行政院國家科學委員會補助專題研究計畫成果報告

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※ 氧化壓力下綠藻之抗氧化功能評估 ※

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計畫類別：☑個別型計畫 □整合型計畫

計畫編號：NSC－89－2320－B038－051

執行期間：89 年 8 月 1 日至 90 年 7 月 31 日

計畫主持人：楊素卿 副教授

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- □國際合作研究計畫國外研究報告書一份

執行單位：臺北醫學大學保健營養學系

中 華 民 國 90 年 10 月 31 日

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Preparation of NSC Project Reports

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一、中文摘要

本研究延續去年「綠藻之抗氧化功能評估」之研究，利用 CCl_4 誘發大白鼠產生氧化傷害後，評估氧化壓力下綠藻是否仍具有提高體內抗氧化能力之作用。實驗期間訂為 6 週，而實驗組分為三組：第一組前三週及後三週給予酪蛋白基礎飼料；第二組前三週給予酪蛋白基礎飼料及後三週給予 0.5%的綠藻飼料；第三組前三週及後三週給予 0.5%的綠藻飼料。另外，以 CCl_4 作為誘發氧化傷害的誘導劑(CCl_4 與 Olive oil 以 1:1 的比例配製)，於實驗期第四週開始每星期的星期一與星期四，給予兩次的腹腔注射(大白鼠每 100 克給予 0.05ml 的劑量)。血液樣品於第 1、3 週時，從尾靜脈抽取 2ml 的血液；肝臟及實驗末血液樣本，則由犧牲動物取得。所得之血液及肝臟樣本進行以下之分析：(1)抗氧化狀態、(2)抗氧化酵素活性、(3)抗氧化能力、(4)總抗氧化狀態、(5)肝臟功能、(6)肝臟組織病變等。結果發現，在 CCl_4 誘發氧化壓力下，0.5%綠藻之添加並不會提高體內的抗氧化狀態及抗氧化力。

關鍵詞：綠藻、抗氧化能力、氧化壓力、抗氧化酵素活性、肝臟功能、肝臟組織病變、大白鼠

Abstract

According to the results of our previous study in "The evaluation of the antioxidant function of chlorella", chlorella could indeed increase antioxidative capacity in rats, especially supplemented with 0.5 and 4% chlorella had the best efficiency. This study continued the study of "The evaluation of the antioxidant function of chlorella" last year to evaluate whether chlorella can improve antioxidative capacity in rats under the oxidative stress after the oxidative damage induced by CCl_4. The experimental period was six weeks and rats were divided into three groups. The first group was fed a casein-based control diet for the entire 6 weeks. The second group was fed a casein-based control diet for 3 weeks, and then supplemented with 0.5% chlorella diet for the last 3 weeks. The third group was fed a 0.5% chlorella supplemented diet for the entire 6 weeks. Additionally, the oxidative damage was induced by ip injection of 0.05 mL/100 g body weight CCl_4, suspended in olive oil (1:1), in rats twice a day on both Monday and Thursday from week 4. Blood samples (2 ml) were collected from the tail vein of rats on week 1 and week 3. After the end of the experimental period, rats were sacrificed, and liver and blood samples were obtained. The following assays were analyzed in all liver and blood samples: (1) antioxidant status, (2) antioxidant enzyme activities, (3) antioxidative capacity, (5) total antioxidant status, (5) hepatic function, and (6) hepatic pathology. The results showed that 0.5% chlorella diets can not improve antioxidantive capacity in the rats treated with CCl_4 under oxidative stress.

