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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※探討PPAR-α在飲食油脂油之中對肝前癌細胞形

※ 成過程中所扮演脂角色 ※

※To Explore the role of PPAR-α in the effect of dietary

※ Oil in liver foci synthesis ※

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本成果報告包括以下應繳交之附件：

□赴國外出差或研習心得報告一份

□赴大陸地區出差或研習心得報告一份

□出席國際學術會議心得報告及發表之論文各一份

□國際合作研究計畫國外研究報告書一份

執行單位：中山醫學大學營養系

英文摘要

Previous results have shown that dietary fish oil can inhibit breast and colon carcinogenesis. Data from our laboratory have shown that dietary fish oil can decrease rat liver foci formation by using the diethylnitrosamine (DEN)-phenobarbital (PB) model when compared with dietary corn oil. However, the chemopreventive mechanisms of fish oil are not fully understood. Fish oil is high in n-3 fatty acid, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) so it could modify the fatty acid distribution in tissues. Furthermore, fish oil could modify fatty acid metabolites especially, prostaglandin E_2 and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). In rat livers, fish oil decreased the $PGF_{2\alpha}$ synthesis when compared with corn oil and the addition of tumor promoter PB had not effect on the $PGF_{2\alpha}$ synthesis. In this project we would like to explore the possible mechanisms of inhibitory capacity of dietary fish oil in of rat liver foci formation by the diethylnitrosamine-phenobarbital model. Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors and PPAR-α is the predominant isoform in rodent livers. PPARs could be activated by their ligands or activators and then modify the PPAR response gene mRNA expression. WY-14, 643 is a potent ligand of PPAR-α. When compared with WY-14,643 the polyunsaturated fatty acids such as linoleic acid (LA), EPA and DHA are weak activator of PPAR-α. In rat hepatic parenchymal cells, the PPAR-A is activated by EPA but not by LA, linolenic acid, or arachidonic acid (AA). Since LA, AA and the fatty acid derivative, perfluorinated decanoate are weaker than WY-14,643 to bind to PPAR-α, unlike WY-14,643, they may not influence or have little effect on promoting liver cancinogenesis initiated by DEN. This may be due to different capacity of induction of PPAR response gene mRNA expression between strong and weak PPAR-α ligands (or activators). We hypothesize that because amount of PPAR response gene mRNA expression induced by LA and EPA are lower than by WY-14,643, fish oil and corn oil will not induce rodent liver cancer. Furthermore, fish oil and corn oil could influence liver foci formation through a PPAR-α related mechanism. Dietary fish oil increased the CYP4A1 and L-FABP, PPAR response genes, mRNA expression compared with dietary corn oil and clofibrate. However, Dietary fish oil decrease the liver GSH foci synthesis compared with dietary corn oil and clofibrate. The relationship between fish oil and liver carcinogenesis needs more future study.

