行政院國家科學委員會專題研究計畫　成果報告

薑精油對肝癌細胞及正常肝細胞生理機能之影響

計畫類別：個別型計畫
計畫編號：NSC91-2320-B-002-148-
執行期間：91 年 08 月 01 日至 92 年 07 月 31 日
執行單位：國立臺灣大學食品科技研究所

計畫主持人：沈立言

報告類型：精簡報告

處理方式：本計畫可公開查詢

中　華　民　國 93 年 2 月 10 日

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薑精油對肝癌細胞及正常肝細胞生理機能之影響

Effect of ginger essential oil on the physiological functions of hepatoma cells and normal hepatocytes

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一、中文摘要

本研究以南投縣所栽培之生薑（*Zingiber officinale* Roscoe）進行水蒸氣蒸餾法所萃取之薑精油為實驗材料，以 MTT assay 來探討不同濃度的薑精油對於人類肝癌細胞株 Hep G2 與 Hep 3B 之生存力。並採用大白鼠初代肝細胞之分離與培養為實驗模式，探討不同濃度之薑精油對大白鼠初代肝細胞生存力、抗氧化及解毒代謝能力之影響。實驗結果顯示，薑精油平均萃取率為 0.16%。經由 GC-MS 分析其精油成份含有高量的萜烯類碳氫化合物，尤以 Geranial 含量高達 25.2%，Citronellol 次之約為 18.7%。肝癌細胞株 Hep 3B 以高濃度 100~200 μg/ml 薑精油處理 24 小時後細胞生存抑制百分比皆顯著高於控制組($p<0.05$)，IC_{50} 約為 173 μg/ml。而對於肝癌細胞株 Hep G2 而言，50~200 μg/ml 薑精油則能顯著性抑制肝癌細胞 Hep G2 之生長，IC_{50} 為 94.2 μg/ml。可見薑精油對於肝癌細胞 Hep G2 之生存抑制效果較 Hep 3B 佳。在薑精油低濃度（50 μg/ml）情況下，對於肝癌細胞 Hep G2 就有抑制 28.1%生存力，對初代肝細胞則無顯著性影響，並且能夠降低細胞之脂質過氧化。10 μg/ml 濃度之薑精油則可提高大白鼠初代肝細胞之抗氧化及其解毒代謝系統能力。而當薑精油濃度提升至 100 μg/ml 時，對肝癌細胞 Hep 3B、Hep G2 抑制效果達到 22.3 和 54.7%，對於正常初代肝細胞亦有 52.1%的傷害。

關鍵詞：薑精油、細胞生存力、抗氧化、解毒作用、大白鼠初代肝細胞、人類肝癌細胞株

Abstract

Ginger oil was extracted from *Zingiber officinale* Roscoe which was cultivated in Nanto county using the steam distillation method. The cell viability of hepatoma cell line Hep G2 and Hep 3B which were treated with various concentrations of ginger oil was investigated by MTT assay. In addition, the cell viability, antioxidation and detoxification systems of primary rat hepatocytes treated with various concentrations of ginger oil were also investigated. The result showed that the average yield of extraction of fresh ginger was 0.16%. The major components of the ginger essential oil are geranial (25.2 %) and citronellol (18.7 %) in ginger essential oil according to the GC-MS analysis. In terms of the results of MTT assay, when the Hep 3B cells were treated with high concentration of ginger essential oil (100~200 μg/ml) for 24 hours, the inhibition percentages of cell viability were significantly higher than that of the control ($p<0.05$). The IC_{50} of Hep 3B was around 173 μg/ml of ginger essential oil. Of Hep G2, treated with 50~200 μg/ml of ginger essential oil, inhibition percentages of cell viability were also significantly higher than that of the control ($p<0.05$). The IC_{50} of Hep G2 was about 94.2 μg/ml of ginger essential oil. These results showed that the

inhibition effect of ginger essential oil on Hep G2 was much better than on Hep 3B. Under low concentration of ginger essential oil (50 μg/ml) treatment, there was 28.1 % inhibition of cell viability in Hep G2 cells, but no significant effect on primary rat hepatocytes, and also decreasing the lipid peroxidation in hepatocytes. Ten μg/ml of ginger essential oil could increase the capabilities of antioxidation and detoxification systems in primary rat hepatocytes. While 100 μg/ml of ginger essential oil treatment not only could cause 22.3 and 54.7% inhibition of cell viability in Hep 3B and Hep G2, respectively, but also cause 52.1 % inhibition of cell viability in primary rat hepatocytes.
Key words: ginger essential oil, cell viability, antioxidation, detoxification, primary rat hepatocytes, human hepatoma cell lines

