之子實體，著生的木頭有褐腐之現象，因此樟芝可能是褐腐的木材腐生菌。數十年來，有不少人力、物力研究樟芝的人工栽培，卻始終無法成功。樟芝菌種在木層上生長極緩慢甚至停滯，更遑論其子實體的長出。目前食品工業界已成功的利用高科技之生物技術生產出牛樟芝菌絲體發酵過濾液，提供此一高價值、高潛力之保健食品材料。然而，其諸多醫療保健效用當需更多生化、營養、藥物及毒理等基礎研究驗證。

在抑癌機轉方面，牛樟芝菌絲體發酵過濾液究竟對癌細胞之細胞週期如何影響，是一非常重要的研究課題。因此深入瞭解牛樟芝菌絲體發酵過濾液對於細胞週期或細胞凋亡之調控機制，將有助於癌症預防方法之研究及抗癌食物或藥物之開發（Funk, 1999）。

本研究之樣品為 FFMA 由食品工業發展研究所菌種保存及研究中心提供或自行發酵生產 EFMA。探討以不同濃度之 FFMA 及 EFMA 處理人類肝癌細胞株後，對肝癌細胞之細胞生存力、細胞形態之影響，以找出較適當之 FFMA 及 EFMA 處理濃度；並以流式細胞儀（flow cytometer）研究 FFMA 及 EFMA 對於人類肝癌細胞株細胞週期之影響。

三、結果與討論

(一) 篩選自行發酵牛樟芝菌絲體發酵萃取液（EFMA）之條件

本研究採用的牛樟芝菌株為 BCRC 35716，進行發酵的基本條件： 22℃，培養基含 2％ glucose、2％ malt extract、0.1％ peptone，搖瓶轉速為 100rpm 。以 MTT 方法篩選分析抑制 Hep G2 肝癌細胞株生存力。由表一之結果顯示，不同發酵條件下，抑制 Hep G2 肝癌細胞株生存力之較佳發酵時間為二個月。以此條件所生產之 EFMA 處理 Hep G2 肝癌細胞株，其細胞之生存力為 6.9±0.4％。因此後續之研究工作均採用此發酵條件所自行生產之 EFMA 及食工所提供之 FFMA 進行對人類肝癌細胞株 Hep G2 與 Hep 3B 影響之探討。

(二)在肝癌細胞生存力方面

圖一及圖二為探討食工所 FFMA 及自行發酵 EFMA 對於抑制 Hep G2 及 Hep 3B 生存力的影響。以食工所 FFMA 處理濃度為 100、300、500、600μg/ml 時，結果發現以食工所 FFMA 處理 Hep G2 與 Hep 3B 細胞，其 IC_{50} 分別為 447 及 244μg/ml，與對照組有顯著性差異($p < 0.01$)。而以自行發酵 EFMA 10、30、50、60μg/ml 處理 Hep G2 及 Hep 3B 肝癌細胞時，IC_{50} 分別為 24.6 及 48.1μg/ml (圖三與圖四)。

(三）在肝癌細胞形態方面

探討食工所 FFMA 及自行發酵 EFMA 對於 Hep G2 及 Hep 3B 細胞形態之影響。以食工所 FFMA 100、300、500、600μg/ml 處理時，結果如圖五及圖六所示，發現以 100 及 300μg/ml FFMA 處理 72 小時後，其細胞形態與控制組之形態並無顯著改變。然而，以 500 與 600μg/ml FFMA 處理 72 小時後，Hep G2 及 Hep 3B 之細胞形態發生了相當程度之變化，包括：形狀變形(shape deformation)、單層細胞崩潰(destruction of cell monolayer)、細胞核模糊且細胞皺縮死亡與懸浮，此結果與上述之細胞生存力結果相呼應。而以自行發酵 EFMA 30 與 50μg/ml 的劑量處理時，Hep G2 及 Hep 3B 細胞在培養 72 小時後，其細胞形態也發生類似的變化(圖七與圖八)。

(四）在肝癌細胞週期方面

圖九、圖十、圖十一及圖十二為細胞流式儀(Flow cytometer)分析食工所 FFMA 及自行發酵 EFMA 對 Hep G2 及 Hep 3B 細胞週期影響之結果。以 500μg/ml 的食工所 FFMA 處理時，Hep G2 肝癌細胞株有明顯被滯留在 G0/G1 期的現象，此表示細胞在染色體合成時受到抑制；而在 Hep 3B 肝癌細胞株方面，以食工所 FFMA 處理濃度為 300 及 500μg/ml 時，發現大量細胞壞死 (necrosis)之現象，少部分細胞被滯留於 S 期，顯示食工所 FFMA 對於 Hep 3B 肝癌細胞株有很強的毒殺效果，並使少部分細胞無法進行細胞分裂。另外，在自行發酵 EFMA 方面，如圖十一及圖十二所示，隨著 EFMA 處理濃度增加，Hep G2 肝癌細胞株被滯留於 G2/M 期的細胞有增加之趨勢，這表示細胞是處於染色體為 4N 但不進行有絲分裂期之狀態（圖十一）；而 Hep 3B 肝癌細胞株之細胞則有被滯留於 S 期之趨勢。

四、計畫成果自評

目前本研究室已成功篩選出具有較佳抑制肝癌細胞生存力之牛樟芝菌絲體過濾液之發酵條件，並已瞭解食工所FFMA及自行發酵EFMA對人類肝癌細胞株Hep G2及Hep 3B在生存力、細胞形態及細胞週期等方面之影響。

