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(72) **Inventeur/Inventor:**
MAJEED, MUHAMMED, US

(73) **Propriétaire/Owner:**
MAJEED, MUHAMMED, US

(74) **Agent:** NORTON ROSE FULBRIGHT CANADA LLP/S.E.N.C.R.L., S.R.L.

(54) **Titre : ACTIVITE HEPATOPROTECTRICE DU GARCINOL**
(54) **Title: HEPATOPROTECTANT ACTIVITY OF GARCINOL**

(57) **Abrégé/Abstract:**
The present invention discloses the hepatoprotective potential of garcinol.

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(72) Inventor; and
(71) Applicant : **MAJEED, Muhammed** [US/US]; Founder And Managing Director, Sabinsa Corporation, 20 Lake Drive, East Windsor, NJ 08520-5321 (US).

(72) Inventor *(for US only)*: **BANI, Sarang**; 19/1 And 19/2, I Main, Ii Phase, Peenya Industrial Area, Bangalore, Karnataka 560058 (IN).

(74) Agent: **NAGABHUSHANAM, Kalyanam**; Sabinsa Corporation, 20 Lake Drive, East Windsor, NJ 08520-5321 (US).

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(54) **Title**: HEPATOPROTECTANT ACTIVITY OF GARCINOL

(57) **Abstract**: The present invention discloses the hepatoprotective potential of garcinol.

HEPATOPROTECTANT ACTIVITY OF GARCINOL

FIELD OF THE INVENTION

[Para 002] The invention in general relates to medicaments for hepatotoxicity (hepatic toxicity) management. More specifically, it relates to the hepatoprotective potential of garcinol.

DESCRIPTION OF PRIOR ART

[Para 003] The term "hepatotoxicity" refers in general to the chemical induced liver damage. Such damage occurs mainly when the liver discharges its innate function of transformation and clearance of chemicals in the body.

[Para 004] The close association of the liver to the gastrointestinal tract and the spleen through the portal venous system, as part of its metabolic function further exacerbates the impact of toxicity due to circulating drugs.

[Para 005] There have been reports that 75% of the idiosyncratic drug reactions lead to liver transplantation or death. In the United States, approximately 2000 cases of acute liver failure occur annually and 50% of such cases could be attributed to the effects of drugs. The pathophysiological mechanisms underlying drug induced include,

(i) Hepatocyte disruption that follows actin fibril disassembly caused due to the covalent binding of the drug to the intracellular proteins and the decrease in ATP levels.

(ii) Drug induced blockage of transport pumps preventing normal bile excretion to cause cholestasis.

(iii) Immune response mediated by the binding of the drug to P-450 enzyme.

(iv) TNF-α mediated apoptosis of hepatocytes.

(v) Mitochondrial disruption due to decreased ATP production brought about by the drug induced inhibition of NAD and FAD in the beta-oxidation energy production mechanism.

(vi) Toxic metabolites induced bile duct injury.

(vii) Activation of liver cell types like the Browicz-Kuppfer cells and receptors like toll-like receptor 4 (TLR4) and CD14 thereof during early ethanol induced liver damage, leading to internalization of the lipopolysaccaride fraction of the cell walls of gram negative bacteria that flourish in the gut in an alcohol rich environment. This then leads to activation of pro-inflammatory cytokines like TNF-α and superoxides which would enter the stellate cells in the liver, leading to collagen synthesis, fibrosis and eventually liver cirrhosis.

[Para 006] Hepatoprotection as an ongoing therapeutic (both preventive and prophylactic) means, thus assumes tremendous importance in conditions where there is interplay of one or more of the aforesaid mechanisms in causing liver damage. Such conditions include,

(i) Nonalcoholic steatohepatitis (NASH) which represents fat in the liver and ensuing inflammation thereof. Though it resembles alcoholic liver disease, it occurs in people who are non-alcoholics or who consume very little alcohol. NASH is enigmatic in the sense that it may regress on its own or worsen in a slow manner leading to fibrosis and subsequently life threatening cirrhosis. Further, no specific therapies exist for this condition except life style management methods.

(ii) Alcohol abuse resulting from habitual or prolonged consumption;

(iii) Chemotherapy for cancer.

(iv) Conditions such as non-alcoholic fatty liver disease where there is fat deposition in the liver without signs and symptoms of inflammation or liver damage.

(v) Viral induced acute or chronic hepatitis, where the latter condition may lead to fibrosis and life threatening cirrhosis/liver cancer.

[Para 007] Garcinol, isolated from Garcinia sp. fruit rind is known in the art as an anti-oxidant and chemo protective agent. (Tanaka, T. et.al. Prevention of colonic aberrant crypt foci by dietary feeding of garcinol in male F3444 rats. Carcinogenesis, June 2000: 21 (6): 1183-9).

[Para 008] Garcinol and isogarcinol were evaluated for their antibacterial activity against methicillin-resistant Staphylococcus *aureus* (Linuma M et al, Antibacterial activity of some Garcinia benzophenone derivatives against methicillin-resistant Staphylococcus *aureus*. Biol Pharm Bull 1996 February; 19(2): 311-4).

[Para 009] Garcinol's role as a potent inhibitor of histone acetyltransferases (HATs) both *in vitro* and *invivo* was reported by Tapas et al in 2004 ("Polyisoprenylated Benzophenone, Garcinol, a Natural Histone Acetyl transferase Inhibitor, Represses Chromatin Transcription and Alters Global GeneExpression", The Journal of Biological Chemistry, Vol. 279, No. 32, Issue of August 6, pp.33716–33726, 2004).

[Para 0010] The present inventors add further to the medical potential of garcinol in disclosing the molecule's hepatoprotectant activity through modulation of one or more pathophysiological effects highlighted herein above. In specific, the present inventors have sought to study the effects of garcinol in modulating the biochemical markers associated with hepatotoxicity.

SUMMARY OF THE INVENTION

[Para 0011] The present invention discloses the potential of garcinol as a hepatoprotectant. Protection of cultures of Hep-2 cells in the presence of effective concentrations of garcinol has been demonstrated. Also demonstrated is garcinol mediated modulation of biochemical markers in animal models of toxin-CCl_4 induced, drug-Paracetamol induced and alcohol induced hepatoxicity.

BRIEF DESCRIPTION OF DRAWINGS

[Para 0012] FIG 1: shows the graphical representation of the effect of different doses of garcinol on TGF-beta 1 expression (pg/ml) in the liver homogenates from toxin (CCl_4) induced hepatotoxic animal models. Values are expressed as Mean ± SE, n = 6); P value * : <0.001.

[Para 0013] FIG 2: shows the graphical representation of the effect of different doses of garcinol on Inter-Cellular Adhesion Molecule 1 (ICAM-1 or CD52) expression (pg/ml) in the liver homogenates from toxin (CCl_4) induced hepatotoxic animal models. Values are expressed as Mean ± SE, n = 6); P value * : <0.001.

[Para 0014] FIG 3: shows the graphical representation of the effect of different doses of garcinol on C-reactive protein expression (mg/ml) in the liver homogenates from toxin (CCl_4) induced hepatotoxic animal models. Values are expressed as Mean ± SE, n = 6); P value * : <0.001.

[Para 0015] FIG 4: shows the graphical representation (flow cytometric studies) of the effect of different doses of garcinol on Tumor Necrosis Factor-α (TNF-α) in the blood drawn from toxin (CCl_4) induced hepatotoxic animal models.

[Para 0016] FIG 5: shows the graphical representation (flow cytometric studies) of the effect of different doses of garcinol on Interleukin-2 (IL-2) in the blood drawn from toxin (CCl_4) induced hepatotoxic animal models.

[Para 0017] FIG 6: shows the graphical representation of the effect of different doses of garcinol on Interleukin-4 (IL-4) in the blood drawn from toxin (CCl_4) induced hepatotoxic animal models.

[Para 0018] FIG 7: shows the graphical representation of the effect of different doses of garcinol on TGF-beta 1 expression (pg/ml) in the liver homogenates from drug (Paracetamol) induced hepatotoxic animal models. Values are expressed as Mean ± SE, n = 6); P value * : <0.01., **: <0.001. GC : Garcinol; APAP : Paracetamol.

[Para 0019] FIG 8: shows the graphical representation of the effect of different doses of garcinol on Inter-Cellular Adhesion Molecule 1 (ICAM-1 or CD52) expression (pg/ml) in the liver homogenates from drug (Paracetamol) induced hepatotoxic animal models. Values are expressed as Mean ± SE, n = 6); P value * : <0.01., **: <0.001. GC : Garcinol; APAP : Paracetamol.

[Para 0020] FIG 9: shows the graphical representation (flow cytometric studies) of the effect of different doses of garcinol on Tumor Necrosis Factor-α (TNF-α) in the blood drawn from drug (Paracetamol) induced hepatotoxic animal models.

[Para 0021] FIG 10: shows the graphical representation of the effect of different doses of garcinol on Tumor Necrosis Factor-α (TNF-α) and Interleukin-1β (IL-1β) in the serum of alcohol (Ethyl alcohol) induced hepatotoxic animal models. Values are expressed as Mean ± SE, n = 6); P value * : <0.01., **: <0.001., GC : Garcinol.

[Para 0022] FIG 11: shows the graphical representation of the effect of different doses of garcinol on Interleukin-12 (IL-12) in the serum of alcohol (Ethyl alcohol) induced hepatotoxic animal models.

[Para 0023] FIG 12: shows the graphical representation of the cytotoxic potential (% cytotoxicity) of garcinol in Hep G2 liver cancer cell line in comparison with Silymarin, a known hepatoprotective agent.

[Para 0024] FIG 13: shows the graphical representation of the hepatoprotective effect of garcinol in comparision with the known hepatoprotective agent Silymarin in Hep G2 liver cancer cell line.

DETAILED DESCRIPTION (FIGS 1-13)-PREFERRED EMBODIMENT

[Para 0025] In the most preferred embodiment, the present invention pertains to a method of mammalian hepatocyte protection, said method comprising step of bringing into contact mammalian hepatocytes with an effective concentration of garcinol. More specifically, the effective concentration of garcinol is from about 0.78µg/ml to about 6.25 µg/ml (FIGS.12 and 13). In an alternate most preferred embodiment, the present invention relates to a method of providing hepatoprotection, said method comprising step of administering a therapeutically effective amount of garcinol to a subject in need thereof. More specifically, the subject is a mammal.

[Para 0026] In another preferred embodiment, the present invention relates to a method of reducing increased levels of cytokine expression in mammalian models of liver damage (hepatotoxicity), said method comprising step of administering an effective amount of garcinol to said models **(FIGS. 1, 4, 5, 6, 7, 9, 10 and 11)**. In specific embodiments, the cytokines are Transforming Growth Factor G-β1 (TGF- β1), Tumor Necrosis Factor-α, Interleukin-2 (IL-2), Interleukin-4 (IL-4) and Interleukin-12 (IL-12). In further specific embodiment, hepatotoxicity in mammalian models may be induced by toxin, drug or ethyl alcohol. In an alternate specific embodiment, liver damage is induced by combinations of toxin, drug and ethyl alcohol.

[Para 0027] In yet another preferred embodiment, the present invention relates to a method of reducing increased levels of adhesion molecule expression in mammalian models of liver damage (hepatotoxicity) induced by toxins and/or drugs, said method comprising step of administering an effective amount of garcinol to said models **(FIGS.2 and 8)**. In a specific embodiment, the adhesion molecule is Intracellular Adhesion Molecule-1 (ICAM-1 or CD 52).

[Para 0028] In yet another preferred embodiment, the present invention relates to a method of reducing elevated levels of liver enzymes and/or bile pigments in mammalian models of liver damage (hepatotoxicity), said method comprising step of administering an effective amount of garcinol to said models. In specific embodiments, the liver enzymes are Alanine Transaminase, Aspartate aminotransferase and Alkaline phosphatase **(Tables 3, 4 and 5)**. In another specific embodiment, the bile pigment is bilirubin. In further specific embodiment, liver damage in mammalian models may be induced by toxin, drug or ethyl alcohol. In an

alternate specific embodiment, liver damage is induced by combinations of toxin, drug and ethyl alcohol.