Keywords: chlorella, antioxidative capacity, oxidative stress, antioxidant enzyme activity, hepatic function, hepatic pathology, rat

二、緣由與目的

現代人類追求的不單只是長壽，而且要活得健康。Fujiwara 等人(1990)；Parker 與 Goodrum (1990)之流行病學調查研究中發現：慢性疾病的發生，大多是在日積月累的飲食不均衡及不良的生活習慣下所導致，攝食的習慣和人類的健康及壽命有著不可分的關係。由於近年來國民生活以及知識水準提高，健康意識日漸抬頭，「預防重於治療」之健康觀念漸漸受到重視，也由於如此而有所謂的「健康食品」或「機能性食品」之產生。這些健康食品通常為天然食品，經過特殊的加工製成，只要適量攝取不會導致中毒毒性，因其安全性高故可長期服用。目前市售健康食品價格高昂，標示不清之健康食品充斥，所發生之糾紛層出不窮，因此為了保護消費大眾不受誇大不實之健康食品廣告所蒙騙，健康

食品的機能性與安全性之評估是目前相當重要且不容忽視之研究主題。

近年來的研究發現，氧化傷害是人類疾病、老化的主要因素，如果從日常的飲食生活習慣中加以留意，不難達成其預防效果，而且藉由飲食的攝取來預防疾病是最有效且經濟的方法。Palozza 與 Krinsky (1992)及呂(1995)指出癌症、白內障、動脈粥狀硬化及老化等疾病與自由基所引發的氧化反應有關，Frei(1994)亦指出慢性發炎、缺血、心肌梗塞及一些自體免疫的慢性疾病發生與自由基氧化反應也具相關性；含有抗氧化物質的食品具有保護生物體免於自由基之傷害，並可抵抗慢性病的產生。由於這些醫學研究報導，食品研究專家及業者亦致力於開發「消除自由基，預防慢性成人疾病」之天然健康食品，「綠藻」就是其中最受注目的產品之一。

綠藻，是一種單細胞藻類，大小約只有 50 μm，與人體紅血球體積相當。綠藻是生長在 30 億年前的藻類，含豐富完整的營養，東海綠藻(1997)分析出其主要成分為：蛋白質 60%；脂肪 8%；碳水化合物 15%；粗纖維 3%；灰份 6.8%，並富含維生素、礦物質，其中又以維生素 A、類胡蘿蔔素、維生素 B 群、鐵的含量最為豐富，以及些許酵素(如超氧化歧化酶)與生長因子(如菌落刺激因子；colony-stimulating factor)，其功效有：改變酸性的體質為弱鹼性、增強抵抗力、加強造血功能、活化蛋白質合成、促進新陳代謝正常化及防止老化等，在歐美及日本的健康食品市場上，佔有舉足輕重的地位。有關綠藻的研究多是以單細胞生物觀點出發，來探討單細胞生物內的酵素、遺傳基因、植物化學系統、分子生物及化學結構等方向，而對生理方面之影響的研究尚不多見。如同綠藻含有豐富之抗氧化營養素，但是探討綠藻之抗氧化功能的文獻卻少之又少。直到近年，Anjali(1998)等之報告指出母鼠攝取綠藻(每日投予綠藻 500 毫克/公斤體重) 14 天後，胎鼠和初生鼠肝中脂質過氧化物濃度降低，結果顯示綠藻可經由懷孕的母體至子體的途徑抑制子鼠體內過氧化物之生成。另外，根據本研究室去年所執行「綠藻之抗氧化功能評估」之研究結果顯示，綠藻的確具有增加大白鼠體內抗氧化力的生理機能，而且以添加 0.5% 與 4% 綠藻時的效果最佳。

以上之研究均為大白鼠均處於正常狀況下之結果，若模擬現代人處於充滿氧化壓力環境的狀況，給予大白鼠一外來之氧化壓力後，綠藻是否仍能發揮其抗氧化效果，則需要再進一步深入的探討。許多評估天然食物之生理機能的研究中，常常會利用外來的方式來誘發實驗動物產生氧化性的傷害，而其中又以 CCl_4 所造成之化學性肝臟傷害，為最常用且最有效的氧化傷害手段。根據 Fanelli 等人的報告，以 CCl_4 藉由破壞肝臟功能之途徑，誘發大白鼠產生氧化傷害後，可以有效地促進血液及肝臟中過氧化物之堆積及降低抗氧化酵素之合成(Fanelli, 1998)。因此，本研究擬採用此法，製作具有氧化傷害之動物實驗模型後，具體評估氧化壓力下綠藻之抗氧化功能。