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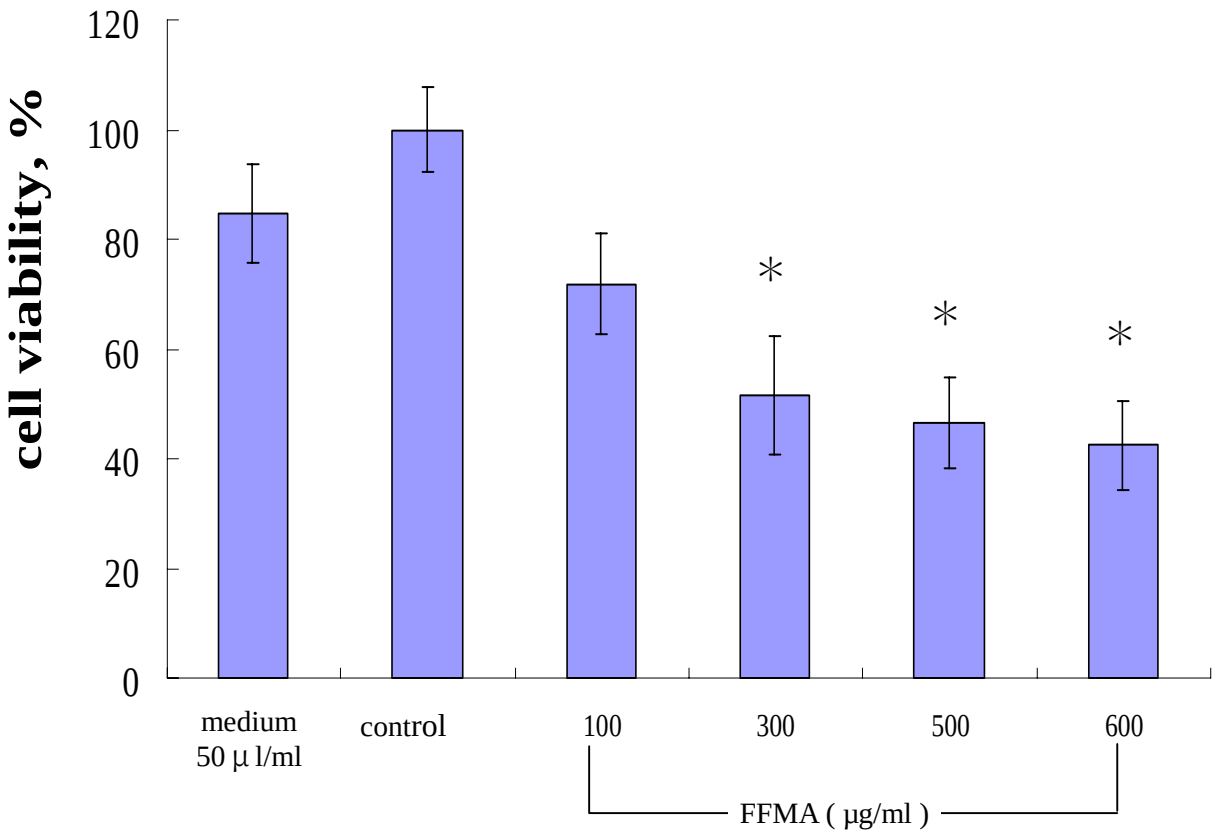
表一、不同發酵條件生產之牛樟芝菌絲體發酵萃取液對 Hep G2 肝癌細胞株生存力的影響

Table 1. Effects of extract of fermented mycelia of *Antrodia camphorata* from various fermentation conditions on the cell viability of Hep G2

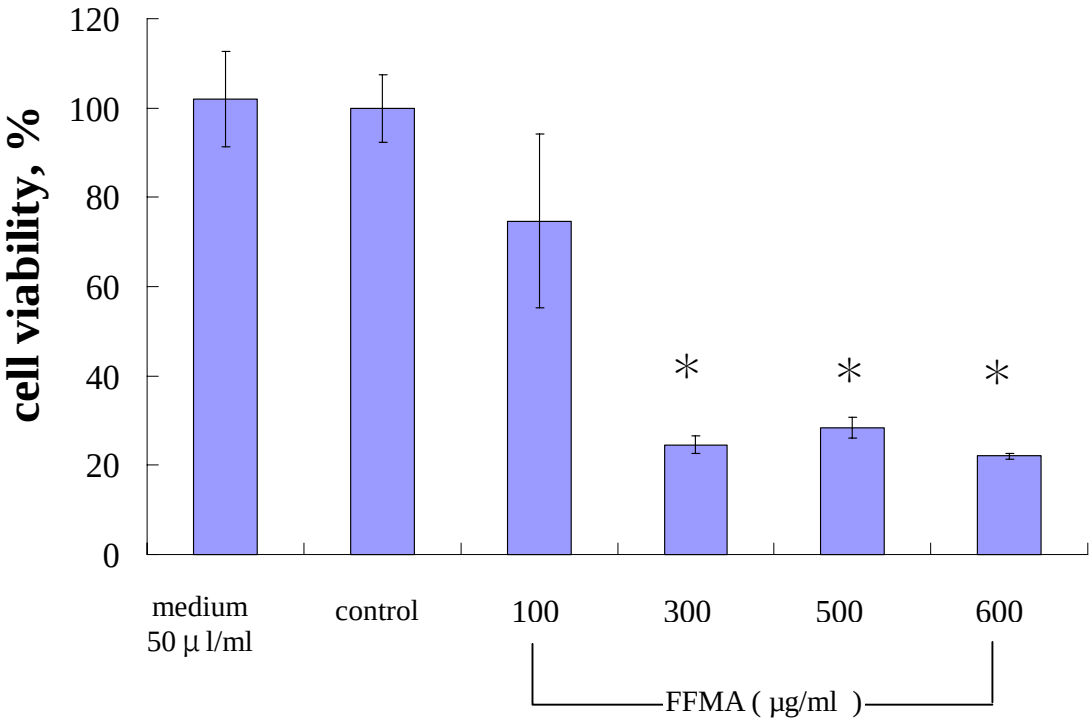
Fermentation conditions	cell viability (%)
Fermentation time 1 week	114.9
2	54.5
3	40.4
1 month	12.6
2	6.9
2% lactose instead of 2% glucose	17.5
30℃ instead of 22℃	70.1

1. 100ml fermentation broth was added into the 250 ml flask.
2. Standard fermentation condition: 22℃; fermented medium containing 2% glucose, 2% malt extract, and 0.1% peptone; 100rpm