[PARA 0029] The potential therapeutic value of garcinol as a hepatoprotectant may be understood through examples elucidated herein below.

EXAMPLE 1

[PARA 0030] Acute Oral Safety of Garcinol: No mortality was observed up to 2000 mg/kg. p. o. in mice up to two weeks of observation. The parameters studied and observations recorded are included in **Table 1**. (OECD Guidelines for Testing of Chemicals. Guideline 423, Acute Oral Toxicity - Acute Toxic Class Method, Adopted, (1996). Organization for Economic Cooperation and Development).

Table 1: Parameters studied on acute oral safety of garcinol

General Behavior	**Dermal**
Aggressiveness: Nil Fearful: Nil Passive: Nil General Movement: Normal General Locomotor Activity: Normal	Blanching: Nil Hyperemia: Nil Cyanosis: Nil
Central Nervous System	**General Observations**
Excitation: Nil Motor Activity: Normal Tremors: Nil Clonic convulsions: Nil	Muscular Weakness: Nil Salivation: Normal Pilo erection: Nil Diarrhea: Nil
Respiratory System	**Reflexes**
Respiration rate: Normal Respiration Depth: Normal	Corneal: No Effect Pinnal: No Effect
Autonomic Nervous System	**Food and Water (Intake/Excretion)**
Motor Activity: Normal Atexia: Nil Respiration Rate: Normal Diarrhea: Nil	Fecal Output: Normal Urine Output: Normal

EXAMPLE 2

Effect of different doses of Garcinol (GC) on TGF-β1, ICAM-1 and CRP expressions in the liver homogenates of carbon tetrachloride induced liver damage in rats.

[Para 0031] Animals used in the experiment: Male Wistar Rats

[Para 0032] Weight of the animals: 140-160 grams

[Para 0033] Doses of Garcinol (GC) used for the study: 1.25, 2.5, 5, 10 mg/kg p.o.

[Para 0034] Standard drug: Silymarin (50 mg/kg) p.o.

[Para 0035] Procedure: Liver injury was induced by administration of carbon tetrachloride (CCl_4) mixed with liquid paraffin (5 fold dilution). Animals were given single dose of CCl_4 at 1ml/kg, p.o. followed by the administration of garcinol at different time intervals **(Table 2).** [(i) Bramanti G, Murmann W, Pierini P and Comporti M (1978). Effect of cicloxilic acid on CCl_4 – induced liver injury. Drug Research 28: 1112-1217. (ii) B Singh, A K Saxena, B K Chandan, K K Anand (1998). Hepatoprotective activity of Verbenalin on experimental liver damage in rodents. Fitoterapia LXIX 2: 135-140. **(iii) B.K. Chandan, A.K. Sharma, K.K. Anand** *Boerhaavia diffusa*: A study of its hepatoprotective activity. Journal of Ethnopharmacology Volume 31, Issue 3, March 1991, Pages 299-307].

Table 2: provides details of day and time schedule of CCl_4 and garcinol administration

Day and time Schedule of toxin/drug (GC) Administration	**Treatment**	**Time elapsed since toxin administration**
Day 1 10 A.M.	CCl_4	0h
4 P.M.	GC	6h after CCl_4
Day 2 10 A.M.	GC	24h after CCl_4
Day 3 10 A.M.	GC	48h after CCl_4
12 Noon	Samples Collection (liver & blood)	50h after CCl_4/2h after last treatment of test material (GC)

[Para 0036] Liver tissue was homogenized on ice with a polytron and homogenate was centrifuged at 5000 g for 15 min. Aliquots of the supernatant were separated and used for biochemical analysis. Supernatants were stored at −80 °C until cytokine analysis. TGFβ, ICAM-1, C-reactive protein (CRP) were estimated using commercially available kits based

on sandwich and competitive ELISA technique according to the manufacturers' instructions. All cytokine concentrations were carried out by means of colorimetric measurement at 450 nm on an ELISA plate reader by interpolation from a standard curve. [(i) Magari K, Miyata S, Ohkubo Y, Mutoh S, (2004). Inflammatory cytokine levels in paw tissues during development of rat collagen-induced arthritis: Effect of FK506, an inhibitor of T cell activation. Inflammation Research. 53: 469–474 (Magari *et al.*, 2004); and (ii) Modulation of Th1/Th2 cytokines and inflammatory mediators by hydroxychavicol in adjuvant induced arthritic tissues. Anjali Pandey , Sarang Bani, Prabhu Dutt, Krishna Avtar Suri. Cytokine 49 (2010) 114–121]

[Para 0037] Results : Garcinol inhibited increased levels of TGFβ (FIG 1) ICAM-1 (FIG 2), C-reactive protein (CRP) (FIG 3) associated with acute hepatitis resulting from carbon tetrachloride induced liver damage in rats.

EXAMPLE 3

Effect of different doses of Garcinol (GC) on TNF-α, IL-2 and IL-4 in the blood of carbon tetrachloride induced liver damage in rats.

[Para 0038] Animals used in the experiment: Male Wistar Rats

[Para 0039] Weight of the animals: 140-160 grams

[Para 0040] Doses of Garcinol (GC) used for the study: 1.25, 2.5, 5, 10 mg/kg p.o.

[Para 0041] Standard drug: Silymarin (50 mg/kg) p.o.

[Para 0042] Procedure: Liver injury was induced by administration of carbon tetrachloride (CCl_4) mixed with liquid paraffin (5 fold dilution). Animals were given single dose of CCl_4 at 1ml/kg, p.o. followed by the administration of garcinol (See aforesaid Table 2). [(i) Bramanti G, Murmann W, Pierini P and Comporti M (1978). Effect of cicloxilic acid on CCl_4 – induced liver injury. Drug Research 28: 1112-1217. (ii) B Singh, A K Saxena, B K Chandan, K K Anand (1998). Hepatoprotective activity of Verbenalin on experimental liver damage in rodents. Fitoterapia LXIX 2: 135-140. (iii) B.K. Chandan, A.K. Sharma, K.K. Anand *Boerhaavia diffusa*: A study of its hepatoprotective activity. Journal of Ethnopharmacology, Volume 31, Issue 3, March 1991, Pages 299-307].

[Para 0043]The animals were bled retro-orbitally and blood was collected in EDTA-coated tubes for the estimation of PE-labeled anti-rat TNF-alpha, IL-2 ad IL-4 monoclonal antibody expression. Analysis was done on flow cytometer (BD-FACS CANTO II). These

fluorochrome-labeled monoclonal antibodies were added directly to 100 µl of whole blood, which was then lysed using whole blood lysing reagent. Following the final centrifugation, samples were resuspended in phosphate-buffered saline (pH, 7.4) and analyzed directly on the flow cytometer. A fluorescence trigger was set on the PE (FL2) parameter to collect the events. [(i) Bani S, Kaul A, Khan B, Ahmad SF, Suri KA, Gupta BD, Satti NK, Qazi GN, (2006).Suppression of T lymphocyte activity by lupeol isolated from Crataeva *religiosa.* Phytotherapy Research; 20(4): 279-287. And (ii) Bani S, Kaul A, Khan B, Ahmad SF, Suri KA, Satti NK, (2005). Immunosuppressive properties of an ethyl acetate fraction from Euphorbia *royleana*. Journal of Ethnopharmacology; 99: 185–192].

[Para 0044] Results: Garcinol (GC) caused a dose based inhibition of T-Cell immune response marked by inhibition of TNF-α (FIG 4), IL-2 (FIG 5) and IL-4 (FIG 6) expressed in the blood of carbon tetrachloride induced liver damage in rats.

EXAMPLE 4

Effect of different doses of Garcinol (GC) on bilirubin and serum enzymes Alanine Transaminase (ALT), Aspartate Aminotransferase (AST) and Alkaline Phosphatase (ALP) in carbon tetrachloride induced liver damage in rats

[Para 0045] Animals used in the experiment: Male Wistar Rats

[Para 0046] Weight of the animals: 140-160 grams

[Para 0047] Doses of Garcinol (GC) used for the study: 1.25, 2.5, 5, 10 mg/kg p.o.

[Para 0048] Standard drug: Silymarin (50 mg/kg) p.o.

[Para 0049] Procedure: Liver injury was induced by administration of carbon tetrachloride (CCl_4) mixed with liquid paraffin (5 fold dilution). Animals were given single dose of CCl_4 at 1ml/kg, p.o. followed by the administration of garcinol (Table 2). [(i) Bramanti G, Murmann W, Pierini P and Comporti M (1978). Effect of cicloxilic acid on CCl_4 – induced liver injury. Drug Research 28: 1112-1217. (ii) B Singh, A K Saxena, B K Chandan, K K Anand (1998). Hepatoprotective activity of Verbenalin on experimental liver damage in rodents. Fitoterapia LXIX 2: 135-140. (iii) B.K. Chandan, A.K. Sharma, K.K. Anand *Boerhaavia diffusa*: A study of its hepatoprotective activity. Journal of Ethnopharmacology, Volume 31, Issue 3, March 1991, Pages 299-307].

[Para 0050] Blood was collected from the retro-orbital plexus of experimental animals and no anti-coagulant was added. Blood was made to stand at room temperature for 1 hour. It was

then centrifuged and clear serum was separated. The serum was then stored for analysis. Reference: (Magari *et al.*, 2004).

[Para 0051] Serum biochemistry I (ASPARTATE AMINOTRANSFERASE and ALANINE AMINOTRANSFERASE), Reference: Ritman S, Frankel S, (1957). A colorimeteric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. American Journal of Clinical Pathology; 28: 56-63.

[Para 0052] Procedure: 0.2ml of serum, collected from test and control samples, was mixed with 1.0ml of buffer solution and incubated for 60 min at 37°C in a water bath. After the addition of 1.0ml of chromogen solution, the samples were kept at room temperature for 20minutes for the reaction to proceed and 10ml NaOH was added. The optical density was red at 546nm after 5min. To the blank, serum was added after the addition of chromogen solution.

[Para 0053] Serum Biochemistry II (ALKALINE PHOSPHATASE), Reference: Klaus Walter and Schutt C. (1974). Acid and alkaline phosphatase in serum. In: Methods of Enzymatic Analysis.Eds. Hans Ulrich Bergmeyer, Verlag ChemieWeinheim,Academic press, Inc. New York, 2nd Edition, Vol.2, pp. 855-64.

[Para 0054] Procedure: Alkaline phosphatase activity measurement is based on the ability of the enzyme to hydrolyze *p*-nitrophenol phosphate under alkaline conditions. The cleaved product *p*-nitrophenol is yellow in alkaline solution and is measured at 400-420 nm (Klaus and Schutt, 1974). 2.0 ml of buffered substrate was taken in the tubes 'Test' 'Control' and 'Blank' followed by addition of serum and distilled water (0.1ml) in 'Test' and 'Blank' respectively and incubated in a water bath for 30 minutes at 25^0C. After incubation sodium hydroxide was added (0.25 N, 2 ml) to all the tubes, which was followed by serum (0.1ml) to the tubes marked 'Control'. The yellow colour formed was measured spectrophotometrically against blank at 410nm.

[Para 0055] Serum Biochemistry III [BILIRUBIN (BRBN)], Reference: Malloy H T and Evelyn K A (1937). The determination of bilirubin with photoelectric colorimeter. Journal of Biological Chemistry 119: 481-490.

[Para 0056] Procedure: Serum bilirubin was estimated by the method of Malloy and Evelyn (1937). In the two sets of test tubes marked 'Test' and 'Control', serum (0.2 ml) and distilled water (1.8 ml) were added. To the tubes marked 'Test' and 'Standard' diazo reagent (0.5 ml) was added. To the test tubes marked 'Control' and 'Blank' diazo blank (0.5 ml) was added. Finally methanol (2.5 ml) was added into each test tube. The tubes were mixed and allowed to stand for 30 minutes in dark. The tubes were read after 10 minutes at 540nm.