三、結果與討論

1. 血中抗氧化相關物質濃度

實驗組血漿中尿酸濃度於第六週時控制組低，顯示尿酸作為自由基的清除者，作用於清除因 CCl_4 所引起之自由基。另外，血漿中鐵離子濃度兩組無明顯變化。血漿中維生素 C 濃度兩組間亦無明顯變化，而實驗組之血漿維生素 E 濃度則高於控制組 2 倍。由此推測，綠藻之添加可使體內維生素 E 濃度提高，進而作用於清除因 CCl_4 所引起之自由基(表一、表二)。

2. 紅血球與肝臟中抗氧化酵素

實驗組之紅血球 GSH-Px 活性於第六週時有下降傾向，但無統計上之差異。第六週時，實驗組中紅血球 SOD 活性明顯高於控制組，但肝臟中 SOD 不會因攝取綠藻而增加，反而因 CCl_4 之注射而降低。因此推測，綠藻在健康或有氧化壓力之狀況下均能提高紅血球中 SOD 活性(表三、表四)。

3. 肝臟及 LDL 中脂質過氧化物

實驗組之肝臟及 LDL 中脂質過氧化物

含量均無明顯變化(表五)。

4. 血中總抗氧化狀態

在實驗組中體內總抗氧化狀態明顯降低。推測可能原因為綠藻添加量不足、實驗期間過短等因素，有進一步探討之需要(表六)。

5. 肝功能指數

第三及六週因 CCl_4 之注射造成 GOT 之升高，但在攝取綠藻後，下降了 36%。GPT 亦於三及六週因 CCl_4 之注射而升高，而攝取綠藻後，亦有下降之趨勢(表七)。

四、計畫成果自評

綠藻飼料對於處於氧化壓力狀態下之大白鼠體內抗氧化物濃度、抗氧化酵素活性、抗氧化力與總抗氧化狀態的影響並不大，僅明顯提高血漿中維生素 E 濃度及紅血球中 SOD 活性。顯示 0.5%的綠藻添加不足以因應 CCl_4 所誘發的氧化傷害。

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(以上包括測量方法的參考文獻)

表一、綠藻飲食在四氯化碳誘發氧化壓力下，對大白鼠血中抗氧化物質濃度的影響*

Table 1 The effect of chlorella on the concentration of plasma antioxidants in rats treated with CCl_4 under oxidative stress

Variables	Control	Exam
Uric acid(μ mole/L)		
Baseline	0.80 ± 0.06[a]	0.80 ± 0.06[a]
3 weeks	0.65 ± 0.02[a]	0.52 ± 0.04[a]
6 weeks	1.52 ± 0.55[a']	0.75 ± 0.04[b]
	Two-way ANOVA	
Dosage	Time	Dosage*Time
NS	<0.05	NS
Vitamin C(μ mole/L)		
Baseline	37.56 ± 3.97[a]	37.56 ± 3.98[a]
3 weeks	32.26 ± 4.46[a]	59.29 ± 23.38[a]
6 weeks	44.95 ± 17.97[a]	58.92 ± 31.43[a]
	Two-way ANOVA	
Dosage	Time	Dosage*Time
NS	NS	NS
Vitamin E(μ mole/L)		
Baseline	6.01 ± 0.29[a]	6.01 ± 0.29[a]
3 weeks	4.97 ± 1.47[a]	5.98 ± 0.37[a]
6 weeks	2.26 ± 0.93[a']	5.43 ± 1.25[b]
	Two-way ANOVA	
Dosage	Time	Dosage*Time
NS	NS	NS

*All values are means±S.D. (n=8).
Different superscripts(a,b) indicate significant differences (P<0.05) between the groups.
'Indicates significant differences compared to the baseline at the same dosage of chlorella (P<0.05).
NS indicates no significant differences among the groups(P>0.05).