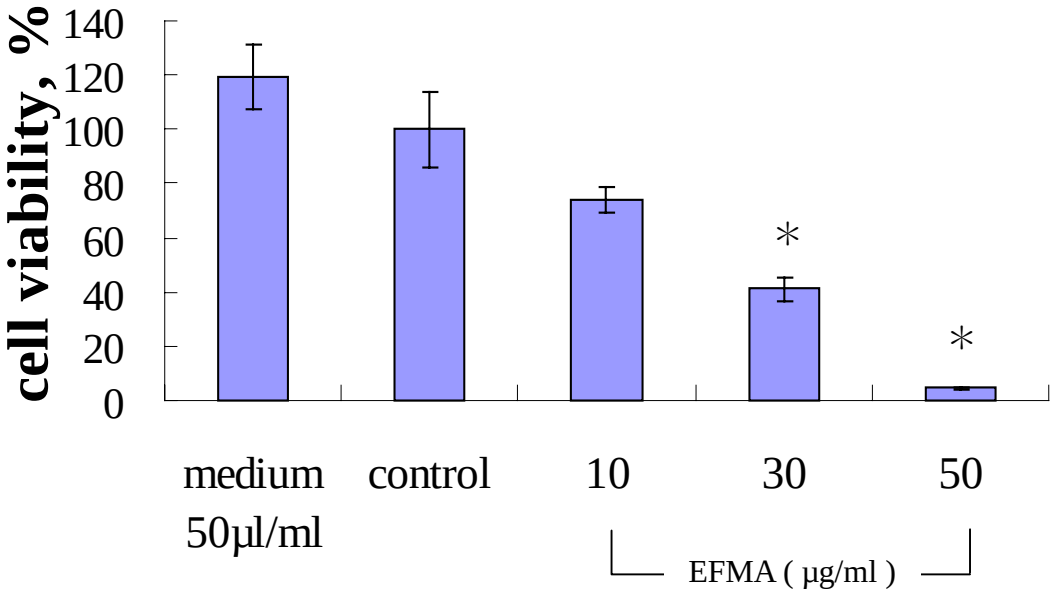
圖一、不同濃度食工所 FFMA 處理 Hep G2 細胞 72 小時後對其生存力的影響

Figure 1. Effects of various concentrations of FFMA from FIRDI on the cell viability of Hep G2 cells for 72 hr treatment. ＊Significantly different (p<0.01) between treatment and control.



圖二、不同濃度食工所 FFMA 處理 Hep 3B 細胞 72 小時對其生存力的影響

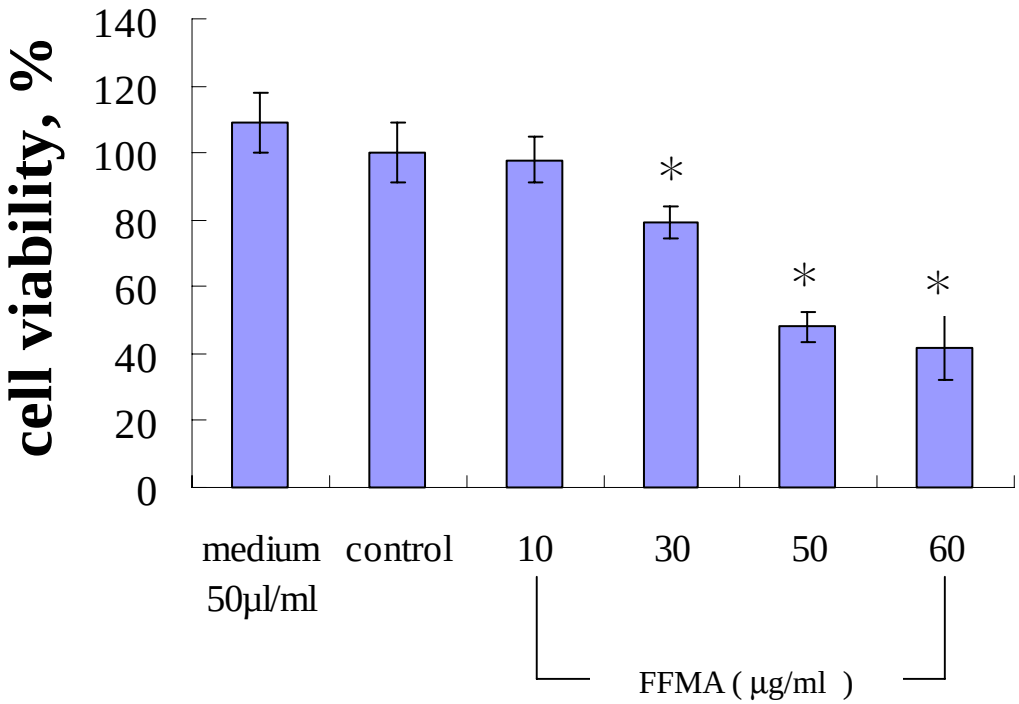
Figure 2. Effects of various concentrations of FFMA from FIRDI on the cell viability of Hep 3B cells for 72 hr treatment. ＊Significantly different (p<0.01) between treatment and control.



圖三、不同濃度自行發酵 EFMA 處理 Hep G2 細胞 72 小時對其生存力的影響

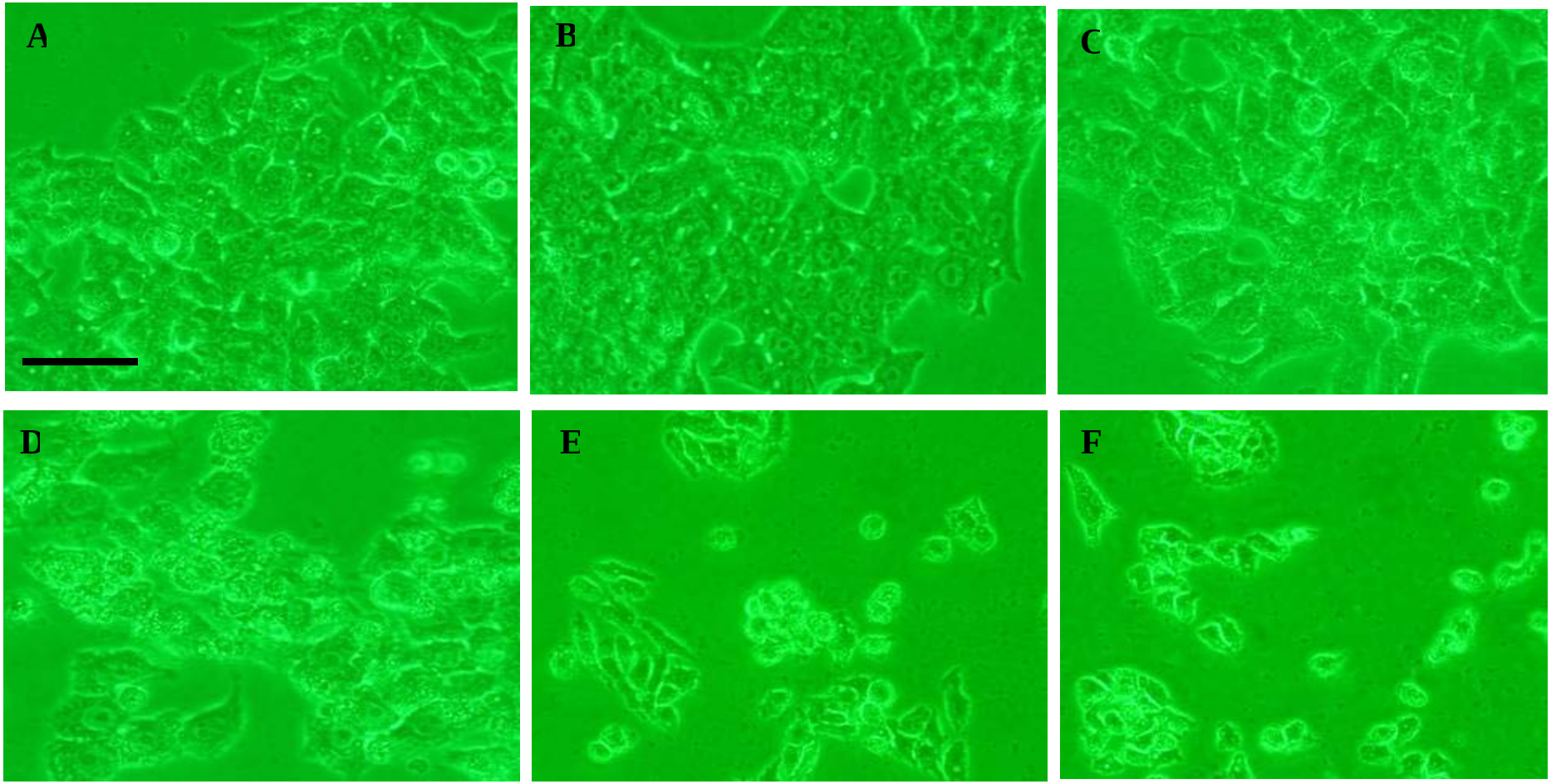
Figure 3. Effects of various concentrations of EFMA from our lab on the cell viability of Hep G2 cells for 72 hr treatment. ＊Significantly different (p<0.01)