二、緣由與目的

薑（*Zingiber officinale* Roscoe）在中國自古栽培為藥用及蔬菜用（胡，1978），而其以水蒸氣蒸餾法所萃取之薑精油更廣泛應用於食品、化妝品、藥品等工業上（Lawrence, 1984）。薑在台灣菜餚與中國傳統藥膳保健食品之食物療法中亦扮演著相當重要的角色。現今科學性的文獻報告中亦顯示，薑的確具有許多不錯的生理機能，例如可以降低噁心嘔吐的症狀（Yamahara et al., 1989; Bone et al., 1990; Arfeen et al., 1995; Meyer et al., 1995; Visalyaputra et al., 1998; Aikins Murphy, 1998; Ernst and Pittler, 2000; Vutyavanich et al., 2001; Fugh-Berman and Kronenberg 2003,）、提升腸胃功能（Al-Yahya et al., 1989; Wu et al., 1990; Yamahara et al., 1990; Stewart et al., 1991; Platel and Srinivasan, 1996; Sharma and Gupta, 1998; Micklefield et al., 1999; Pin et al., 2002）、對心血管疾病之預防有助益（Srivastava, 1984, 1986, 1989; Srinivasan and Sambaiah, 1991; Tanabe et al., 1993; Verma et al., 1993; Lumb, 1994; Bordia et al., 1997; Bhandari et al., 1998; Fuhrman et al., 2000）、在預防癌症方面亦具有潛力（Hashim et al., 1994; Soudamini et al., 1995; Katiyar et al., 1996; Vimala et al., 1999; Bode et al., 2001）。由於癌症是台灣十大死亡原因之首，而肝癌又是癌症中名列第一的頭號殺手。且肝臟是人體中重要的解毒代謝器官，因此探討如何利用食物來預防癌症、護肝達到養生保健的目的，的確是非常重要的研究課題。故本研究採用細胞模式，探討薑精油對肝癌細胞及正常肝細胞生理機能的影響。以期尋找出在何種條件下薑精油能發揮抑制肝癌細胞的效果，並能提升正常肝細胞之解毒代謝與抗氧化能力，達到人類防癌養生保健之目的。

目前動物細胞或組織之培養技術已廣泛的使用於各種有關生物醫學的領域。其中已知肝臟是具有代謝控制、酵素誘導、對激素反應及解毒抗氧化等特殊生理功能的器官，而這些功能的執行大部份都在肝的實質細胞（parenchymal cell）中進行。以肝灌注分離新鮮肝細胞配合細胞培養（cell culture）具有專一性、成本低、實驗條件容易控制、免於其他內生性因子的影響及大量樣品易取得等優點。因此，目前此技術已廣泛被應用於多種研究中（McQueen and Williams, 1987; Davila, 1991），包括肝細胞之毒性試驗、藥物代謝、肝細胞的功能、控制因子間交互作用，以及抗癌及抗突變等研究。所以培養肝實質細胞，是一項重要的實驗技術。自 Berry 和 Friend 於 1973 年發展出利用膠原蛋白酶分離肝細胞之技術以來，並經 Kreamer 等人於 1986 年修正後，使得肝細胞培養技術更趨成熟，成功率與產量均獲長足進步。

因此，本研究擬以薑精油為實驗材料，作用於人類肝癌細胞株Hep G2與Hep 3B，了解不同濃度薑精油對肝癌細胞之抑制率。再利用肝灌注手術分離大白鼠初代肝細胞配合細胞培養為實驗模式，探討活體外不同濃度的薑精油對肝

細胞之生存力、細胞形態變化、抗氧化及解毒代謝系統之影響，以便有系統地瞭解薑精油生理活性。

三、結果與討論

（一）薑精油之萃取與分析

以水蒸氣蒸餾法進行薑精油之萃取，平均萃取率為 0.16%。

薑精油以 Hewlett Packard 5973 GC-MS 進行氣相層析質譜分析，分析圖譜結果如圖一所示，共有 14 個主要吸收波峰，其各代表成分如表一所示，薑精油組成中以 Geranial 含量高達 25.2%，Citronellol 次之為 18.7%。這些成分多為萜烯類碳氫化合物，也是薑精油香味的主要來源。