中文摘要

已有証據顯示，魚油可抑制乳癌及結腸癌的形成，至於魚油與肝癌的關係，也有資料指出高魚油飲食組相較與高玉米油飲食組可減少 diethylnitrosamine (DEN)-phenobarbital (PB)模式下所產生的肝前癌細胞，但魚油抑制癌症的機制仍不清楚。 魚油因富含 eicosapentaenoic acid (EPA)及 docosahexaenoic (DHA)，因此多食魚油將改變組織中脂肪酸組成，進而改變脂肪酸代謝產物，減少 prostaglandin E_2 及 prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$)生合成但該模式下並無充分證據支持 $PGF_{2\alpha}$與魚油抑制肝前癌細胞形成有關。由於相當多研究指出 Peroxisome proliferator-activated receptors (PPARs)是脂質調控細胞代謝與細胞分化的重要調節者，因此是否飲食油脂調節肝前癌細胞生成與其活化 PPAR-α有關，值得進一步研究。 PPARs，是 ligand-activated transcription factors，而在鼠類肝臟中以 PPAR-α較多。PPARs 被 PPAR 活化劑活化後，將進而調節 PPAR 反應基因轉錄作用 (PPAR response gene mRNA transcription)。 雖然脂肪酸可與 PPAR-α結合，但不同脂肪酸與 PPAR-α間的親和性並不一致，例如老鼠肝實質細胞 (hepatic parenchymal cells) PPAR-α可被 EPA 活化，但亞麻油酸，次亞麻油酸和花生油四烯酸則無此作用。 雖然 EPA 比其他脂肪酸對 PPAR-α的活性強，但與一降血脂質藥物，WY-14,643 相較下，WY-14,643 與 PPAR-α間的親和力又較 EPA 強多了。 雖然長期給予鼠類 WY-14,643 可誘發肝癌之形成，但是証據也顯示與 PPAR-α親和力較弱之 activators，例如:亞麻油酸和花生油四烯酸，以及 perfluorinated decanoate (脂肪酸衍生物)對於 DEN 誘發鼠類肝癌形成並沒有影響或影響很小，因此我們假設這可能與其對 PPAR-response genes mRNA 轉錄的強弱有關。 所以本計畫我們假設，魚油與玉米油可經由與 PPAR-α相關機制來影響由 DEN-PB 模式下所產生的肝前癌細胞，同時其作用機制可能與 WY-14,643 誘發肝癌不同。與以往實驗結果一致，與餵食玉米油組老鼠相比較，餵食玉米油組老鼠肝臟中 GSH Foci 顯著減少，而餵食 clofibrate 組老鼠肝臟中 GSH Foci 顯著增加。但是餵食魚油組老鼠肝臟中 GST-P 之 mRNA 及蛋白質量較餵食玉米油組老鼠及餵食 clofibrate 組老鼠高。PPAR Response genes 例如 CYP4A1, L-FABP 之 mRNA 在餵食魚油組老鼠肝臟中量大於餵食玉米油組老鼠及餵食 clofibrate 組老鼠。CYP4A1 蛋白質量亦與 mRNA 結果相似，餵食魚油組老鼠肝臟中大於餵食玉米油組老鼠及餵食 clofibrate 組老鼠。

計畫緣由與目的

近年來癌症在大部分已開發國家已漸居死亡原因的榜首，台灣亦不例外。從流行病學或是實驗動物模式，結果都顯示飲食中油脂含量及種類與某些癌症發生有密切的相關 (Miller et al., 1990)。多元不飽合脂肪酸 (例如，大部分的蔬菜油)較飽合脂肪酸 (例如，一般動物性油脂)易刺激癌症形成。相反的，若飲食富含超長鏈多元不飽合脂肪酸 (例如，某些魚油)，則不會促進乳癌的形成，有時還呈現抑制作用。魚類油脂中富含超長鏈脂肪酸，例如，eicosapentaenoic acid (EPA)以及 docosahexaenoic acid (DHA)，均屬於 n-3 多元不飽合脂肪酸。以往研究結果以證實，與玉米油相較，魚油具有抑制乳癌及直腸腫瘤生成的作用 (Abou-El-Ela et al., 1989; Lee et al., 1993; Reddy et al., 1991; Chen et al., 1997)。

在飲食油脂與肝癌形成的相關研究方面，Chen 等人 (1997)即發現高魚油飲食 (10% by weight)與高玉米油飲食 (15% by weight)比較下，魚油可有效抑制 diethylnitrosamine (DEN;誘發劑)-phenobarbital (PB; 促進劑)模式下肝前癌細胞之形成。 在該實驗中，雖然魚油組 $PGF_{2\alpha}$濃度較玉米油組低，但並無證據支持 $PGF_{2\alpha}$濃度高低與肝癌生成有關，因此我們推論魚油應是經由其他機制抑制肝前癌細胞的形成。