between treatment and control.



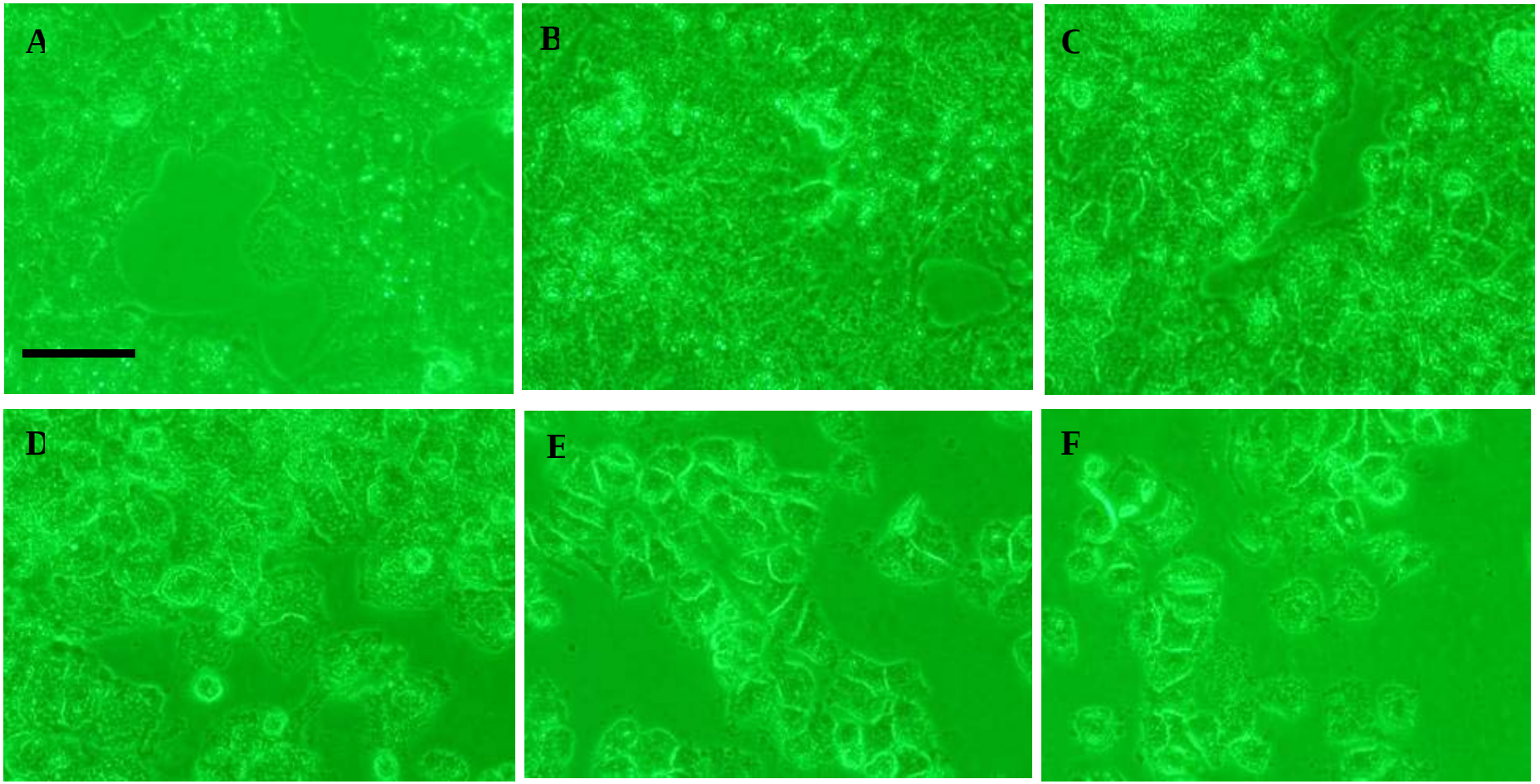
圖四、不同濃度自行發酵 EFMA 處理 Hep 3B 細胞 72 小時對其生存力的影響

Figure 4. Effects of various concentrations of EFMA from our lab on the cell viability of Hep 3B cells for 72 hr treatment. ＊Significantly different (p<0.01) between treatment and control.