（二）在肝癌細胞株 Hep G2 與 Hep 3B 生存力方面

肝癌細胞在薑精油處理 24 小時後以 MTT assay 與倒立式位相差顯微鏡來探討肝癌細胞生存力。在薑精油低濃度 50 μg/ml 處理 24 小時後，肝癌細胞株 Hep 3B 生存力為 96.5%（$p>0.05$）。當薑精油處理濃度達到 100 μg/ml 時，經由 Dunnett's test 統計分析結果顯示，與控制組比較下，則顯著地抑制 22.3% 肝癌細胞株 Hep 3B 之生存力（$p<0.05$）。而高濃度薑精油 175-200 μg/ml 處理下則抑制 50%肝癌細胞株 Hep 3B 的生存力，且形態上可明顯看出肝癌細胞發生皺縮現象（Fig. 5D），肝癌細胞株 Hep 3B 之 IC_{50} 約為 173 μg/ml（Fig. 2）。而在肝癌細胞株 Hep G2 方面，在低濃度 50 μg/ml 薑精油處理下已能夠顯著降低 28.1%肝癌細胞株 Hep G2 的生存力（$p<0.05$）。當薑精油處理濃度達到 100 μg/ml 以上時，肝癌細胞株 Hep G2 的生存力只剩 26.8~45.3%，即表示濃度達到 100 μg/ml 以上時可抑制 50%以上肝癌細胞株 Hep G2 的生存力，觀察其形態也發現肝癌細胞產生皺縮之現象（Fig. 6C, 6D），其 IC_{50} 為 94.2 μg/ml（Fig. 3）。由以上結果可知，薑精油對於 Hep G2 抑制生存力效果較 Hep 3B 佳，而且在低濃度下即對 Hep G2 之生存力具有抑制效果。

（三）大白鼠正常初代肝細胞方面

大白鼠初代肝細胞生存力：

大白鼠初代肝細胞在薑精油處理後以 MTT assay 與倒立式位相差顯微鏡來探討其生存力。由圖四之結果顯示，大白鼠初代肝細胞經由 10 μg/ml 薑精油處理後，不但無抑制細胞之生存力，反而有促進 16.2%生存力之效果。在細胞形態方面，圖七之結果顯示經由薑精油 30 μg/ml 處理 24 小時後，仍可清楚看見大白鼠初代肝細胞的細胞膜及細胞核相當完整（Fig. 7B），生長狀況與控制組相似（Fig. 7A）。當薑精油的處理濃度達到 70 μg/ml 時，開始有抑制 28.4%正常初代肝細胞生存力，隨著薑精油處理濃度之增加初代肝細胞之生存力也隨之下降。在薑精油濃度為 100 μg/ml 以上時，正常初代肝細胞生存力僅剩 15.2～52.3%，可見此濃度已對正常初代肝細胞已有相當大的抑制生存力的作用，同時細胞形態也已經漸漸有細胞膜破損（Fig. 7D, 7E），在 200 μg/ml 濃度薑精油處理 24 小時後，細胞膜不但破損，細胞萎縮，細胞核也消失，可見肝細胞已受嚴重的傷害（Fig. 7F）。

脂質過氧化測定：

參考 Fraga 等人（1998）之方法。利用脂質過氧化產物 malondialdehyde（MDA）在酸及高熱環境下，可與二分子 thiobarbituric acid（TBA）縮合成一粉紅色物質（TBA chromogen），在 excitation 515 及 emission 555 下，以螢光分光光度計測定 MDA 濃度，即得 TBARS 值。結果顯示，正常大白鼠初代肝細胞在薑精油處理組其 TBARS 質皆低於控制組，即表示薑精油有降低脂質過氧化之效果（Fig. 8）。此項目會進一步深入探討。

抗氧化及解毒代謝之影響：

過氧化氫或有機過氧化物可藉由麩

胱甘肽（GSH）與麩胱甘肽過氧化酶（GSH peroxidase）催化代謝形成氧化態麩胱甘肽（GSSG），之後氧化態麩胱甘肽再利用麩胱甘肽還原酶（GSH reductase）還原成還原態麩胱甘肽（GSH），在細胞內不斷代謝利用，維持抗氧化系統保護細胞，避免氧化性傷害。而當外來異物質侵入細胞時，麩胱甘肽亦參與其代謝。外來異物會在麩胱甘肽硫轉移酶（GST）催化下與麩胱甘肽反應形成結合物（GST S-conjugates）達到解毒之目的。實驗結果顯示，在低濃度薑精油（10 μg/ml）處理初代肝細胞時，能增進麩胱甘肽還原酶之活性（Fig. 9），GSH peroxidase 的結果與麩胱甘肽還原酶結果相似（Fig. 10）。可見薑精油能增進正常初代肝細胞抗氧化系統之活性，保護肝細胞避免受到氧化性傷害。在解毒代謝方面，低濃度薑精油處理（10 μg/ml）下，能增進初代肝細胞解毒代謝酵素麩胱甘肽硫轉移酶（GST）之活性（Fig. 11），與初代肝細胞生存力之結果相呼應。