就以分子生物層面的研究來看，peroxisome proliferators 誘發 peroxisome proliferation 因而引起一群新的接受器的發現，統稱爲 peroxisome proliferator-activated receptors (PPARs)(Issemann et al., 1990)。 PPARs 可歸屬於 superfamily of nuclear hormone receptors，並獨立自成一個 PPAR subfamily。 到目前爲止，PPARs 至少含有三個不同的 isoforms (α，β，以及γ1 和 γ2) (Dreyer et al., 1992; Sher et al., 1993; Kliewere et al., 1994)，就鼠類肝臟而言，其 PPAR-α isoform 以 PPAR-α爲主 (Issemann et al., 1990)。 PPARs 與其他在 superfamily of nuclear hormone receptors 的成員一樣是 ligand-activated transcription factors (reviewed in Tsai et al., 1994; Lemberger et al., 1996)。 PPARs 可被 PPAR 的活化劑 (ligands 或 activators)活化後，將進而調節對 PPAR 有反應的基因 (PPAR-response genes)的轉錄 (mRNA transcription)(Reviewed in Cattley et al., 1998)。PPARs 與 retinoic X receptor (RXR)分別由 PPAR 的活化劑及 9-cis retinoic acid 活化後形成 heterodimer (Keller et al., 1993)。 PPAR-RXR heterodimer 在與位於 PPAR-response genes 上 promoter 區域的一個特殊 DNA motif 結合而調節 mRNA transcription。這個特殊 DNA motif 被稱爲 peroxisome proliferator response element (PPRE)(Dreyer et al., 1992)。 目前所知的 PPAR-response genes 大多和 peroxisomes 內以及粒腺體內的脂肪代謝有關 (Kliewer et al., 1992)，例如 ACO、L-FABP、adipocyte lipid binding protein (aP2)、cyclooxygenase-2、cytochrome P450 4A1 (CYP 4A1)(Aldridge et al., 1995)、hydroxymethylglutaryl-CoA synthase (Rodriguez et al., 1994)、apolipoprotein AI and CIII 等。 近來研究也已趨向探討 PPAR-response genes 與細胞生長，分化 (differentiaiton)及癌症形成 (carcinogenesis)之間的關係，例如 aP2 已經證實與脂肪細胞的分化有密切的關係 (Spiegelman et al., 1997)，而 ACO (Chu et al., 1995; Gonzalez et al., 1998)、CYP4A1 和 L-FABP(reviewed in Bentley et al., 1993)則與老鼠類肝癌生成有關。

PPAR 的活化劑，除了已知的 peroxisome proliferators 外，近來由於 binding assay 的發展，一些脂肪酸 (例如: LA，LNA，AA， EPA，DHA)

及 eicosanoids (prostaglandin J_2，8-hydroxyeicosatetraenoic acid，leukotriene B4 等) (Forman et al., 1997; Kliewer et al., 1997; Krey et al., 1997)也被證實具有活化 PPARs 的能力，並可調節 PPAR-response gene mRNA 的轉錄。 PPARs 可將脂肪酸與基因調節連接在一起，是一個相當重要的發現，因爲這可解釋爲何脂肪酸可調控細胞的生長，分化，進而影響一些疾病的發展，例如，癌症的形成。也有實驗證實，老鼠肝實質細胞 (hepatic parenchymal cells) PPAR-α可被 EPA 活化，但 LA，LNA 和 AA 則無此作用 (Ren et al., 1997)。雖然 EPA 比其他脂肪酸的活性，但與一降血脂質藥物-WY-14,643 相比較 WY-14,643 與 PPAR-α間的親和力較 EPA 強多了，但 EPA 可活化 PPAR-α，增加 ACO 以及 CYP 4A1 mRNA 的轉錄(Krey et al., 1997) 。