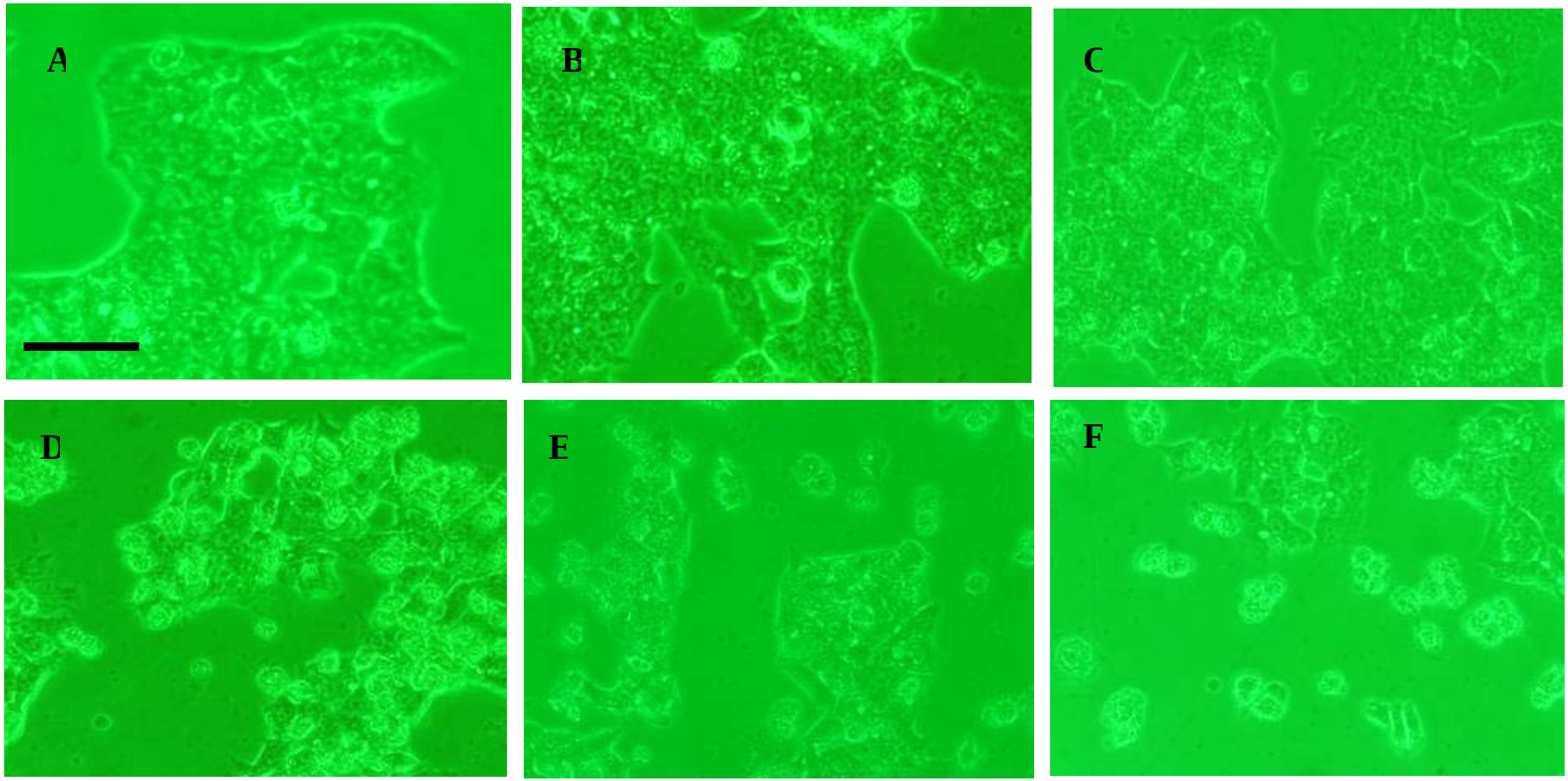
圖五、不同濃度食工所 FFMA 對 Hep G2 細胞形態的影響。

Figure 5. Effects of various concentrations of FFMA from FIRDI on the morphology of Hep G2 cells (200X under inverted stage microscope equipped with phase contrast). A-F, Hep G2 cells were cultured for 72 hr after treatment with 50μl/ml medium and 0, 100, 300, 500 or 600μg/ml FFMA, respectively. ㄧ = 50 μm.