（四）結論

由多種萜烯類碳氫化合物組成的薑精油在低濃度（50 μg/ml）下，對於肝癌細胞 Hep G2 即有抑制 28.1%生存力之效果，且對大白鼠初代肝細胞無不良效果。此外，10 μg/ml 濃度薑精油能夠降低其脂質過氧化，提高抗氧化及解毒代謝系統能力。而當薑精油濃度提升至 100 μg/ml 時，對肝癌細胞株 Hep 3B、Hep G2 生存力之抑制效果達到 22.3 和 54.7%，對於正常初代肝細胞亦有 52.1%之抑制作用。

四、計畫成果自評

目前本研究室已完成不同薑精油濃度對於人類肝癌細胞株Hep 3B、Hep G2與大白鼠正常初代肝細胞之生存力影響，並已完成不同濃度之薑精油對大白鼠正常初代肝細胞之脂質過氧化、肝細胞內麩胱甘肽及其相關酵素活性分析。

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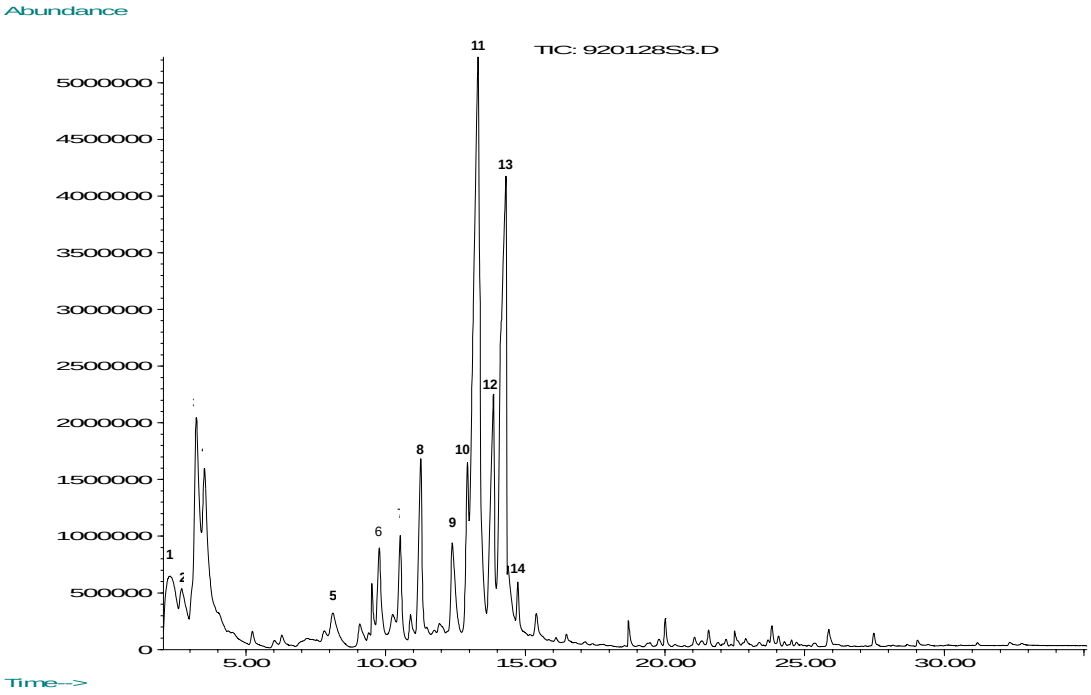


Fig. 1. Gas chromatogram of ginger essential oil.