長期給予鼠類與 PPAR-α親和力較強之 peroxisome proliferators，例如 WY-14,643，卻可誘發肝癌之形成 (Peters et al., 1997; Gonzalez et al., 1998)，然而也有文獻指出如投予小鼠 PPAR-α親和力較弱之 activators，LA，AA 以及 perfluorinated decanoate (脂肪酸衍生物)等，則不會影響 DEN 處理後之鼠類肝癌形成 (Klaunig et al., 1990)。 這可能顯示 PPAR-response genes mRNA 轉錄之的強弱與肝癌形成過程有關。 我們假設，雖然均是 PPAR-α的活化劑，但高度表現 PPAR-response genes 將促成癌生成，但如僅有低度表現，則可能沒致癌作用。 所以經由對 PPAR-α活化與 PPAR-response genes mRNA 轉錄在不同飲食油脂調節 DEN-PB 模式下大鼠肝前癌細胞形成中所扮演角色的探討，將可增加我們對調控癌生成之瞭解。

結果與討論

所有不同實驗組老鼠之進食量、體重及肝臟重皆沒有顯著差異 (data not shown)。與餵食玉米油組老鼠相比較，餵食魚油組老鼠肝臟磷脂質中 EPA 及 DHA 顯著增加而 AA 顯著減少。餵食 clofibrate 組老鼠肝臟磷脂質脂肪酸分布與餵食玉米油組老鼠相似 (Table 1)。與以往實驗結果一致，與餵食玉米油組老鼠相比較，餵食玉米油組老鼠肝臟中 GSH Foci 顯著減少，而餵食 clofibrate 組老鼠肝臟中 GSH Foci 顯著增加 (Table 2)。但是餵食魚油組老鼠肝臟中 GST-P 之 mRNA 及蛋白質量較餵食玉米油組老鼠及餵食 clofibrate 組老鼠高 (Fig 1-B, Fig 2-B)。PPAR Response genes 例如 CYP4A1, L-FABP 之 mRNA 在餵食魚油組老鼠肝臟中量大於餵食玉米油組老鼠及餵食 clofibrate 組老鼠 (Fig1-A, C)。CYP4A1 蛋白質量亦與 mRNA 結果相似，餵食魚油組老鼠肝臟中大於餵食玉米油組老鼠及餵食 clofibrate 組老鼠(Fig2-A)。

計畫成果自評

本計畫在探討魚油與 Liver Foci 形成之關係。研究在探討魚油是否經由活化 PPAR 來影響 Genes 表現。本研究結果顯示魚油活化 PPAR 來影響 Genes 表現能力比玉米油及 Clofibrate 強。本研究結果亦顯示，魚油抑制 Liver Foci 形成。由以往研究結果得知，Proxisome Proliferators 可誘發老鼠肝癌形成。由於本研究結果得知魚油可活化 PPAR，但卻可抑制 Liver Foci 形成，故對於魚油與肝癌形成之關係及其相關機轉仍需繼續探討。

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Table 1. Effect of experimental diet on fatty acid composition[a] of rat liver

Treatment	16:0	18:0	18:1©	18:2©	20:4	20:5	22:6
CO	18.46	28.24	7.22	9.10	32.56	0.00	4.43
FO	17.44	24.37	6.26	7.10	23.14	8.59	13.76
CF	18.80	28.46	6.43	8.39	33.68	0.20	4.05
CO/DEN	15.98	28.41	5.90	9.61	34.87	0.24	4.99
FO/DEN	19.36	23.35	5.59	7.14	23.45	5.45	15.67
CF/DEN	16.63	27.15	8.11	8.73	33.99	0.01	5.39
CO/DEN/PB	16.00	29.13	6.95	7.88	36.12	0.10	3.82
FO/DEN/PB	18.34	24.47	6.99	6.58	23.01	4.80	15.81
CF/DEN/PB	15.82	30.06	5.79	7.87	35.09	0.31	5.06

[a]Values are means expressed as percent total fatty acid yield. n≥ 4 liver samples per

CO (corn oil), FO (fish oil), CF (clofibrate), DEN (diethylnitrosamine), PB (phenobarbital)