[Para 0057] Results: Garcinol reduced increased levels of bilirubin and serum enzymes Alanine Transaminase (ALT), Aspartate Aminotransferase (AST) and Alkaline Phosphatase (ALP) in carbon tetrachloride induced liver damage in rats (Table 3).

Table 3: Effect of Garcinol on bilirubin, ALT, AST and ALP in carbon tetrachloride induced liver damage in rats

SERUM PARAMETERS						
Treatments	Dose (mg/kg)	ALT µmol/min/lt	AST µmol/min/lt	ALP µmol/min/lt	Bilirubin mg%	Average Protection %
Vehicle	-	110.30±13.70	107.70±14.00	30.90±0.72	0.40±0.05	-
Vehicle + CCl_4	-	1460.03±78.30	878.10±41.52	87.99±3.35	1.17±0.07	-
GC+ CCl_4	1.25	1272.50±109.19 (12.84)	746.81±36.93 (14.95)	78.49±6.58 (10.79)	1.03±0.04 (11.96)	12.63
GC+ CCl_4	2.50	1027.07±68.26* (29.65)	646.90±50.53* (26.32)	70.32±3.89* (20.08)	0.89±0.05** (23.93)	24.99
GC+ CCl_4	5.00	828.59±46.56** (43.24)	500.66±49.22** (42.98)	59.19±4.45** (32.73)	0.78±0.04** (33.33)	**38.07**
GC+ CCl_4	10.00	891.36±45.72** (38.94)	522.43±57.50** (40.50)	59.70±3.69** (32.15)	0.75±0.04** (35.89)	36.87
Silymarin + CCl_4	50	744.76±40.16** (48.99)	460.76±25.60** (47.52)	49.84±2.80** (43.35)	0.64±0.04** (48.71)	47.14
(Values as Mean ± SE, n = 6) ; Percent change in parenthesis; P value * : < 0.01; ** : <0.001. (ALT) Alanine transaminase ; (AST) Aspartate aminotransferase ; (ALP) Alkaline phosphatase .						

EXAMPLE 5

Effect of different doses of Garcinol (GC) on TGF-beta 1 and ICAM-1 expression in liver homogenate of Paracetamol induced liver damage in rats.

[Para 0058] Animals used in the experiment: Male Wistar Rats

[Para 0059] Weight of the animals: 140-160 grams

[Para 0060] Doses of Garcinol (GC) used for the study: 1.25, 2.5, 5, 10 mg/kg p.o.

[Para 0061] Standard drug: Silymarin (50 mg/kg) p. o.

[Para 0062] Procedure: Experimental animals orally received paracetamol (400 mg/kg body weight) for seven days. The animals from drug treated group received 400 mg/kg body weight of paracetamol dissolved in water orally along with graded doses of test drug Garcinol for seven days. The standard group animals received 50 mg/kg body weight of standard drug silymarin and 400 mg/kg body weight of paracetamol for seven days and served as standard control. [N.Kanchana and A.Mohamed Sadiq, Hepatoprotective effect of Plumbago *zeylanica* on paracetamol induced liver toxicity in rats, *Int J Pharm Pharm Sci, Vol 3, Issue1, 151-154 (2011)*].

[Para 0063] Liver tissue from experimental animals was homogenized on ice with a polytron and homogenate was centrifuged at 5000 g for 15 min. Aliquots of the supernatant were separated and used for biochemical analysis. Supernatants were stored at −80 °C until cytokine analysis. TGFβ and ICAM-1were estimated using commercially available kits based on sandwich and competitive ELISA technique according to the manufacturers' instructions. All cytokine concentrations were carried out by means of colorimetric measurement at 450 nm on an ELISA plate reader by interpolation from a standard curve. [(i) Magari K, Miyata S, Ohkubo Y, Mutoh S, (2004). Inflammatory cytokine levels in paw tissues during development of rat collagen-induced arthritis: Effect of FK506, an inhibitor of T cell activation. Inflammation Research. 53: 469–474 (Magari *et al.*, 2004); and (ii) Modulation of Th1/Th2 cytokines and inflammatory mediators by hydroxychavicol in adjuvant induced arthritic tissues. Anjali Pandey , Sarang Bani, Prabhu Dutt, Krishna Avtar Suri. Cytokine 49 (2010) 114–121].

[Para 0064] Results: Garcinol caused a dose dependent inhibition of increased levels of TGF-beta and ICAM-1 expressions in the liver associated with paracetamol induced acute hepatitis (FIG 7 and FIG 8).

EXAMPLE 6

Effect of different doses of Garcinol (GC) on serum enzymes Alanine Transaminase (ALT), Aspartate Aminotransferase (AST) and Alkaline Phosphatase (ALP) in paracetamol (acetyl-para-aminophenol-APAP) induced liver damage in rats

[Para 0065] Animals used in the experiment: Male Wistar Rats

[Para 0066] Weight of the animals: 140-160 grams

[Para 0067] Doses of Garcinol (GC) used for the study: 1.25, 2.5, 5, 10 mg/kg p.o.

[Para 0068] Standard drug: Silymarin (50 mg/kg) p.o.

[Para 0069] Procedure: Experimental animals orally received paracetamol (400 mg/kg body weight) for seven days. The animals from drug treated group received 400 mg/kg body weight of paracetamol dissolved in water orally along with graded doses of test drug Garcinol for seven days. The standard group animals received 50 mg/kg body weight of standard drug silymarin and 400 mg/kg body weight of paracetamol for seven days and served as standard control. [N.Kanchana and A.Mohamed Sadiq, Hepatoprotective effect of Plumbago *zeylanica* on paracetamol induced liver toxicity in rats, *Int J Pharm Pharm Sci, Vol 3, Issue1, 151-154 (2011)*].

[Para 0070] Blood was collected from the retro-orbital plexus of experimental animals and no anti-coagulant was added. Blood was made to stand at room temperature for 1 hour. It was then centrifuged and clear serum was separated. The serum was then stored for analysis. Reference: (Magari *et al.*, 2004).

[Para 0071] Serum biochemistry I (ASPARTATE AMINOTRANSFERASE and ALANINE AMINOTRANSFERASE), Reference: Ritman S, Frankel S, (1957). A colorimeteric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. American Journal of Clinical Pathology; 28: 56-63.

[Para 0072] Procedure: 0.2ml of serum, collected from test and control samples, was mixed with 1.0ml of buffer solution and incubated for 60 min at 37°C in a water bath. After the addition of 1.0ml of chromogen solution, the samples were kept at room temperature for 20 minutes for the reaction to proceed and 10ml NaOH was added. The optical density was red at 546nm after 5min. To the blank, serum was added after the addition of chromogen solution.

[Para 0073] Serum Biochemistry II (ALKALINE PHOSPHATASE), Reference: Klaus Walter and Schutt C. (1974). Acid and alkaline phosphatase in serum. In: Methods of Enzymatic Analysis.Eds. Hans Ulrich Bergmeyer, Verlag ChemieWeinheim,Academic press, Inc. New York, 2nd Edition, Vol.2, pp. 855-64.

[Para 0074] Procedure: Alkaline phosphatase activity measurement is based on the ability of the enzyme to hydrolyze *p*-nitrophenol phosphate under alkaline conditions. The cleaved product *p*-nitrophenol is yellow in alkaline solution and is measured at 400-420 nm (Klaus and Schutt, 1974). 2.0 ml of buffered substrate was taken in the tubes 'Test' 'Control' and 'Blank' followed by addition of serum and distilled water (0.1ml) in 'Test' and 'Blank' respectively and incubated in a water bath for 30 minutes at 25^0C. After incubation sodium hydroxide was added (0.25 N, 2 ml) to all the tubes, which was followed by serum (0.1ml) to the tubes marked 'Control'. The yellow colour formed was measured spectrophotometrically against blank at 410nm.

[Para 0075] Serum Biochemistry III [BILIRUBIN (BRBN)], Reference: Malloy H T and Evelyn K A (1937). The determination of bilirubin with photoelectric colorimeter. Journal of Biological Chemistry **119:** 481-490.

[Para 0076] Procedure: Serum bilirubin was estimated by the method of Malloy and Evelyn (1937). In the two sets of test tubes marked 'Test' and 'Control', serum (0.2 ml) and distilled water (1.8 ml) were added. To the tubes marked 'Test' and 'Standard' diazo reagent (0.5 ml) was added. To the test tubes marked 'Control' and 'Blank' diazo blank (0.5 ml) was added. Finally methanol (2.5 ml) was added into each test tube. The tubes were mixed and allowed to stand for 30 minutes in dark. The tubes were read after 10 minutes at 540nm.

[Para 0077] Results: Garcinol reduced increased levels of bilirubin and serum enzymes Alanine Transaminase (ALT), Aspartate Aminotransferase (AST) and Alkaline Phosphatase (ALP) in paracetamol induced liver damage in rats (Table 4).

Table 4: Effect of Garcinol on bilirubin, ALT, AST and ALP in paracetamol induced liver damage in rats

SERUM PARAMETERS					
Treatments	Dose (mg/kg)	ALT (µmol min/lt)	AST(µmol min/lt)	ALP(µmol min/lt)	Average Protection%
Vehicle	-	98.32±5.20	117.49±8.26	21.42±2.04	-
Vehicle+APAP	-	1102.10±46.98	839.79±59.17	40.54±3.61	-
GC+APAP	1.25	862.97±53.38 (21.69)	761.61±43.96 (9.30)	37.21±3.44 (8.21)	13.06
GC+APAP	2.50	808.64±38.55* (26.62)	650.88±35.84* (22.49)	34.28±1.98 (15.44)	21.51
GC+APAP	5.00	661.67±51.40** (39.96)	463.41±38.56** (44.81)	30.74±2.05** (24.17)	**36.31**
GC+APAP	10.00	590.22±50.98** (46.44)	536.39±44.13** (36.12)	33.60±1.93* (17.11)	33.22
Silymarin + APAP	50	377.04±14.44** (65.78)	379.64±23.22** (54.79)	26.90±2.24** (33.64)	51.40

(Values as Mean ± SE, n = 6); Percent change in parenthesis; P value *: < 0.01; ** : <0.001. (ALT) Alanine transaminase ; (AST) Aspartate aminotransferase ; (ALP) Alkaline phosphatase

EXAMPLE 7

Effect of different doses of Garcinol (GC) on TNF-α in the blood of paracetamol induced liver damage in rats.

[Para 0078] Animals used in the experiment: Male Wistar Rats

[Para 0079] Weight of the animals: 140-160 grams

[Para 0080] Doses of Garcinol (GC) used for the study: 1.25, 2.5, 5, 10 mg/kg p.o.

[Para 0081] Standard drug: Silymarin (50 mg/kg) p.o.

[Para 0082] Procedure: Experimental animals orally received paracetamol (400 mg/kg body weight) for seven days. The animals from drug treated group received 400 mg/kg body weight of paracetamol dissolved in water orally along with graded doses of test drug Garcinol for seven days. The standard group animals received 50 mg/kg body weight of standard drug silymarin and 400 mg/kg body weight of paracetamol for seven days and served as standard control. [N.Kanchana and A.Mohamed Sadiq, Hepatoprotective effect of Plumbago *zeylanica*

on paracetamol induced liver toxicity in rats, *Int J Pharm Pharm Sci, Vol 3, Issue1, 151-154 (2011)*].

[Para 0083] The animals were bled retro-orbitally and blood was collected in EDTA-coated tubes for the estimation of PE-labeled anti-rat TNF-alpha monoclonal antibody expression. Analysis was done on flow cytometer (BD-FACS CANTO II). These fluorochrome-labeled monoclonal antibodies were added directly to 100 µl of whole blood, which was then lysed using whole blood lysing reagent. Following the final centrifugation, samples were resuspended in phosphate-buffered saline (pH, 7.4) and analyzed directly on the flow cytometer. A fluorescence trigger was set on the PE (FL2) parameter to collect the events. [(i) Bani S, Kaul A, Khan B, Ahmad SF, Suri KA, Gupta BD, Satti NK, Qazi GN, (2006).Suppression of T lymphocyte activity by lupeol isolated from Crataeva *religiosa.* Phytotherapy Research; 20(4): 279-287. And (ii) Bani S, Kaul A, Khan B, Ahmad SF, Suri KA, Satti NK, (2005). Immunosuppressive properties of an ethyl acetate fraction from Euphorbia *royleana*. Journal of Ethnopharmacology; 99: 185–192].