Table 1. The composition of ginger essential oil

Peak number	component	M. W.	Conc. (%)	CAS Reg. No.
1	Myrcene	136	4.67	123-35-3
2	Limonene	136	2.60	138-86-3
3	β-phellandrene	136	8.70	555-10-2
4	1,8- cineole	154	10.33	470-86-2
5	Terpinolene	136	1.45	586-62-9
6	2-heptanol＋ 6-methyl-5-hepten-2-one	—	2.61	543-49-7 110-93-0
7	citronellal＋ α-copaene	—	2.27	106-23-0 3856-25-5
8	Linalool	154	3.65	78-70-6
9	Neral	152	2.74	106-26-3
10	Zingiberene	204	3.23	495-60-3
11	Geranial	154	25.24	141-27-5
12	β-sesquiphellandrene ＋ ar-curcumene ＋ geranyl acetate	—	6.69	20307-83-9 644-30-4 105-87-3
13	Citronellol	156	18.69	106-22-9
14	Geraniol	154	0.99	106-24-1

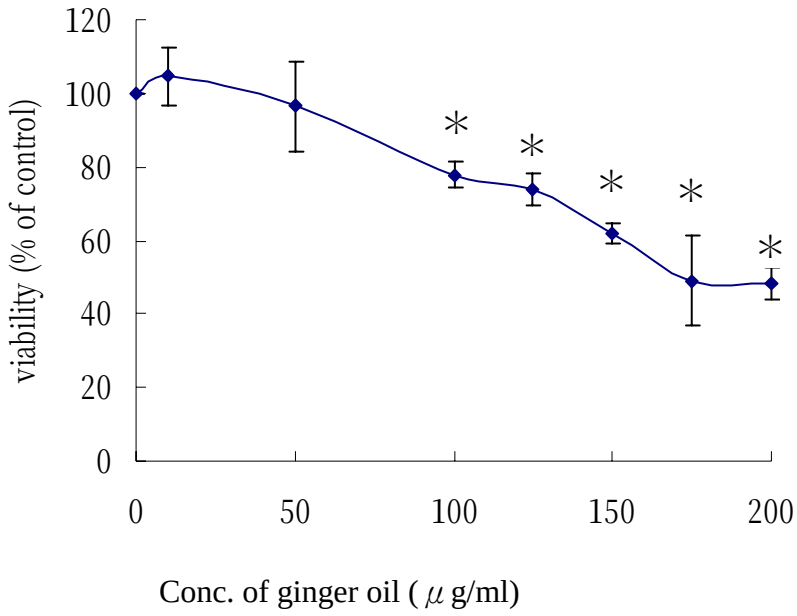


Fig. 2. Effect of various concentrations of ginger oil on the cell viability of hepatoma cell line Hep 3B after 24 hrs treatment. Values are expressed as means±SD from 3 data.* Significant difference ($p<0.05$) by Dunnett's test .

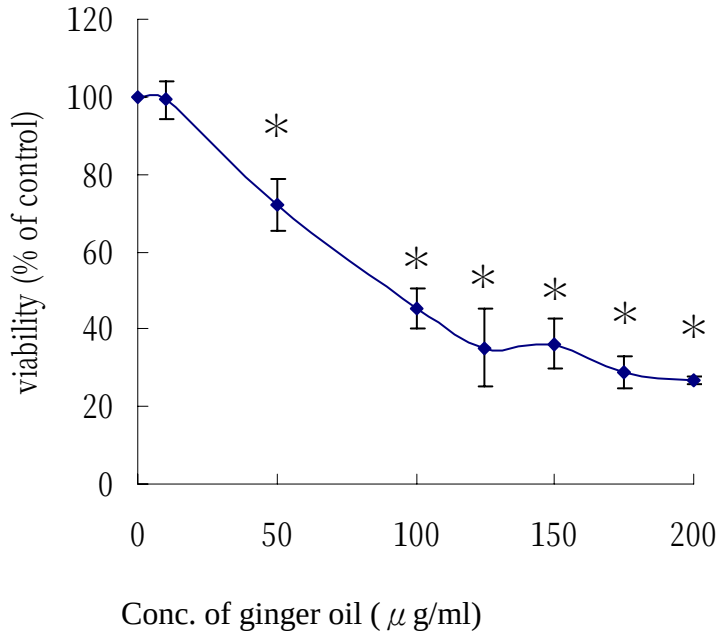
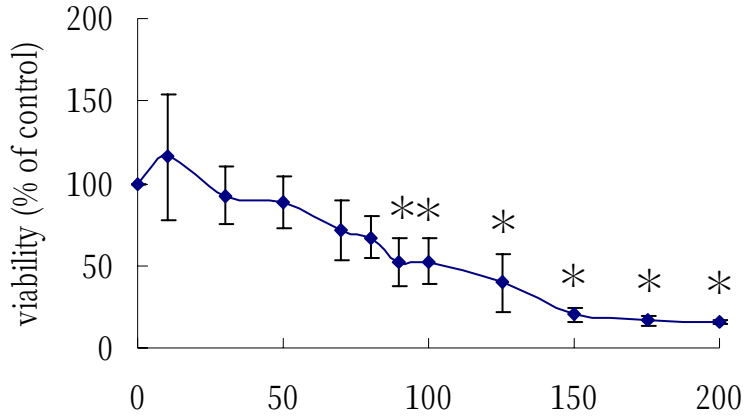