Table 2. Effect of experimental diet on foci synthesis[a] of rat liver

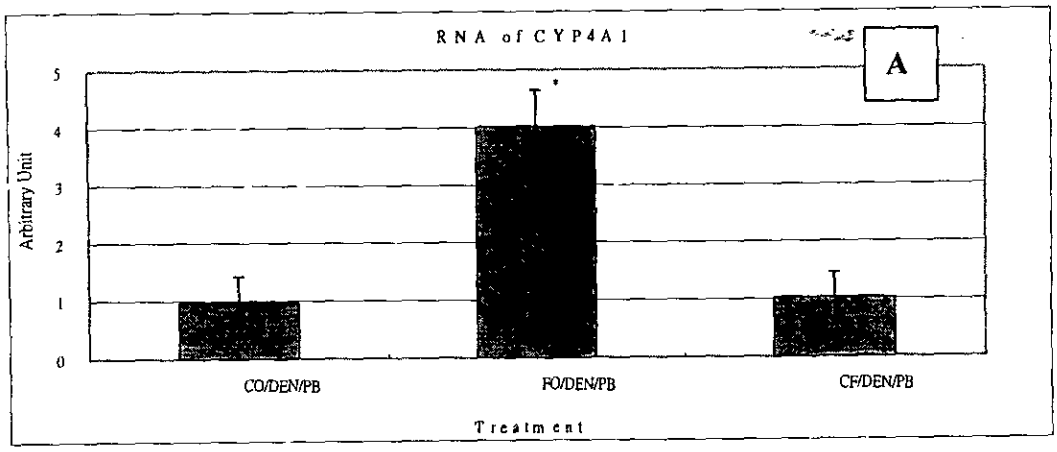
Treatment	%	foci no/mm	foci no/mm
CO/DEN/PB	0.11±0.06	1.34±0.79	13.47±7.85
FO/DEN/PB	0.04±0.03*	0.85±0.36*	20.26±15.23
CF/DEN/PB	0.25±0.14*	2.14±0.92*	7.66±3.07*

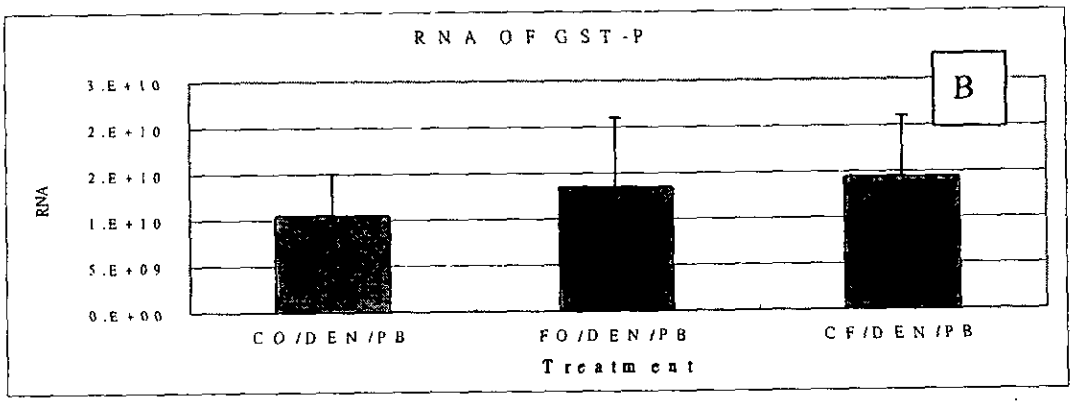
[a]Values are means ± S.D. expressed liver foci. n=9-10 liver samples per diet group.