[Para 0084] Results: FIG 9 shows that Garcinol causes dose dependant reduction of increased levels of TNF-α in the liver occurring in acute hepatitis caused by drug (paracetamol) induced liver damage.

EXAMPLE 8

The effect of different doses of Garcinol (GC) on Tumor Necrosis Factor-α (TNF-α), Interleukin-1β (IL-1β) and IL-12 in the serum of alcohol (Ethyl alcohol-EtOH) induced hepatotoxic animal models.

[Para 0085] Animals used in the experiment: Male Wistar Rats

[Para 0086] Weight of the animals: 140-160 grams

[Para 0087] Doses of Garcinol (GC) used for the study: 1.25, 2.5, 5, 10 mg/kg p.o.

[Para 0088] Standard drug: Silymarin (50 mg/kg) p.o.

[Para 0089] Procedure: Male Wistar rats, weighing 180-200g, were given 0.5-0.6ml ethanol orally. The initial dose of ethanol was 6g/kg/day (solutions maximally containing 56% alcohol), and the dose was progressively increased during week 1 to a maintenance dose of 8 g/kg/day that was continued for 5 more weeks. All rats had regular standard rat chow available throughout the 6-week period. Rats were weighted three times per week. [Guangjin Yuan, Zuojiong Gong *, Xiaorong Zhou, Pin Zhang, Xiaomei Sun and Xi Li.

Epigallocatechin-3-Gallate Ameliorates Alcohol-Induced Liver Injury in Rats. *Int. J. Mol. Sci.* 2006, *7*, 204-219].

[Para 0090] Blood was collected from the retro-orbital plexus of the alcohol treated experimental animals and mixed with EDTA for cytokine estimations. For collecting serum no anti-coagulant was added to the blood and it was made to stand at room temperature for 1h. The blood was then centrifuged and clear serum was separated and stored for analysis. TNF-α, IL-1β and IL-12 were estimated using commercially available kits based on sandwich and competitive ELISA technique according to the manufacturers' instructions. All cytokine concentrations were carried out by means of colorimetric measurement at 450 nm on an ELISA plate reader by interpolation from a standard curve.

[Para 0091] Result: Garcinol (GC) inhibited increased levels of TNF-alpha, Interleukin- 1 beta and Interleukin-12 (FIG 10 and FIG 11) induced by TLR-4 activation of Kupffer cells by LPS of gram negative bacteria in the gut the activation and excessive growth of which is due to ethyl alcohol intake.

EXAMPLE 9

Effect of different doses of Garcinol (GC) on serum enzymes Alanine Transaminase (ALT), Aspartate Aminotransferase (AST) and Alkaline Phosphatase (ALP) in in the serum of alcohol (Ethyl alcohol) induced hepatotoxic animal models

[Para 0092] Animals used in the experiment: Male Wistar Rats

[Para 0093] Weight of the animals: 140-160 grams

[Para 0094] Doses of Garcinol (GC) used for the study: 1.25, 2.5, 5, 10 mg/kg p.o.

[Para 0095] Standard drug: Silymarin (50 mg/kg) p.o.

[Para 0096] Procedure: Male Wistar rats, weighing 180-200g, were given 0.5-0.6ml ethanol orally. The initial dose of ethanol was 6g/kg/day (solutions maximally containing 56% alcohol), and the dose was progressively increased during week 1 to a maintenance dose of 8 g/kg/day that was continued for 5 more weeks. All rats had regular standard rat chow available throughout the 6-week period. Rats were weighted three times per week. [Guangjin Yuan, Zuojiong Gong *, Xiaorong Zhou, Pin Zhang, Xiaomei Sun and Xi Li. Epigallocatechin-3-Gallate Ameliorates Alcohol-Induced Liver Injury in Rats. *Int. J. Mol. Sci.* 2006, *7*, 204-219].Blood was collected from the retro-orbital plexus of experimental animals and no anti-coagulant was added. Blood was made to stand at room temperature for 1

hour. It was then centrifuged and clear serum was separated. The serum was then stored for analysis. Reference: (Magari *et al.*, 2004).

[Para 0097] Serum biochemistry I (ASPARTATE AMINOTRANSFERASE and ALANINA AMINOTRANSFERASE), Reference: Ritman S, Frankel S, (1957). A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. American Journal of Clinical Pathology; 28: 56-63.

[Para 0098] Procedure: 0.2ml of serum, collected from test and control samples, was mixed with 1.0ml of buffer solution and incubated for 60 min at 37°C in a water bath. After the addition of 1.0ml of chromogen solution, the samples were kept at room temperature for 20minutes for the reaction to proceed and 10ml NaOH was added. The optical density was red at 546nm after 5min. To the blank, serum was added after the addition of chromogen solution.

[Para 0099] Serum Biochemistry II (ALKALINE PHOSPHATASE), Reference: Klaus Walter and Schutt C. (1974). Acid and alkaline phosphatase in serum. In: Methods of Enzymatic Analysis.Eds. Hans Ulrich Bergmeyer, Verlag ChemieWeinheim,Academic press, Inc. New York, 2nd Edition, Vol.2, pp. 855-64.

[Para 0100] Procedure: Alkaline phosphatase activity measurement is based on the ability of the enzyme to hydrolyze *p*-nitrophenol phosphate under alkaline conditions. The cleaved product *p*-nitrophenol is yellow in alkaline solution and is measured at 400-420 nm (Klaus and Schutt, 1974). 2.0 ml of buffered substrate was taken in the tubes 'Test' 'Control' and 'Blank' followed by addition of serum and distilled water (0.1ml) in 'Test' and 'Blank' respectively and incubated in a water bath for 30 minutes at 25^0C. After incubation sodium hydroxide was added (0.25 N, 2 ml) to all the tubes, which was followed by serum (0.1ml) to the tubes marked 'Control'. The yellow colour formed was measured spectrophotometrically against blank at 410nm.

[Para 0101] Serum Biochemistry III [BILIRUBIN (BRBN)], Reference: Malloy H T and Evelyn K A (1937). The determination of bilirubin with photoelectric colorimeter. Journal of Biological Chemistry 119**:** 481-490.

[Para 0102] Procedure: Serum bilirubin was estimated by the method of Malloy and Evelyn (1937). In the two sets of test tubes marked 'Test' and 'Control', serum (0.2 ml) and distilled water (1.8 ml) were added. To the tubes marked 'Test' and 'Standard' diazo reagent (0.5 ml) was added. To the test tubes marked 'Control' and 'Blank' diazo blank (0.5 ml) was added. Finally methanol (2.5 ml) was added into each test tube. The tubes were mixed and allowed to stand for 30 minutes in dark. The tubes were read after 10 minutes at 540nm.

Table 5

SERUM PARAMETERS						
Treatments	Dose (mg/kg)	ALT (µmol min/lt)	AST(µmol min/lt)	ALP(µmol min/lt)	Bilirubin mg%	Average Protection%
Vehicle	-	118.10±10.20	106.40±16.60	32.30±0.52	0.44±0.08	-
Vehicle+ EtOH	-	1820.08±42.80	1076.32 ±48.22	104.22±8.60	2.08±0.02	-
GC + EtOH	1.25	1320.40±52.20* (27.45)	858.80±32.64 (20.20)	88.68 ±8.58 (14.91)	1.94±0.02 (6.73)	17.32
GC + EtOH	2.50	1080.50±48.56** (40.63)	726.78±46.50* (32.47)	76.12±4.88* (26.96)	1.68±0.04* (19.23)	29.82
GC + EtOH	5.00	876.58±76.80** (51.83)	650.26±56.20** (39.58)	68.32±5.60** (34.44)	1.42±0.06** (31.73)	**39.39**
GC + EtOH	10.00	870.80±48.40** (52.15)	632.48±44.56** (41.23)	69.00±4.82** (33.79)	1.39±0.04** (33.65)	**40.20**
Silymarin + EtOH	50	854.88±38.46** (53.03)	662.70±38.40** (38.44)	66.54±6.30** (36.15)	1.34±0.02** (35.57)	40.79
(Values as Mean ± SE, n = 6) ; Percent change in parenthesis; P value * : < 0.01; ** : <0.001. (ALT) Alanine transaminase ; (AST) Aspartate aminotransferase ; (ALP) Alkaline phosphatase						

[Para 0103] Result: Garcinol (GC) reduced the increased levels of AST, ALT, ALP and Bilirubin in ethyl alcohol induced hepatotoxic experimental models.

EXAMPLE 10

The cytotoxic potential (% cytotoxicity) of garcinol in Hep G2 liver cancer cell line in comparison with Silymarin, a known hepatoprotective agent (Overall hepatoprotective effect of Garcinol vs Silymarin-Tables 6 and 7, FIG 12 and FIG 13)

[Para 0104] Procedure- HEP G2 cells grown in varying concentrations of the material to be checked for cytotoxicity are taken. The medium is then tapped off gently and 100 ml of working stock solution of MTT (150 mg/well) is added into each well. Then the plate is further wrapped in aluminium foil and incubated for 4 hours in CO_2 incubator at 37^0C. The plate is then washed gently with 100 ml of PBS per well. The washing must be done soon after tapping off the medium to avoid drying, flaking and loss of cells during washing. Solubilize the dye in 100 ml of DMSO per well. The plates are shaken for 5 minutes and absorbance read at 492 nm using a Fluostar® optima (BMG) micro plate reader. The absorbance will be directly proportional to the cell viability. The option of reading at 620nm also can be adopted which would deduct the interference of the cell debris in the samples. The data is analyzed by plotting cell number versus absorbance allowing the quantification of changes in cell proliferation. The rate of tetrazolium reduction is proportional to the rate of cell proliferation.

Calculation:

Cytotoxicity of the sample is expressed as IC_{50} value, the concentration which inhibits 50% of the cell growth.

$$\% \text{ Cytotoxicity} = \frac{E - T}{E} \times 100$$

Where, E = Cell viability in the absence of the sample

T = Cell viability in the presence of the sample.

Hepatoprotective activity of Garcinol in HEP G2 cells

IC_{50} = 13.33ug/ml

95%CL = 11.84 to 15.02ug/ml

Hepatoprotective activity of Silymarin in HEP G2 cells

IC 50 = 36.51ug/ml

95%CL = 32.18 to 41.42ug/ml

Lesser the IC_{50} value, better the efficacy.

Table 6: [Cytotoxicity of Silymarin in Hep G2 cell line]

Concentration (µg/ml)	% Cytotoxicity
100.00	81.77
50.00	64.33
25.00	8.62
12.50	-7.46
6.25	-4.25
3.13	-11.27
1.56	-8.63
0.78	-8.31
0.39	2.34
Sigmoidal Dose Response (variable slope)	
BOTTOM	0
TOP	100
IC_{50}	36.51 µg/ml
95% CL	32.18 to 41.42 µg/ml
R^2	0.9545

Table 7: [Cytotoxicity of Garcinol in Hep G2 cell line]

Concentration (µg/ml)	% Cytotoxicity
200.00	79.50
100.00	83.44
50.00	83.15
25.00	60.90
12.50	35.81
6.25	-1.94
3.13	-14.65
1.56	-12.09
0.78	-3.81
Sigmoidal Dose Response (variable slope)	
BOTTOM	0
TOP	100
IC_{50}	13.33 µg/ml
95% CL	11.84 to 15.02 µg/ml
R^2	0.9756

[Para 0105] Results: Garcinol shows comparative hepatoprotective effect like Silymarin at lower concentrations (values below 6.25 μg/ml).

[Para 0106] While the invention has been described with reference to a preferred embodiment, it is to be clearly understood by those skilled in the art that the invention is not limited thereto. Rather, the scope of the invention is to be interpreted only in conjunction with the appended claims.

CLAIMS :

1. Use of an effective concentration of garcinol in contact with mammalian hepatocytes for the protection of said mammalian hepatocyte.