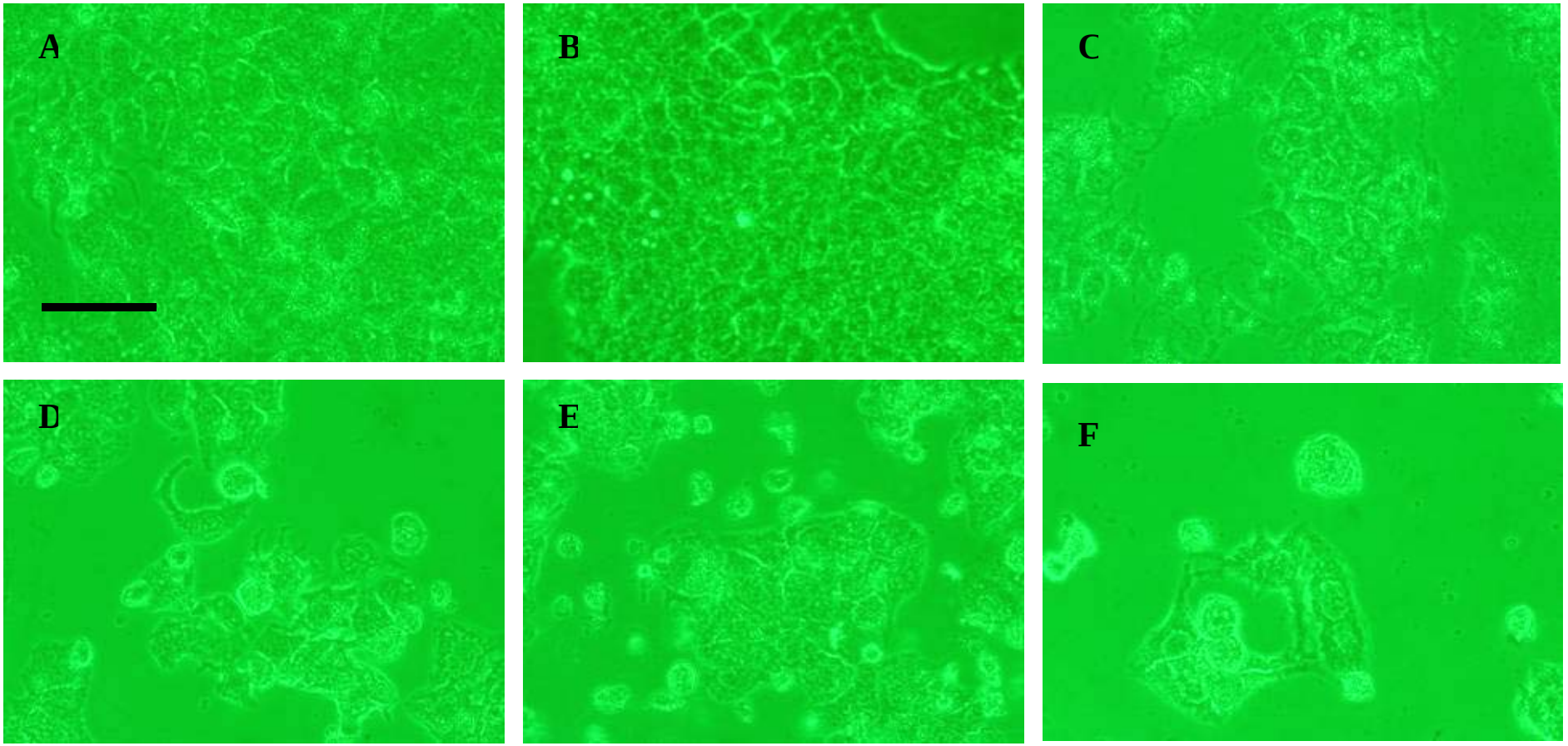
圖六、不同濃度食工所 FFMA 對 Hep 3B 細胞形態的影響

Figure 6. Effects of various concentrations of FFMA from FIRDI on the morphology of Hep 3B cells (200X under inverted stage microscope equipped with phase contrast). A-F, Hep 3B cells were cultured for 72 hr after treatment with 50µl/ml medium and 0, 100, 300, 500 or 600µg/ml FFMA, respectively. ㄧ = 50 µm.



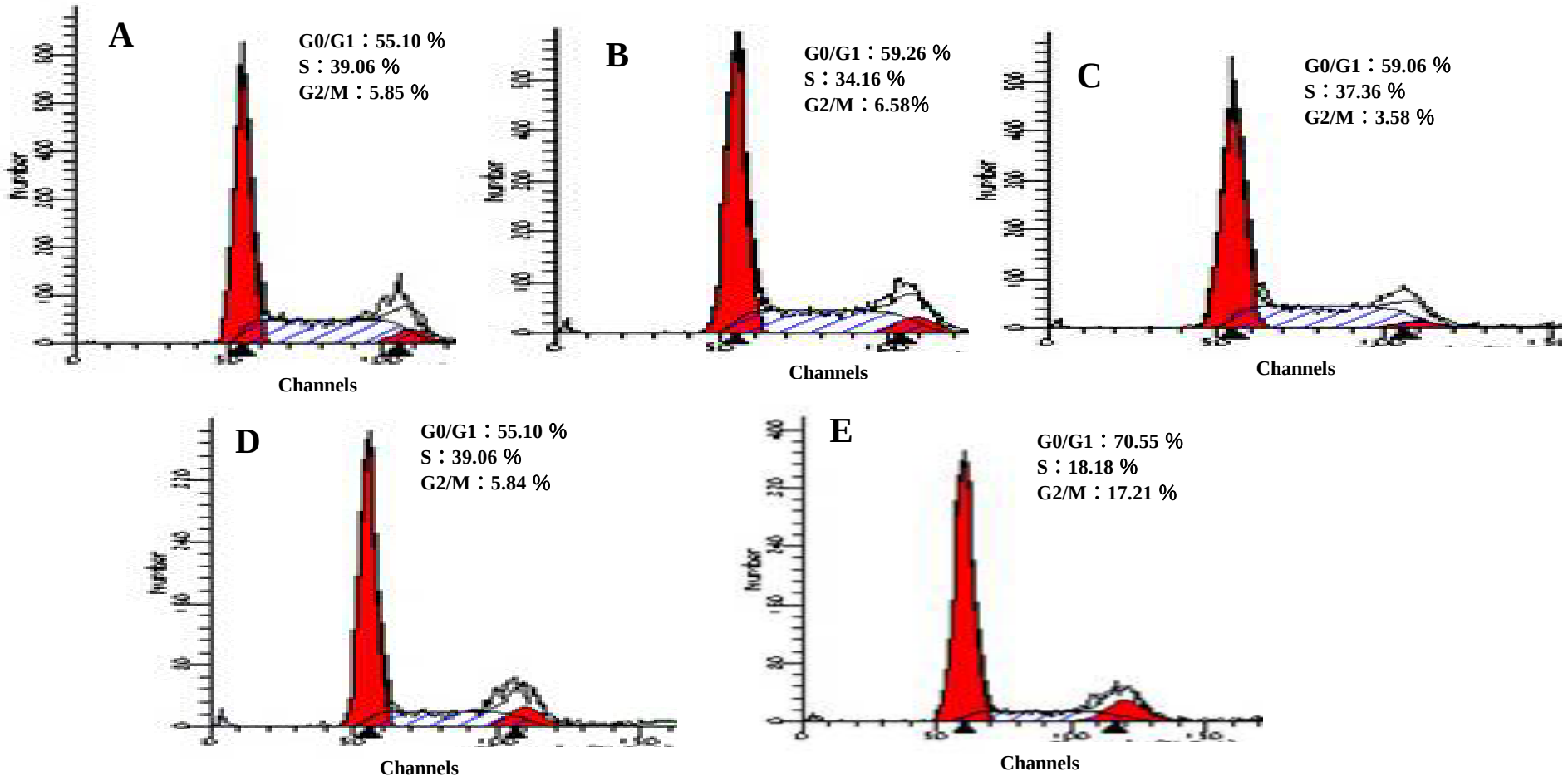
圖七、不同濃度自行發酵 EFMA 對 Hep G2 細胞形態的影響

Figure 7. Effects of various concentrations of EFMA from our lab on the morphology of Hep G2 cells (200X under inverted stage microscope equipped with phase contrast). A-F, Hep G2 cells were cultured for 72 hr after treatment with 50µl/ml medium and 0, 10, 30, 50 or 60µg/ml EFMA, respectively. ㄧ = 50 µm.