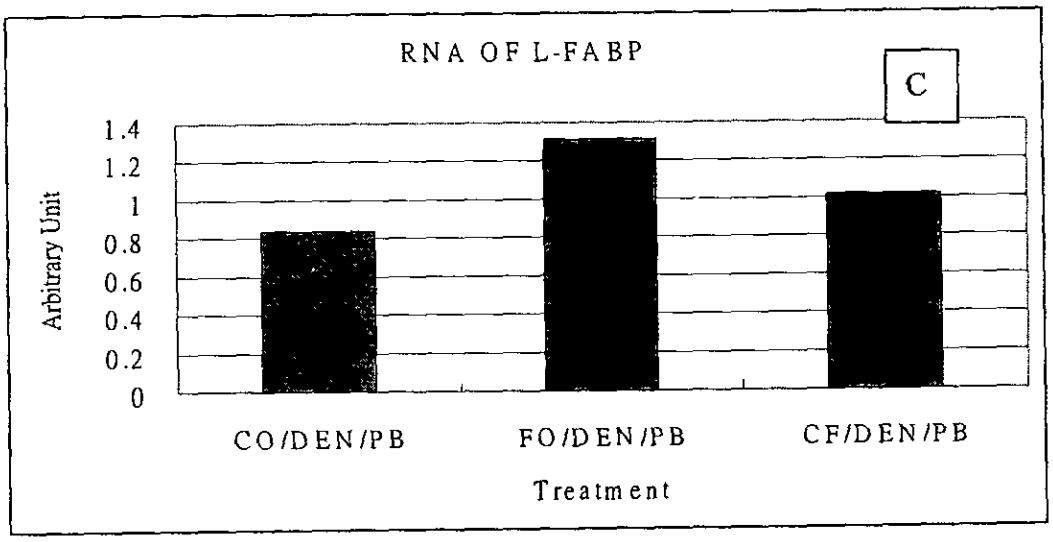
*within the same row are significantly different from CO/DEN/PB treatment group ($p<0.05$).

CO (corn oil), FO (fish oil), CF (clofibrate), DEN (diethylnitrosamine), PB (phenobarbital)