2. The use according to claim 1, wherein the effective concentration of garcinol is from about 0.78µg/ml to about 6.25 µg/ml.

3. Use of an effective concentration of garcinol for reducing increased levels of cytokine expression in mammalian models of liver damage (hepatotoxicity).

4. The use according to claim 3, wherein the cytokine is Transforming Growth Factor G-β1 (TGF- β1).

5. The use according to claim 3, wherein the cytokine is Tumor Necrosis Factor-α.

6. The use according to claim 3, wherein the cytokine is Interleukin-2 (IL-2).

7. The use according to claim 3, wherein the cytokine is Interleukin-4 (IL-4).

8. The use according to claim 3, wherein the cytokine is Interleukin-12 (IL-12).

9. The use according to claim 3, wherein said hepatotoxicity is caused by toxin.

10. The use according to claim 3, wherein said hepatotoxicity is caused by drugs.

11. The use according to claim 3, wherein said hepatotoxicity is caused by ethyl alcohol.

12. Use of an effective concentration of garcinol for reducing increased levels of adhesion molecule expression in mammalian models of liver damage (hepatotoxicity).

13. The use according to claim 12, wherein the adhesion molecule is Intracellular Adhesion Molecule-1 (ICAM-1 or CD 52).

14. The use according to claim 12, wherein said hepatotoxicity is caused by toxin.

15. The use according to claim 12, wherein said hepatotoxicity is caused by drugs.

16. Use of an effective concentration of garcinol for reducing elevated levels of liver enzymes and/or bile pigments in mammalian models of liver damage (hepatotoxicity).

17. The use according to claim 16, wherein said hepatotoxicity is caused by toxin.

18. The use according to claim 16, wherein said hepatotoxicity is caused by drugs.

19. The use according to claim 16, wherein said hepatotoxicity is caused by ethyl alcohol.

20. The use according to claim 16, wherein the liver enzyme is selected from a group comprising Alanine Transaminase, Aspartate aminotransferase and Alkaline Phosphatase.

21. The use according to claim 16, wherein the bile pigment is bilirubin.

22. Use of an effective concentration of garcinol for providing hepatoprotection to a subject in need thereof.

FIG.1

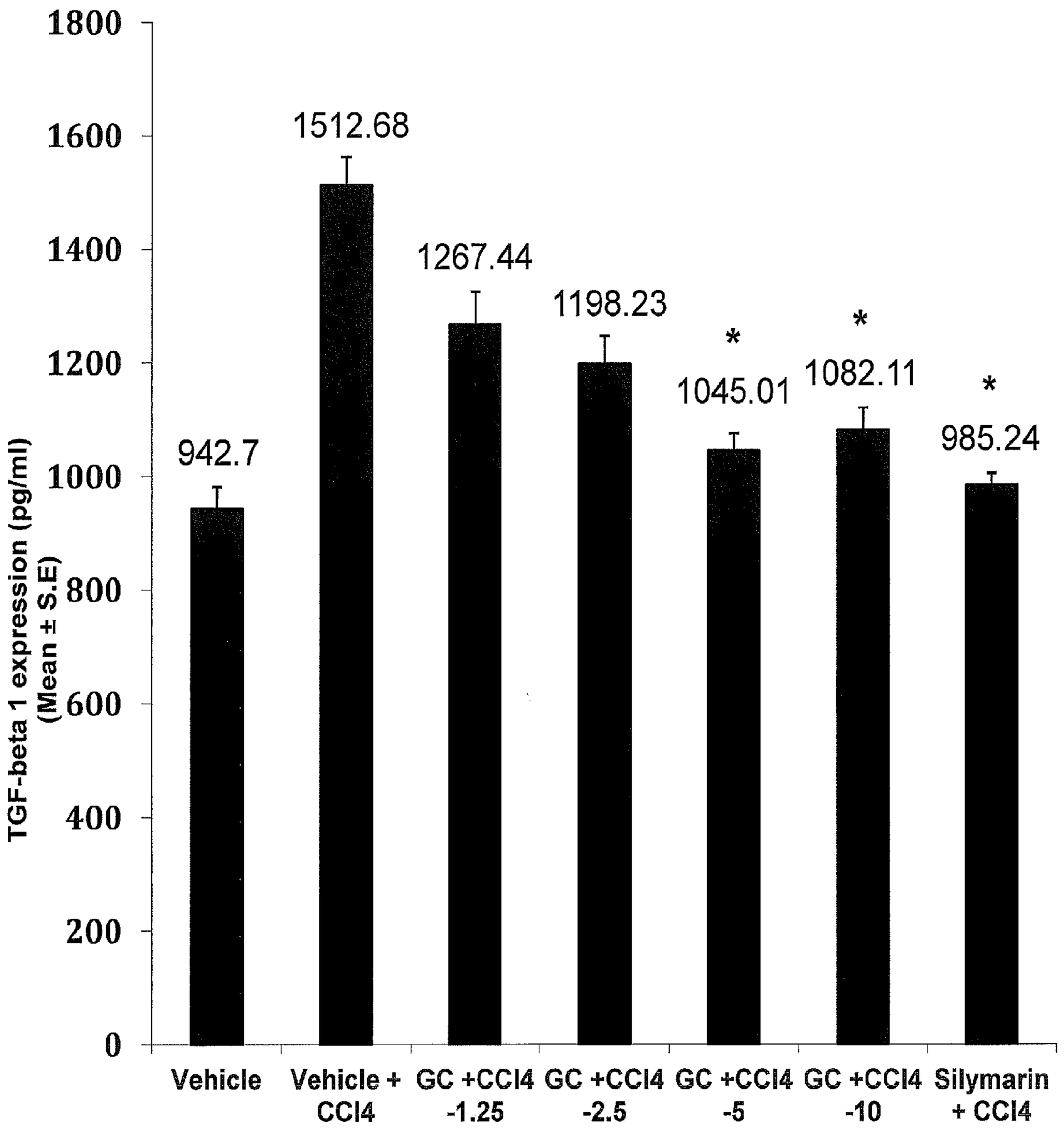

1800
1600
1400
1200
1000
800
600
400
200
0
TGF-beta 1 expression (pg/ml)
(Mean ± S.E)
942.7
1512.68
1267.44
1198.23
*
1045.01
*
1082.11
*
985.24
Vehicle
Vehicle + CCl4
GC +CCl4 -1.25
GC +CCl4 -2.5
GC +CCl4 -5
GC +CCl4 -10
Silymarin + CCl4
[mg / kg] p. o.

FIG.2

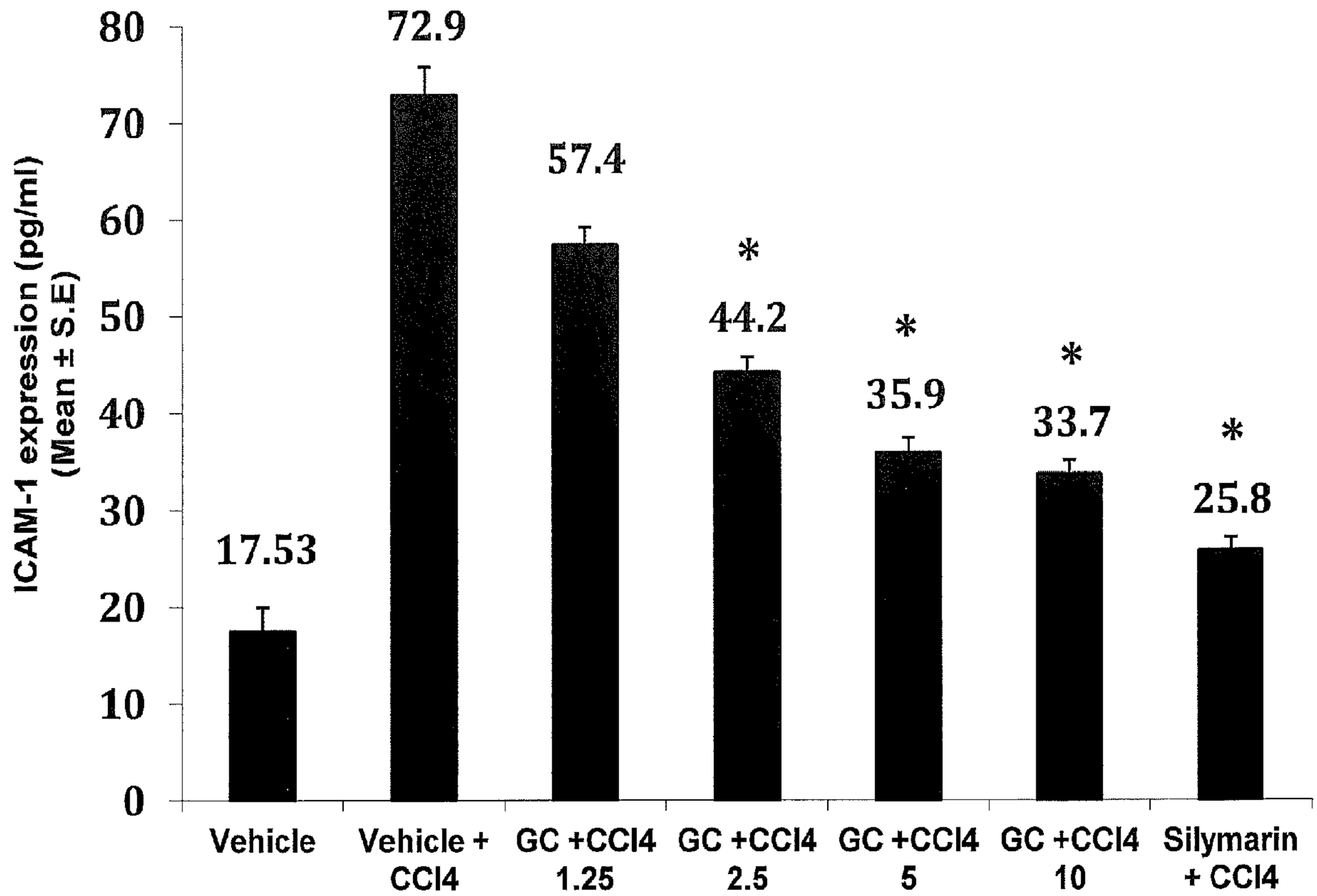

ICAM-1 expression (pg/ml)
(Mean ± S.E)
80
70
60
50
40
30
20
10
0
17.53
72.9
57.4
*
44.2
*
35.9
*
33.7
*
25.8
Vehicle
Vehicle + CCl4
GC +CCl4 1.25
GC +CCl4 2.5
GC +CCl4 5
GC +CCl4 10
Silymarin + CCl4
[mg / kg] p. o.