Fig. 3. Effect of various concentrations of ginger oil on the cell viability of hepatoma cell line Hep G2 after 24 hrs treatment. Values are expressed as means±SD from 3 data.* Significant difference ($p<0.05$) by Dunnett's test.



Conc. of ginger oil (μ g/ml)

Fig. 4. Effect of various concentrations of ginger oil on the cell viability of primary rat hepatocytes after 24 hrs treatment by MTT assay. * Significant difference ($p<0.05$) by Dunnett's test.

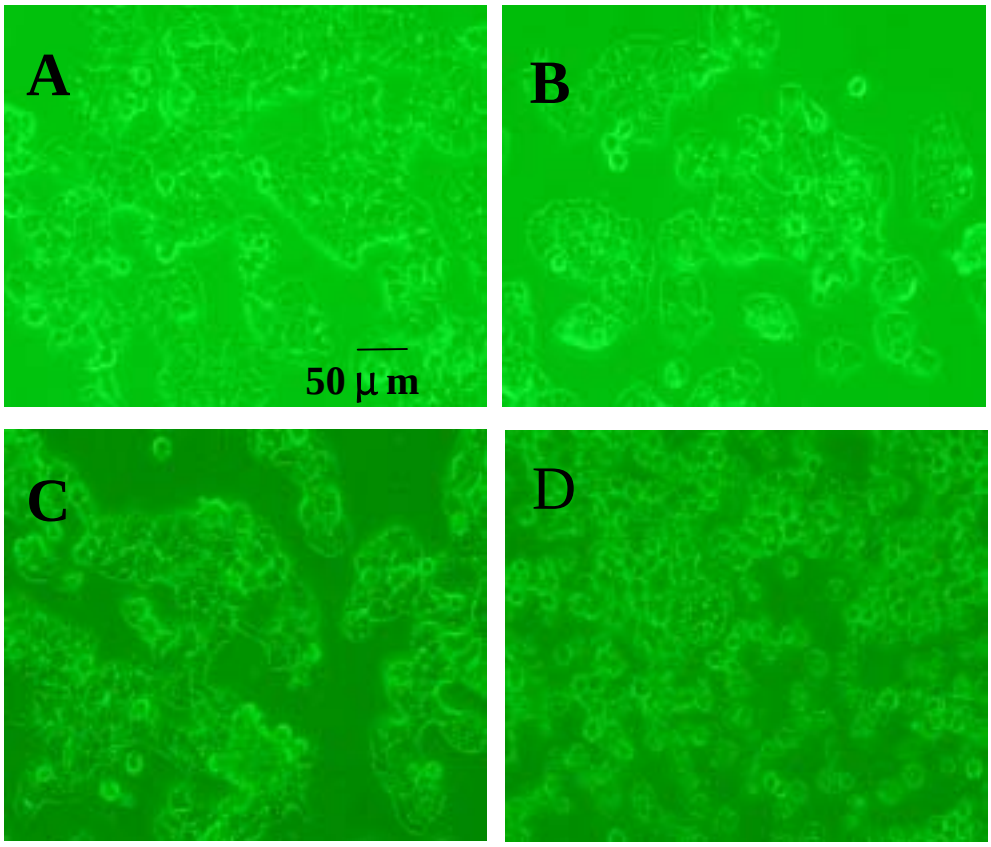


Fig. 5. Effect of various concentrations of ginger oil on the morphology of hepatoma cell line Hep 3B after 24 hrs treatment. A=control, B=50 μ g/ml, C=100 μ g/ml, D=175 μ g/ml. (100X)