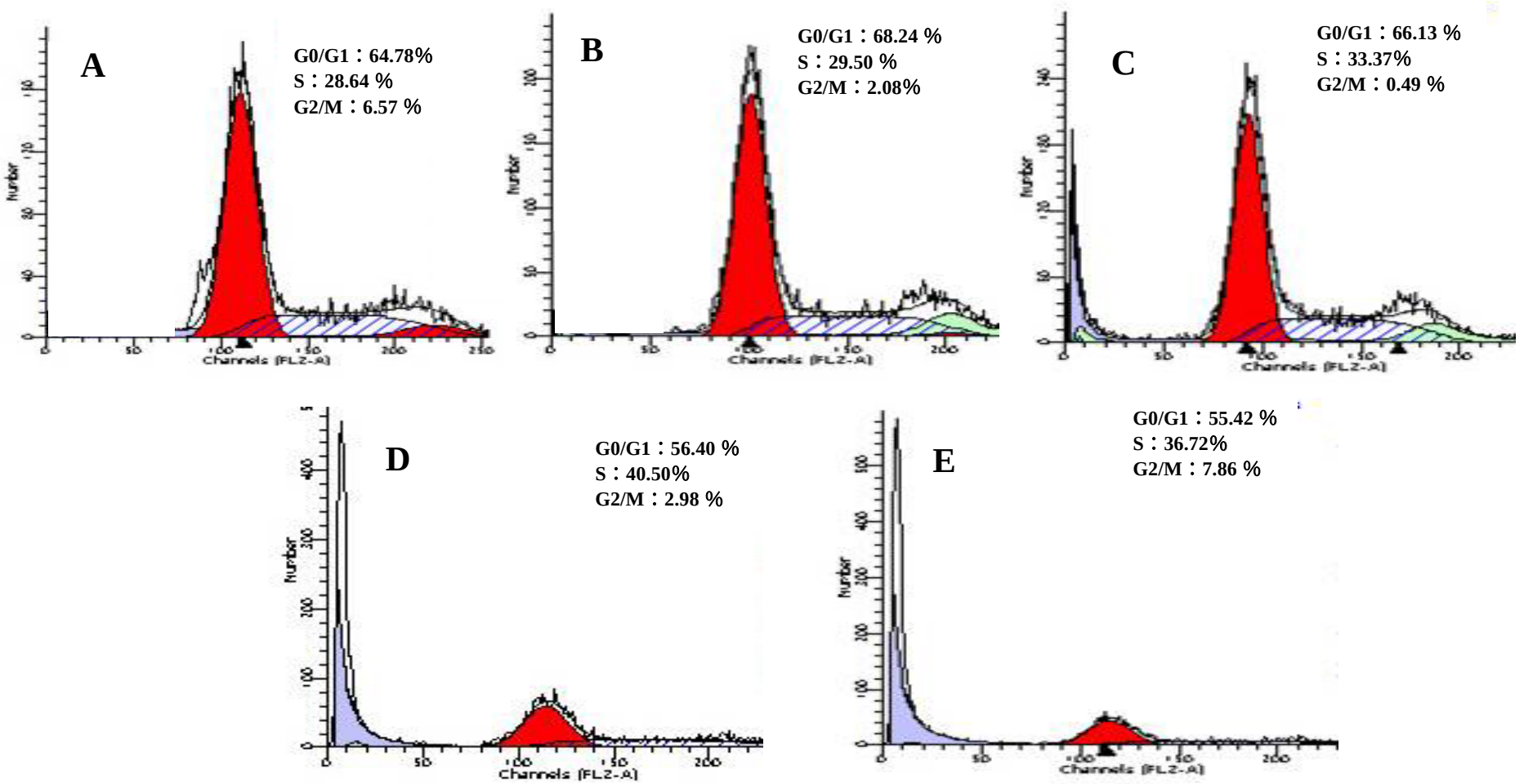
圖八、不同濃度自行發酵 EFMA 對 Hep 3B 細胞形態的影響

Figure 8. Effects of various concentrations of EFMA from our lab on the morphology of Hep 3B cells (200X under inverted stage microscope equipped with phase contrast). A-F, Hep 3B cells were cultured for 72 hr after treatment with 50μl/ml medium and 0, 10, 30, 50 or 60μg/ml EFMA, respectively. ㄧ = 50 μm.