Fig 1. Effect of experimental diet on mRNA expression of rat liver

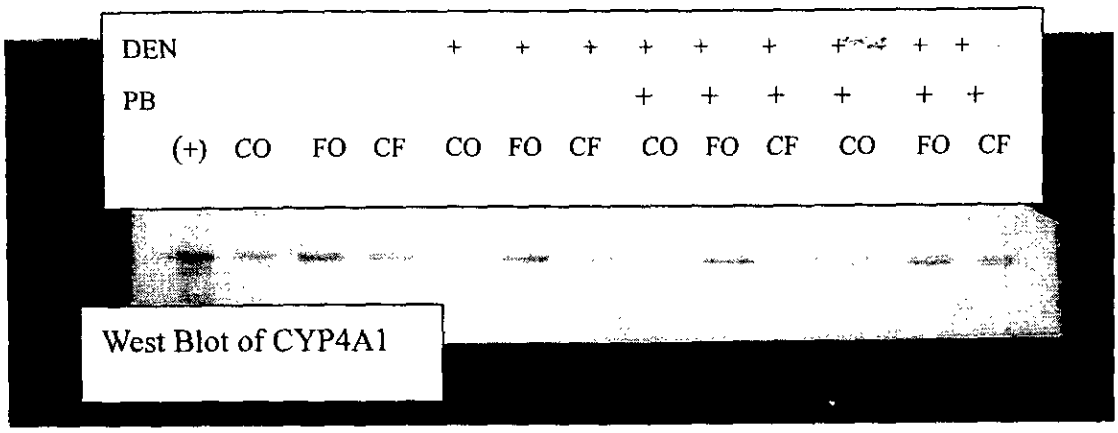


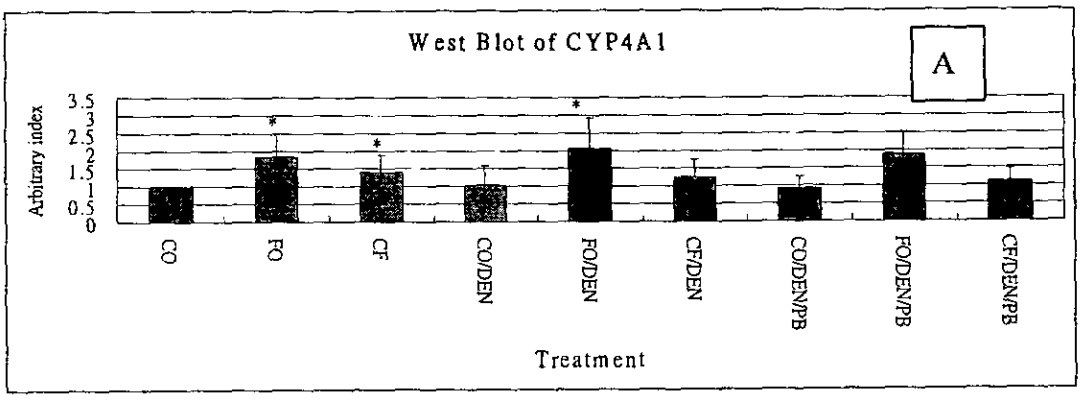


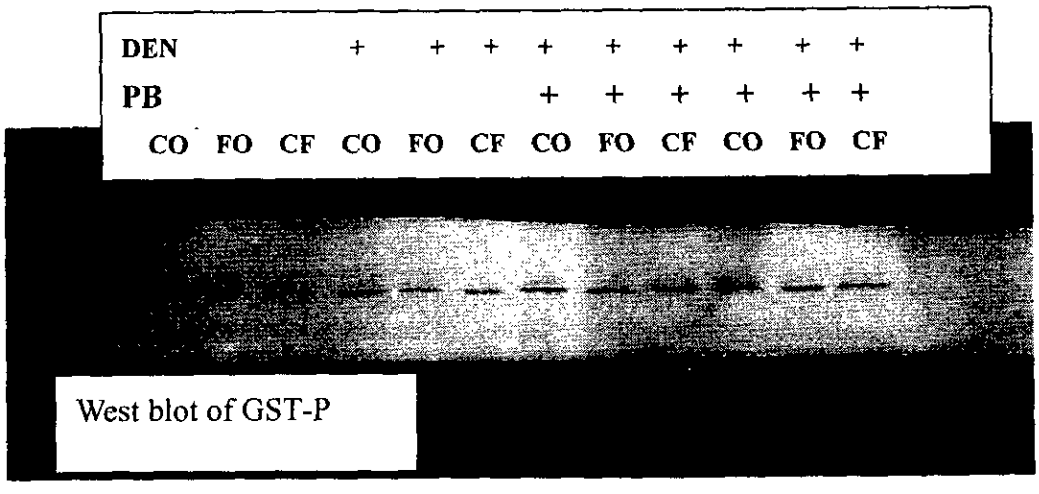


CO (corn oil), FO (fish oil), CF (clofibrate), DEN (diethylnitrosamine), PB (phenobarbital)

* within the same row are significantly different from CO/DEN/PB treatment group ($p<0.05$).







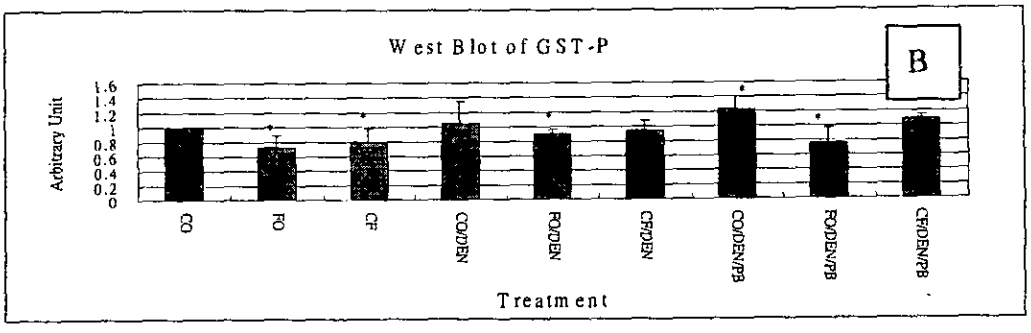


Fig2. Effect of experimental diet on protein expression of rat liver

***within the same row are significantly different from CO treatment group ($p<0.05$).**

CO (corn oil), FO (fish oil), CF (clofibrate), DEN (diethylnitrosamine), PB (phenobarbital)