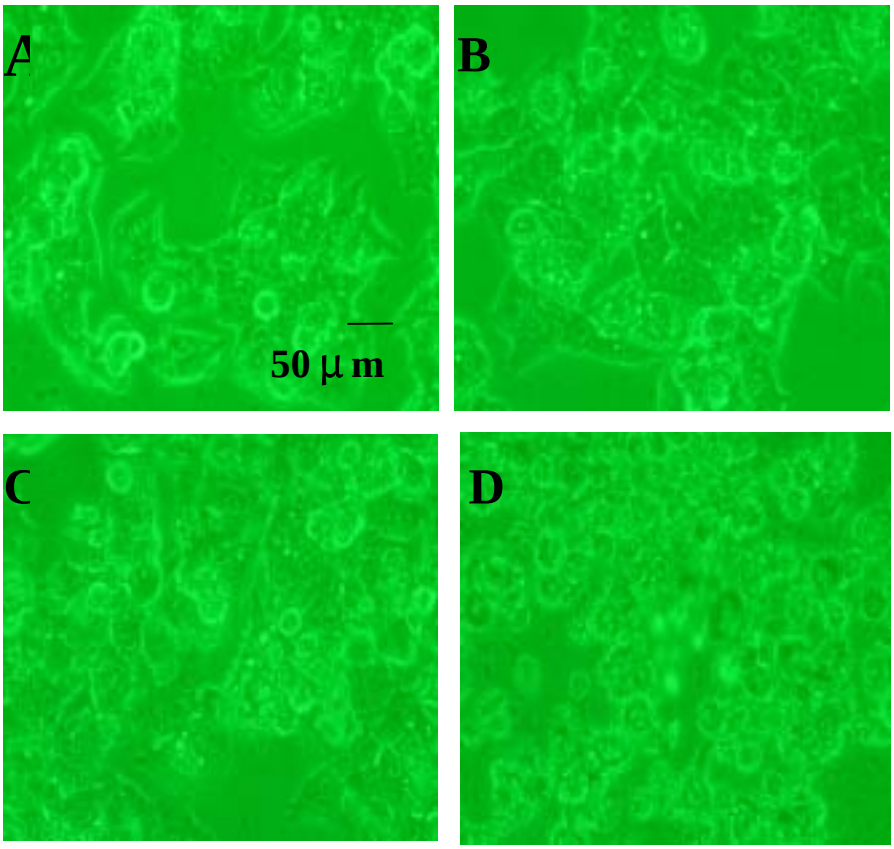


Fig. 6. Effect of various concentrations of ginger oil on the morphology of hepatoma cell line Hep G2 after 24 hrs treatment. A=control, B=30 µ g/ml, C=100 µ g/ml, D=150 µ g/ml. (200X)