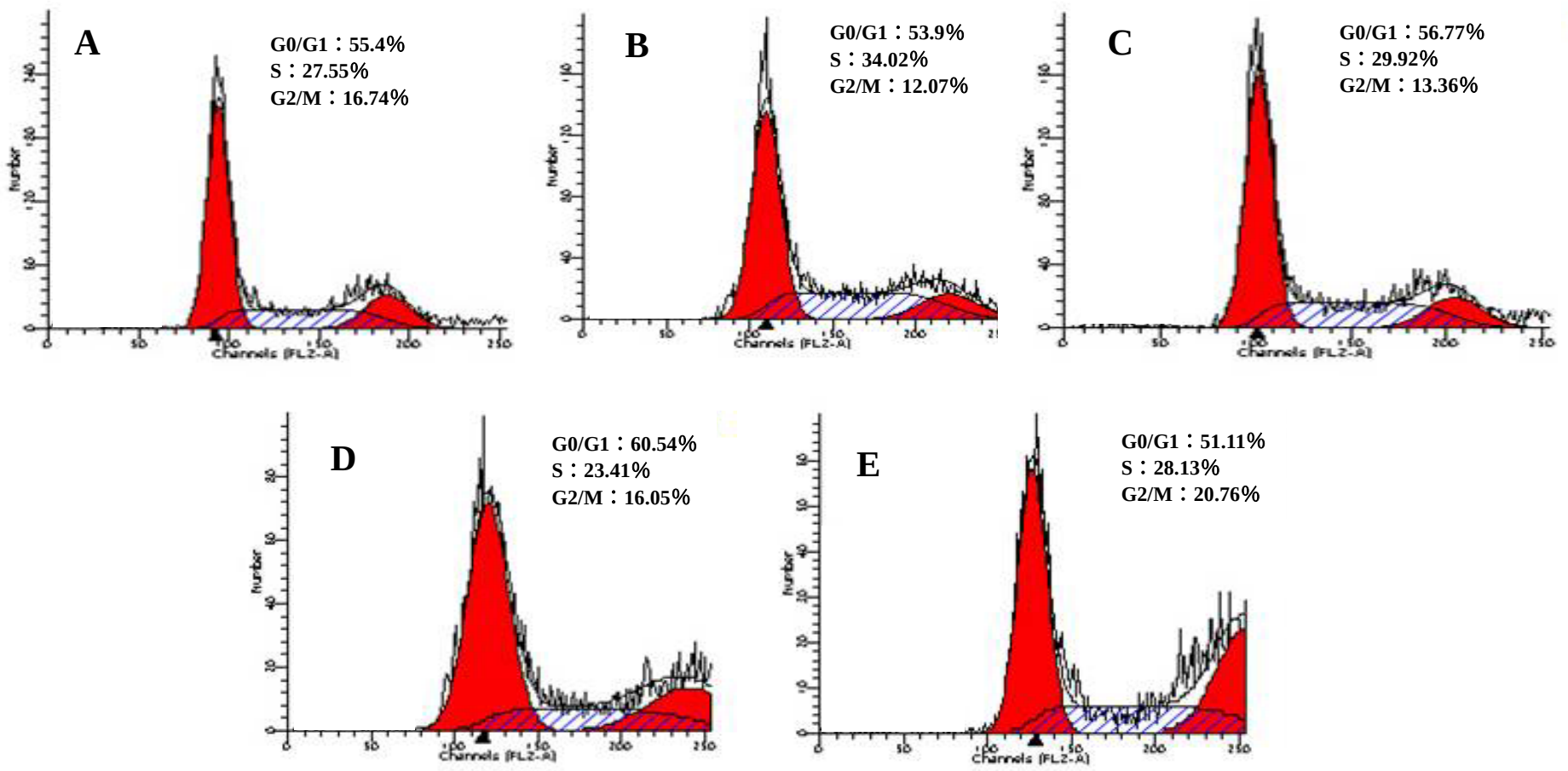
圖九、不同濃度之食工所 FFMA 對 Hep G2 細胞週期之影響

Figure 9. Effects of various concentrations of FFMA from FIRDI on the cell cycle of Hep G2 cells. A-E, Hep G2 cells were cultured for 72 hr after treatment with 0, 50, 100, 300, or 500μg/ml FFMA, respectively.



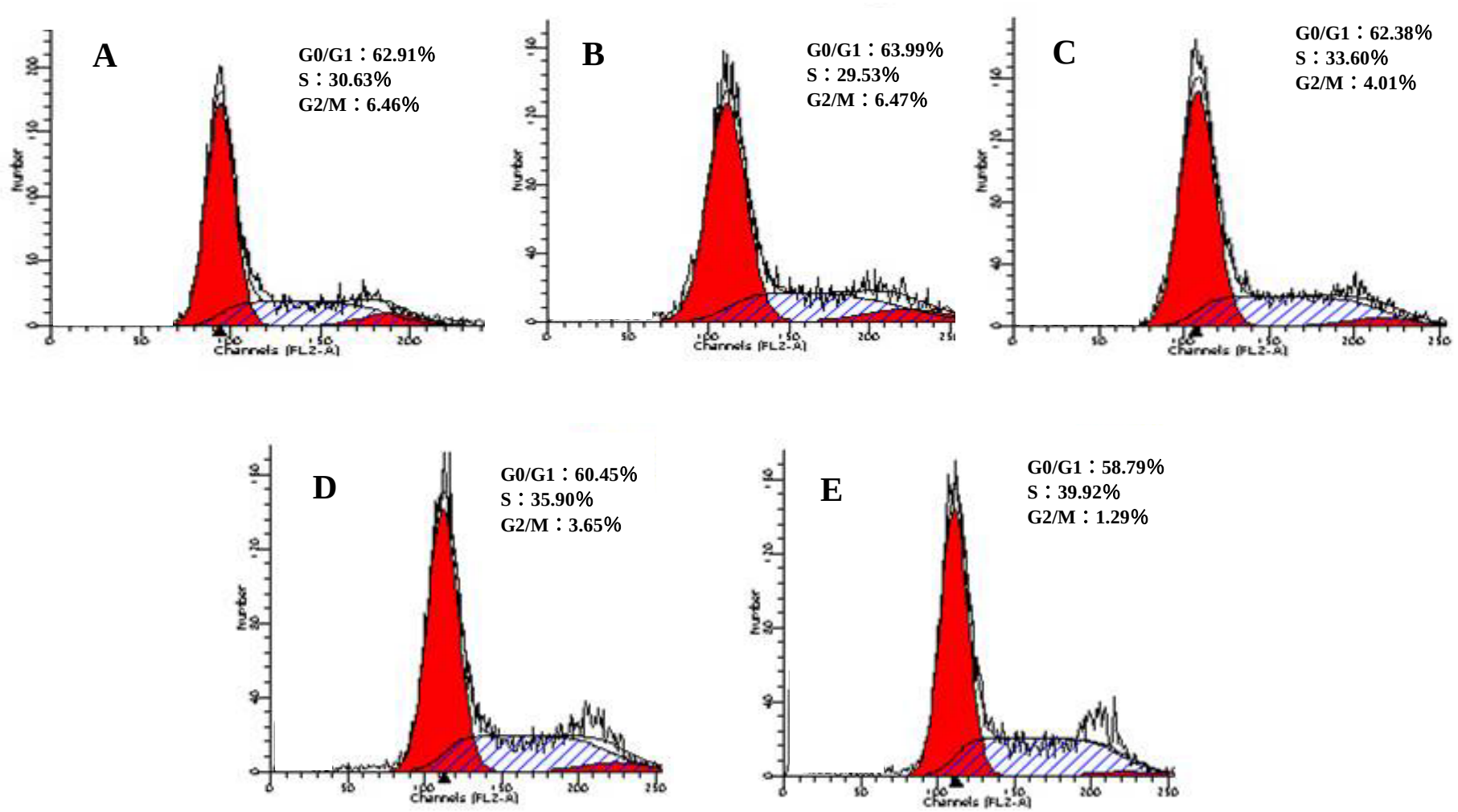
圖十、不同濃度食工所 FFMA 對 Hep 3B 細胞週期之影響

Figure 10. Effects of various concentrations of FFMA from FIRDI on the cell cycle of Hep 3B cells. A-E, Hep 3B cells were cultured for 72 hr after treatment with 0, 50, 100, 300, or 500μg/ml FFMA, respectively.



圖十一、不同濃度之自行發酵 EFMA 對 Hep G2 細胞週期之影響

Figure 11. Effect of various concentrations of EFMA from our lab on the cell cycle of Hep G2 cells. A-E, Hep G2 cells were cultured for 72hr after treatment with 0, 5, 10, 30, or 50μg/ml EFMA, respectively.



圖十二、不同濃度之自行發酵 EFMA 對 Hep 3B 細胞週期之影響

Figure 12. Effects of various concentrations of EFMA from our lab on the cell cycle of Hep 3B cells. A-E, Hep 3B cells were cultured for 72 hr after treatment with 0, 5, 10, 30, or 50μg/ml EFMA, respectively.