表三、綠藻飲食在四氯化碳誘發氧化壓力下，對大白鼠紅血球中抗氧化酵素比活性的影響*

Table 3. The effect of chlorella on the specific activities of erythrocyte antioxidative enzymes in rats treated with CCl_4 under oxidative stress

Variables	Control	Exam
GSH-Px (U/mg protein)		
Baseline	69.21 ± 3.55[a]	69.21 ± 3.55[a]
3 weeks	66.61 ± 3.23[a]	76.84 ± 5.68[a]
6 weeks	99.07 ± 9.20[a']	71.98 ± 3.89[b]
	Two-way ANOVA	
Dosage	Time	Dosage*Time
NS	<0.05	<0.05
SOD (mU/mg protein)		
Baseline	46 ± 6.31[a]	46 ± 6.31[a]
3 weeks	43 ± 5.73[a]	42 ± 4.88[a]
6 weeks	36 ± 5.46[a]	54 ± 6.21[b]
	Two-way ANOVA	
Dosage	Time	Dosage*Time
NS	NS	NS

*All values are means±S.D. (n=8).
Different superscripts(a,b) indicate significant differences (P<0.05) between the groups.
'Indicates significant differences compared to the baseline at the same dosage of chlorella (P<0.05).
NS indicates no significant differences among the groups(P>0.05).

表二、綠藻飲食在四氯化碳誘發氧化壓力下，對大白鼠血漿中二價、三價、總鐵及二價/三價鐵離子濃度的影響*

Table 2. The effect of chlorella on the concentrations of plasma ferrous、ferric ions、total iron and the ratio of ferrous to ferric ion in rats treated with CCl_4 under oxidative stress

Variables	Control	Exam
Ferrous(μ mole/L)		
Baseline	1.43 ± 0.15[a]	1.43 ± 0.15[a]
3 weeks	1.03 ± 0.05[a']	1.10 ± 0.05[a]
6 weeks	2.09 ± 0.24[a']	1.89 ± 0.13[a']
	Two-way ANOVA	
Dosage	Time	Dosage*Time
NS	<0.05	NS
Ferric ion (μ mole/L)		
Baseline	0.43 ± 0.16[a]	0.43 ± 0.16[a]
3 weeks	0.90 ± 0.12[a']	1.13 ± 0.14[a']
6 weeks	0.82 ± 0.15[a]	0.81 ± 0.22[a]
	Two-way ANOVA	
Dosage	Time	Dosage*Time
NS	<0.05	NS
Total ion (μ mole/L)		
Baseline	1.87 ± 0.21[a]	1.87 ± 0.21[a]
3 weeks	1.93 ± 0.11[a]	2.08 ± 0.18[a]
6 weeks	2.54 ± 0.17[a']	2.70 ± 0.14[a']
	Two-way ANOVA	
Dosage	Time	Dosage*Time
NS	<0.05	NS
The ratio of ferrous to ferric ion		
Baseline	2.26 ± 0.33[a]	2.26 ± 0.33[a]
3 weeks	1.29 ± 0.21[a]	1.08 ± 0.15[a]
6 weeks	3.18 ± 0.78[a]	3.54 ± 0.90[a]
	Two-way ANOVA	
Dosage	Time	Dosage*Time
NS	<0.05	NS

*All values are means±S.D. (n=8).

Different superscripts([a,b]) indicate significant differences (P<0.05) between the groups.

'Indicates significant differences compared to the baseline at the same dosage of chlorella (P<0.05).

NS indicates no significant differences among the groups (P>0.05).

表四、綠藻飲食在四氯化碳誘發氧化壓力下，對大白鼠肝中抗氧化酵素比活性的影響*

Table 4. The effect of chlorella on the specific activities of antioxidative enzymes in liver of the rats treated with CCl_4 under oxidative stress

Variables	Control	Exam
GSH-Px (U/mg protein)		
Baseline	12.68 ± 1.50[a]	12.68 ± 1.37[a]
6 weeks	14.41 ± 0.85[a]	16.15 ± 0.88[a']
	Two-way ANOVA	
Dosage	Time	Dosage*Time
NS	<0.05	NS
SOD (mU/mg protein)		
Baseline	1403 ± 153.50[a]	1403 ± 125.75[a]
6 weeks	791 ± 31.29[a']	755 ± 85.35[a']
	Two-way ANOVA	
Dosage	Time	Dosage*Time
NS	<0.05	NS

*All values are means±S.D. (n=8).