FIG.3

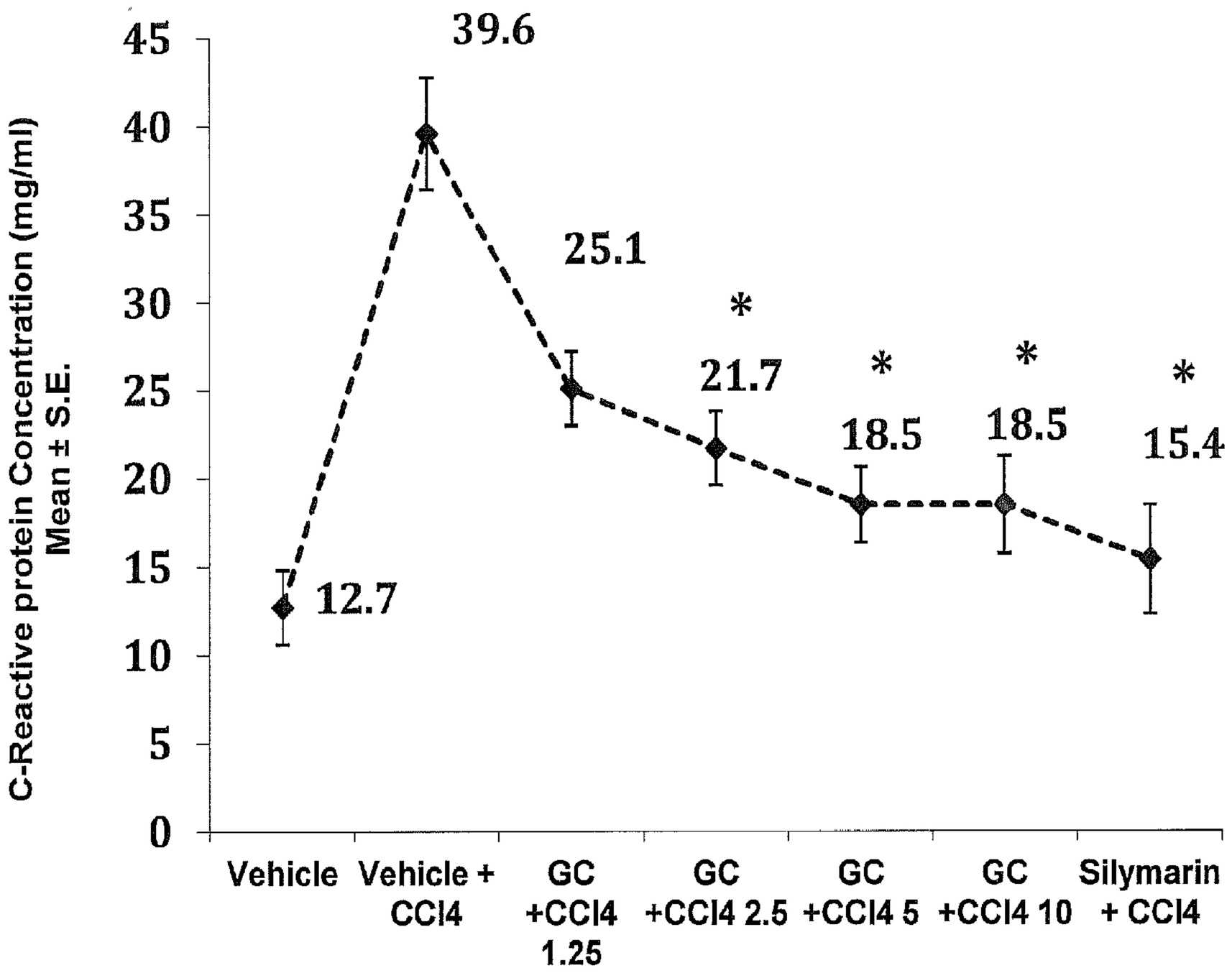

C-Reactive protein Concentration (mg/ml)
Mean ± S.E.
45
40
35
30
25
20
15
10
5
0
39.6
25.1
21.7
18.5
18.5
15.4
12.7
*
*
*
*
Vehicle
Vehicle + CCl4
GC +CCl4 1.25
GC +CCl4 2.5
GC +CCl4 5
GC +CCl4 10
Silymarin + CCl4
[mg / kg] p. o.

FIG.4

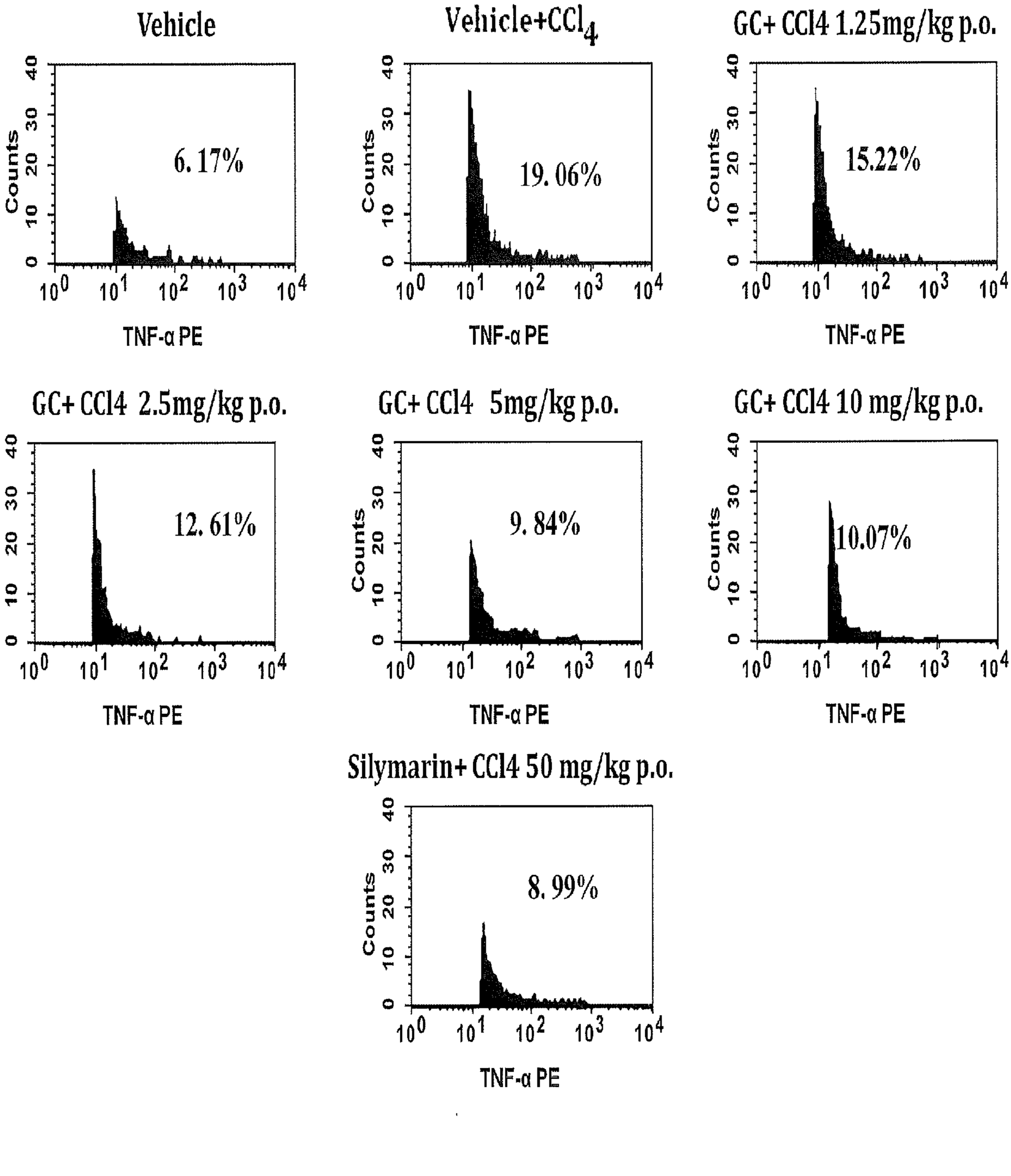


FIG.5

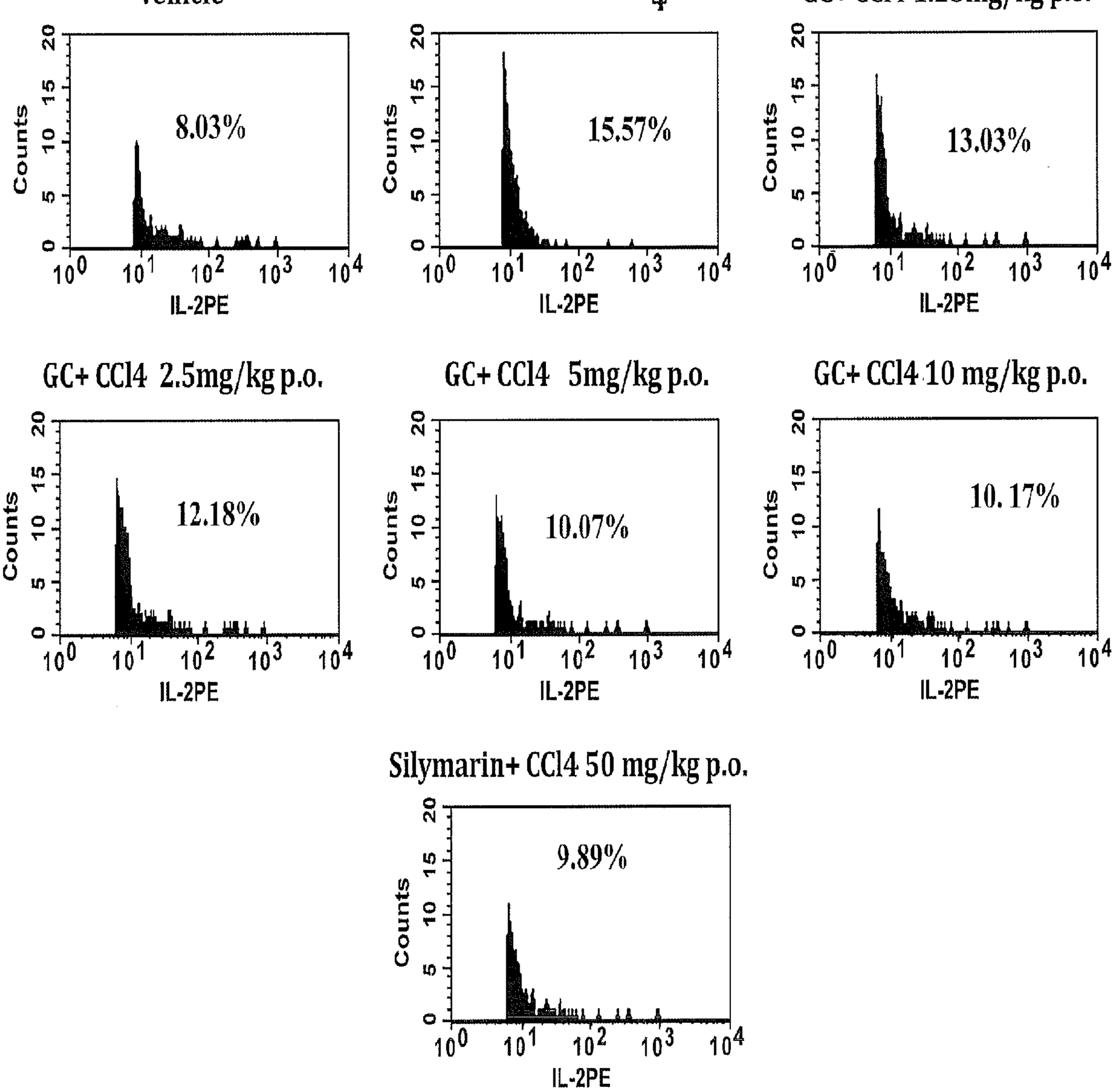

Vehicle
Vehicle+CCl4
GC+ CCl4 1.25mg/kg p.o.
8.03%
15.57%
13.03%
GC+ CCl4 2.5mg/kg p.o.
GC+ CCl4 5mg/kg p.o.
GC+ CCl4 10 mg/kg p.o.
12.18%
10.07%
10.17%
Silymarin+ CCl4 50 mg/kg p.o.
9.89%
Counts
IL-2PE