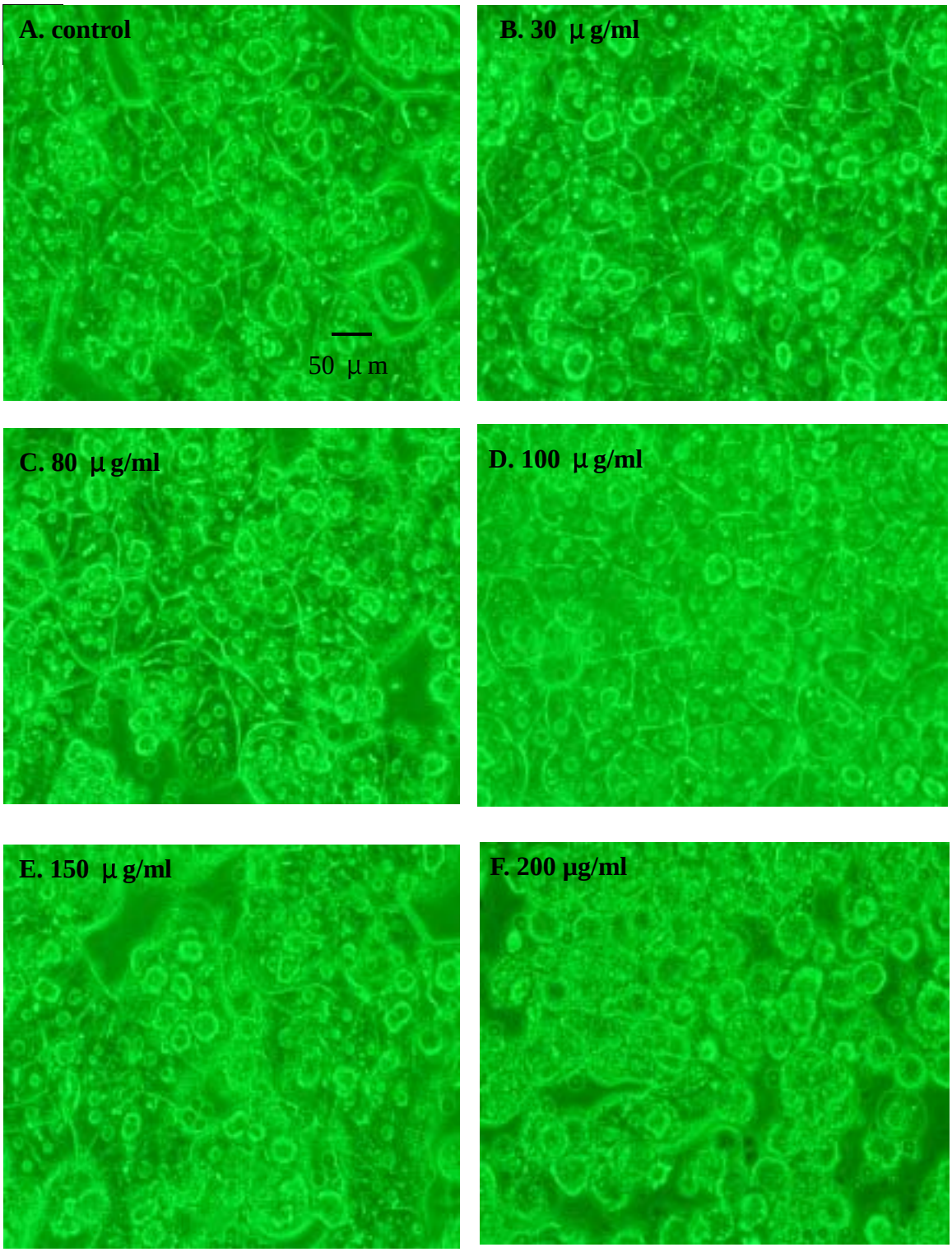


Fig. 7. Effect of various concentrations of ginger oil on the morphology of primary rat hepatocytes after 24 hrs treatment. (200X)

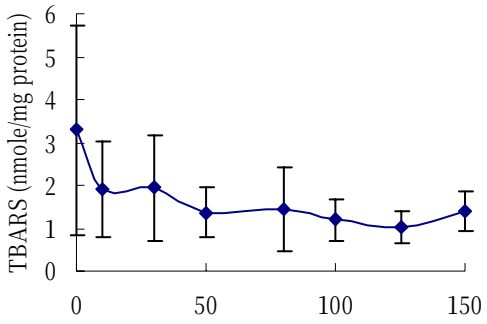


Fig. 8. Effect of various concentrations of ginger oil on thiobarbituric acid-reactive substances (TBARS) production in primary rat hepatocytes after 24 hrs treatment.

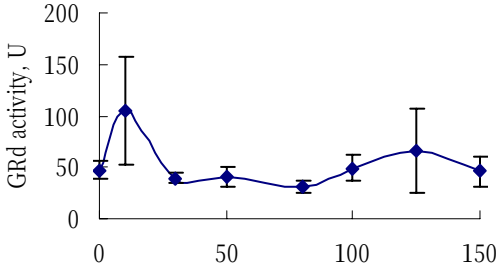
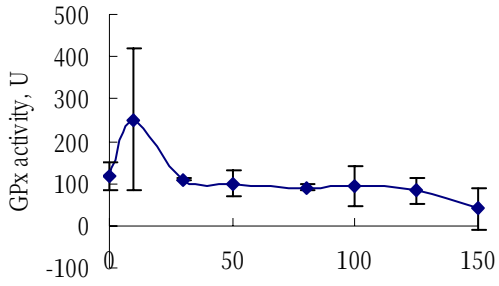
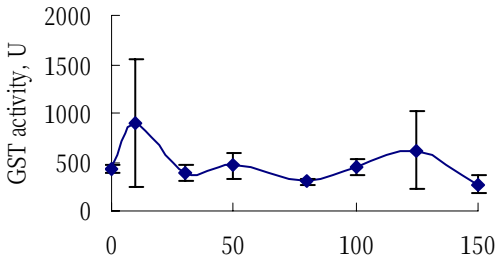


Fig. 9. Changes of GSH reductase activity in primary rat hepatocytes treated with various concentrations of ginger oil for 24 hrs.



Conc. of ginger oil (μ g/ml)

Fig. 10. Changes of GSH peroxidase activity in primary rat hepatocytes treated with various treatment concentrations of the ginger oil for 24 hrs.



Conc. of ginger oil (μ g/ml)

Fig. 11. Changes of GSH S-transferase activity in primary rat hepatocytes treated with various treatment concentrations of the ginger oil for 24 hrs.