Different superscripts([a,b]) indicate significant differences (P<0.05) between the groups.

'Indicates significant differences compared to the baseline at the same dosage of chlorella (P<0.05).

NS indicates no significant differences among the groups(P>0.05).

表七、綠藻飲食在四氯化碳誘發氧化壓力下，對大白鼠血漿中肝功能指數的影響*

Table 7. The effect of chlorella on plasma GOT and GPT in rats treated with CCl_4 under oxidative stress

Variables	Control	Exam
GOT (U/L)		
Baseline	71.49 ± 5.40[a']	71.49 ± 5.40[a']
3 weeks	55.44 ± 1.77[a']	58.29 ± 1.95[a']
CCl_4 treatment		
3 weeks	319.44 ± 17.60[a'*]	332.18 ± 22.19[a'*]
6 weeks	274.66 ± 31.03[a'*]	213.23 ± 43.59[a'*]
	Two-way ANOVA	
Dosage	Time	Dosage*Time
NS	<0.05	NS
GPT (U/L)		
Baseline	31.43 ± 2.45[a']	31.43 ± 2.45[a']
3 weeks	27.74 ± 1.74[a']	31.14 ± 3.36[a']
CCl_4 treatment		
3 weeks	212.43 ± 18.81[a'*]	164.05 ± 33.31[a'*]
6 weeks	290.75 ± 41.49[a'*]	213.38 ± 53.27[a'*]
	Two-way ANOVA	
Dosage	Time	Dosage*Time
NS	<0.05	NS

*All values are means±S.D. (n=8).

Different superscripts([a,b]) indicate significant differences (P<0.05) between the groups.

'Indicates significant differences compared to the baseline at the same dosage of chlorella (P<0.05).

NS indicates no significant differences among the groups(P>0.05).

表五、綠藻飲食在四氯化碳誘發氧化壓力下，對大白鼠肝及低密度脂蛋白中脂質過氧化物濃度的影響*

Table 5. The effect of chlorella on the concentration of lipid peroxidants in liver and LDL of the rats treated with CCl_4 under oxidative stress

Variables	Control	Exam
MDA+4-HNE in liver (μ mole/L)		
Baseline	38.77 ± 1.48[a]	38.77 ± 1.48[a]
6 weeks	39.72 ± 2.33[a]	42.53 ± 2.38[a]
	Two-way ANOVA	
Dosage	Time	Dosage*Time
NS	<0.05	NS
MDA+4-HNE in low density lipoprotein (μ mole/L)		
Baseline	5.09 ± 0.69[a]	5.09 ± 1.16[a]
6 weeks	4.06 ± 0.47[a]	6.21 ± 1.40[a]
	Two-way ANOVA	
Dosage	Time	Dosage*Time
NS	<0.05	NS

*All values are means±S.D. (n=8).

Different superscripts([a,b]) indicate significant differences (P<0.05) between the groups.

'Indicates significant differences compared to the baseline at the same dosage of chlorella (P<0.05).

NS indicates no significant differences among the groups(P>0.05).

表六、綠藻飲食在四氯化碳誘發氧化壓力下，對大白鼠血漿中總抗氧化狀態的影響*

Table 6. The effect of chlorella on the total antioxidant status in plasma of the rats treated with CCl_4 under oxidative stress

Variables	Control	Exam
Total antioxidant status (μ mole/L)		
Baseline	3.63 ± 0.49[a]	3.63 ± 0.49[a]
3 weeks	4.32 ± 0.31[a]	2.12 ± 0.23[b]
6 weeks	6.88 ± 0.98[a']	4.60 ± 1.06[b]
	Two-way ANOVA	
Dosage	Time	Dosage*Time
<0.05	<0.05	NS

*All values are means±S.D. (n=8).

Different superscripts([a,b]) indicate significant differences (P<0.05) between the groups.

'Indicates significant differences compared to the baseline at the same dosage of chlorella (P<0.05).

NS indicates no significant differences among the groups(P>0.05).