FIG.6

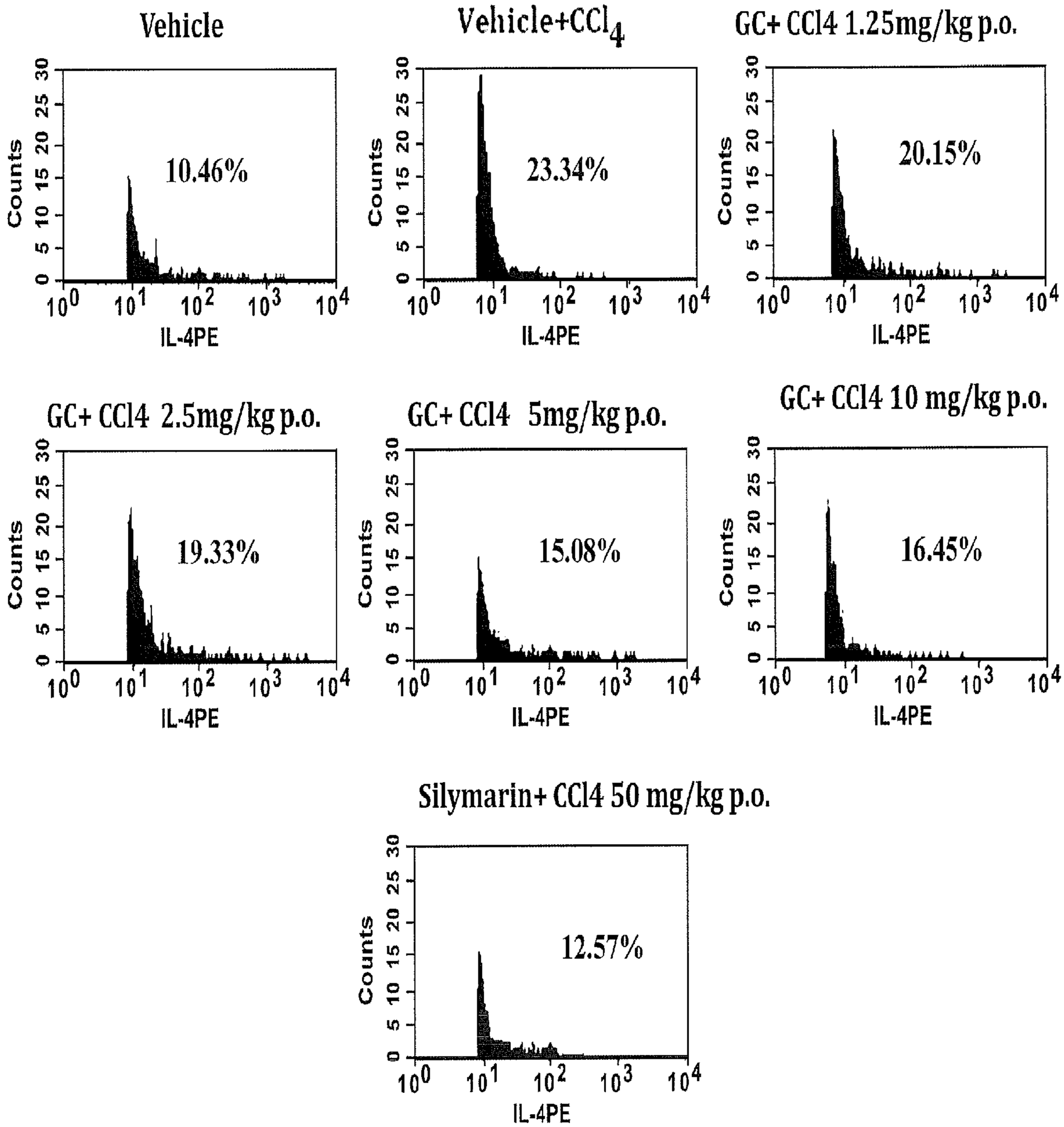

Vehicle
Vehicle+CCl4
GC+ CCl4 1.25mg/kg p.o.
GC+ CCl4 2.5mg/kg p.o.
GC+ CCl4 5mg/kg p.o.
GC+ CCl4 10 mg/kg p.o.
Silymarin+ CCl4 50 mg/kg p.o.
Counts
IL-4PE
10.46%
23.34%
20.15%
19.33%
15.08%
16.45%
12.57%

FIG.7

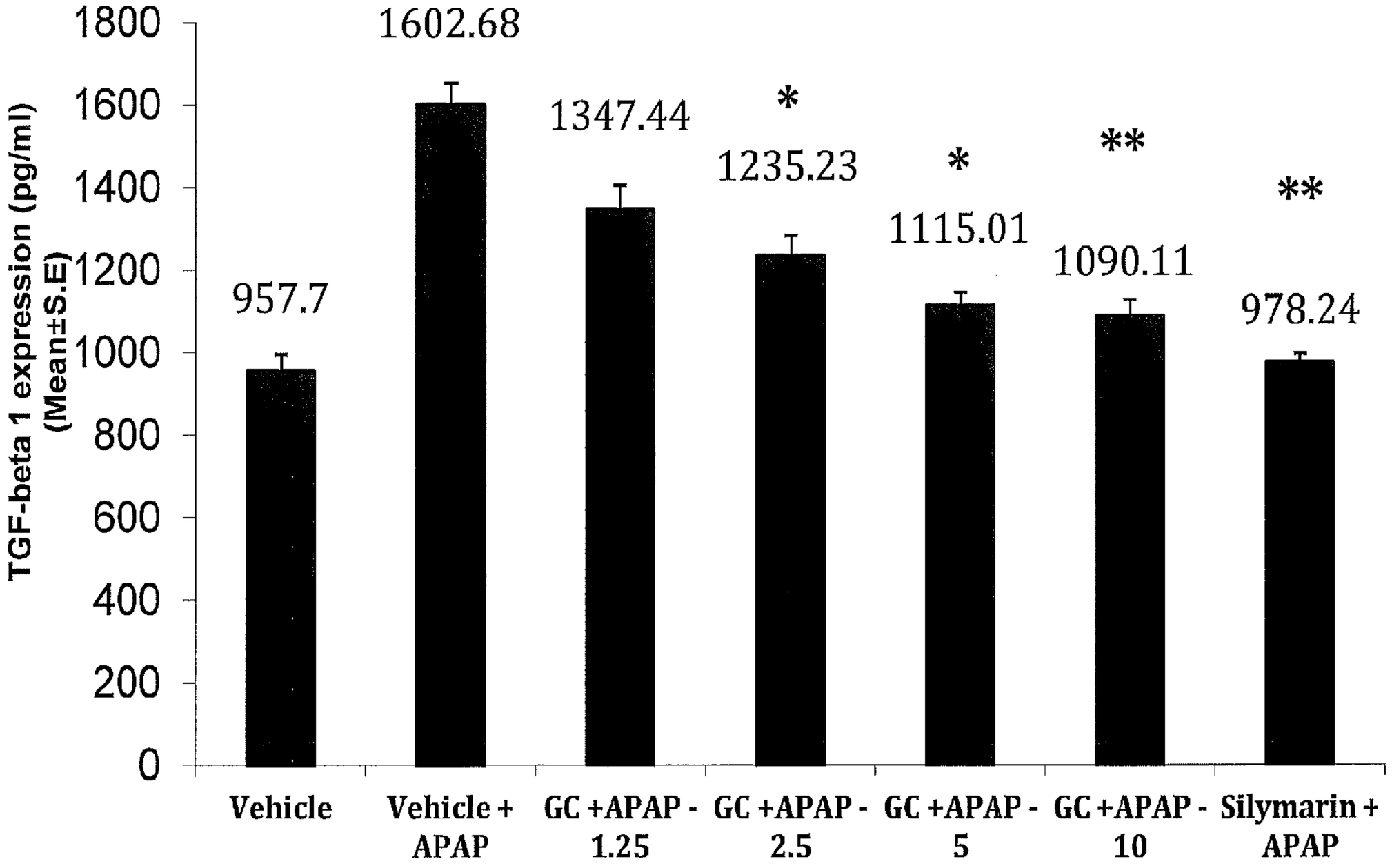

TGF-beta 1 expression (pg/ml) (Mean±S.E)
1800
1600
1400
1200
1000
800
600
400
200
0
957.7
1602.68
1347.44
*
1235.23
*
1115.01
**
1090.11
**
978.24
Vehicle
Vehicle + APAP
GC +APAP - 1.25
GC +APAP - 2.5
GC +APAP - 5
GC +APAP - 10
Silymarin + APAP
[mg / kg] p. o.

FIG.8

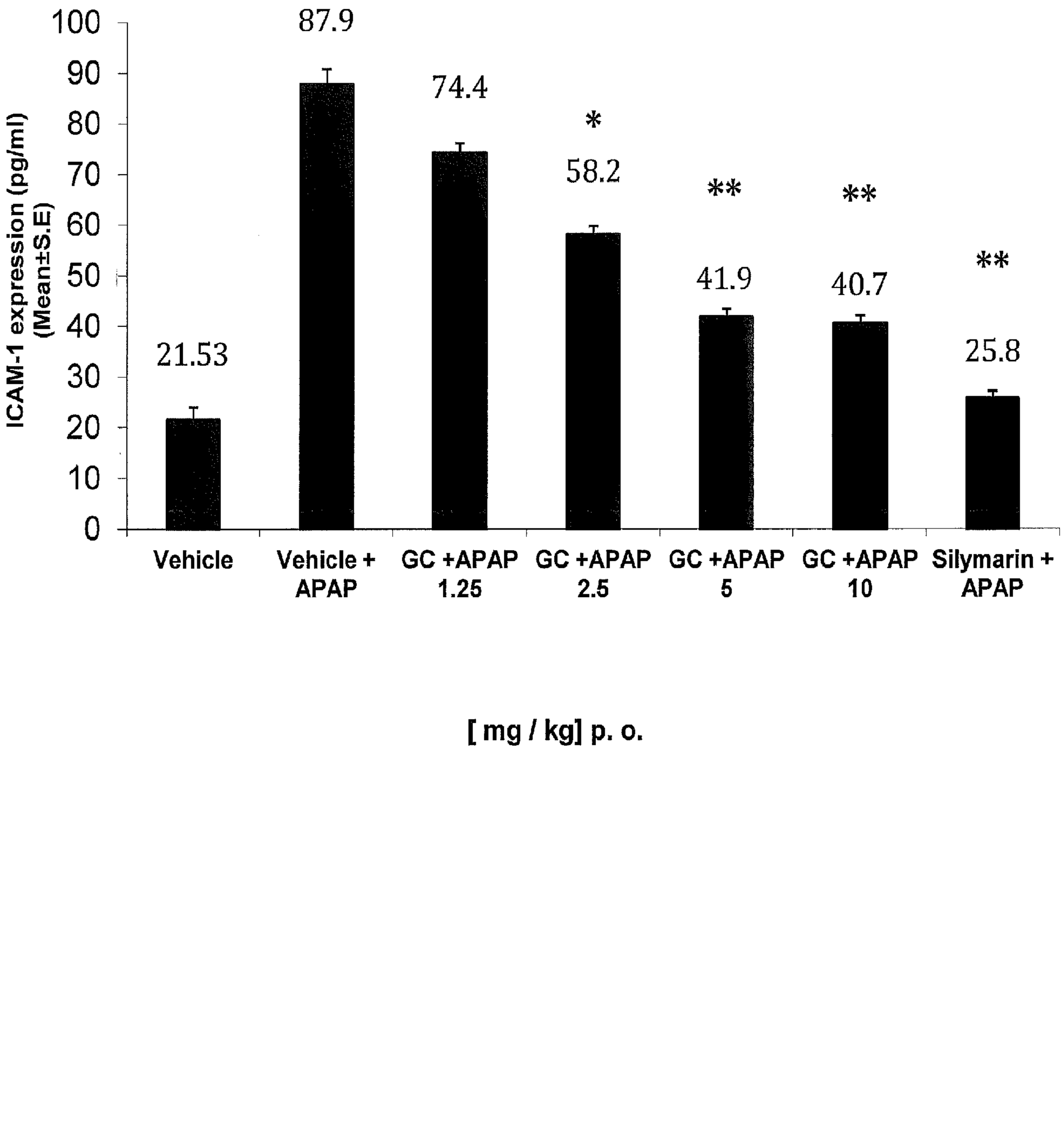

ICAM-1 expression (pg/ml) (Mean±S.E)
100
90
80
70
60
50
40
30
20
10
0
21.53
87.9
74.4
*
58.2
**
41.9
**
40.7
**
25.8
Vehicle
Vehicle + APAP
GC +APAP 1.25
GC +APAP 2.5
GC +APAP 5
GC +APAP 10
Silymarin + APAP
[mg / kg] p. o.

FIG.9

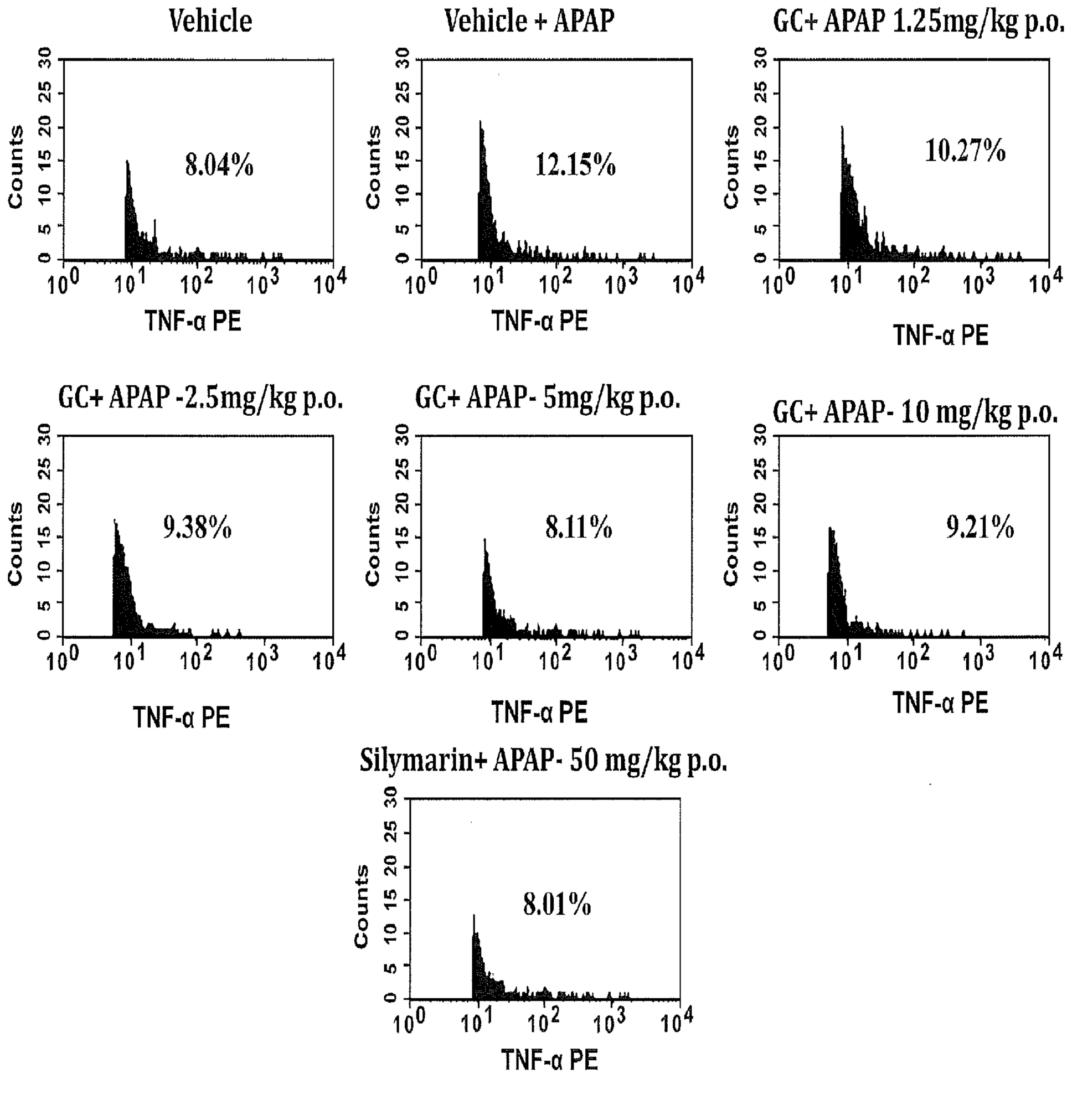

Vehicle
Counts
8.04%
TNF-α PE
Vehicle + APAP
12.15%
GC+ APAP 1.25mg/kg p.o.
10.27%
GC+ APAP -2.5mg/kg p.o.
9.38%
GC+ APAP- 5mg/kg p.o.
8.11%
GC+ APAP- 10 mg/kg p.o.
9.21%
Silymarin+ APAP- 50 mg/kg p.o.
8.01%

FIG.10

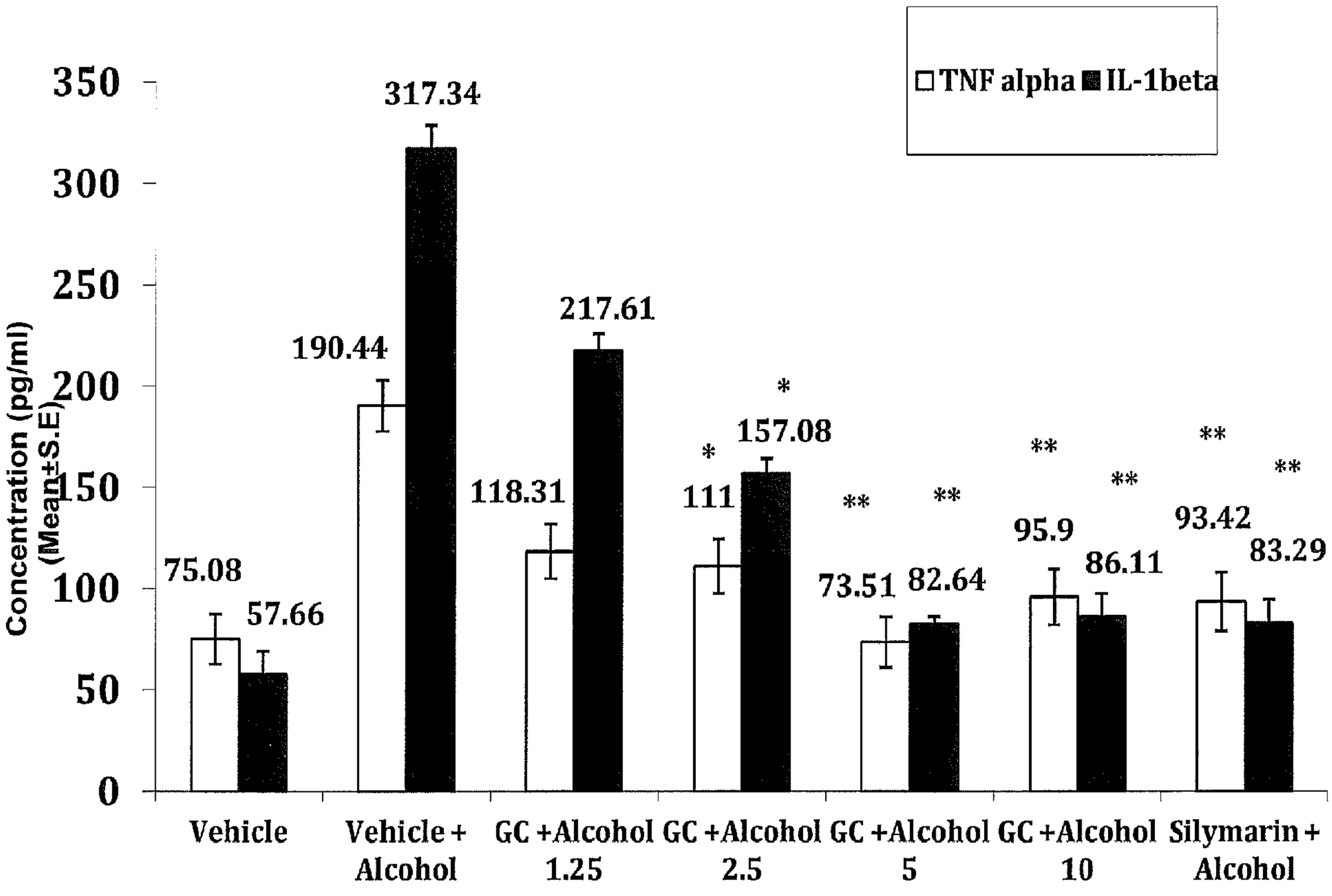

TNF alpha
IL-1beta
Concentration (pg/ml) (Mean±S.E)
350
300
250
200
150
100
50
0
75.08
57.66
190.44
317.34
118.31
217.61
111
157.08
73.51
82.64
95.9
86.11
93.42
83.29
*
**
Vehicle
Vehicle + Alcohol
GC +Alcohol 1.25
GC +Alcohol 2.5
GC +Alcohol 5
GC +Alcohol 10
Silymarin + Alcohol
(mg / kg) p.o

FIG 11

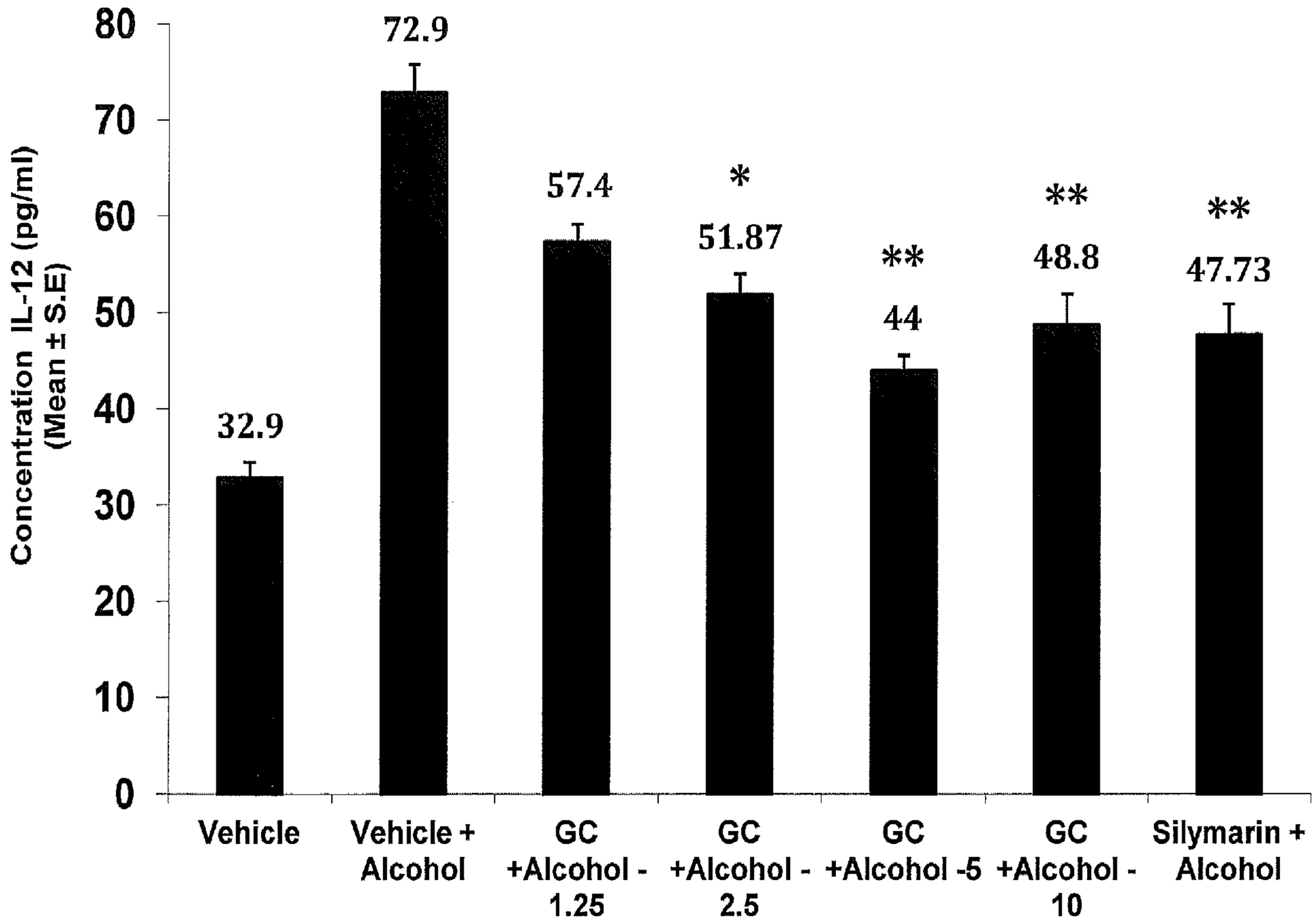
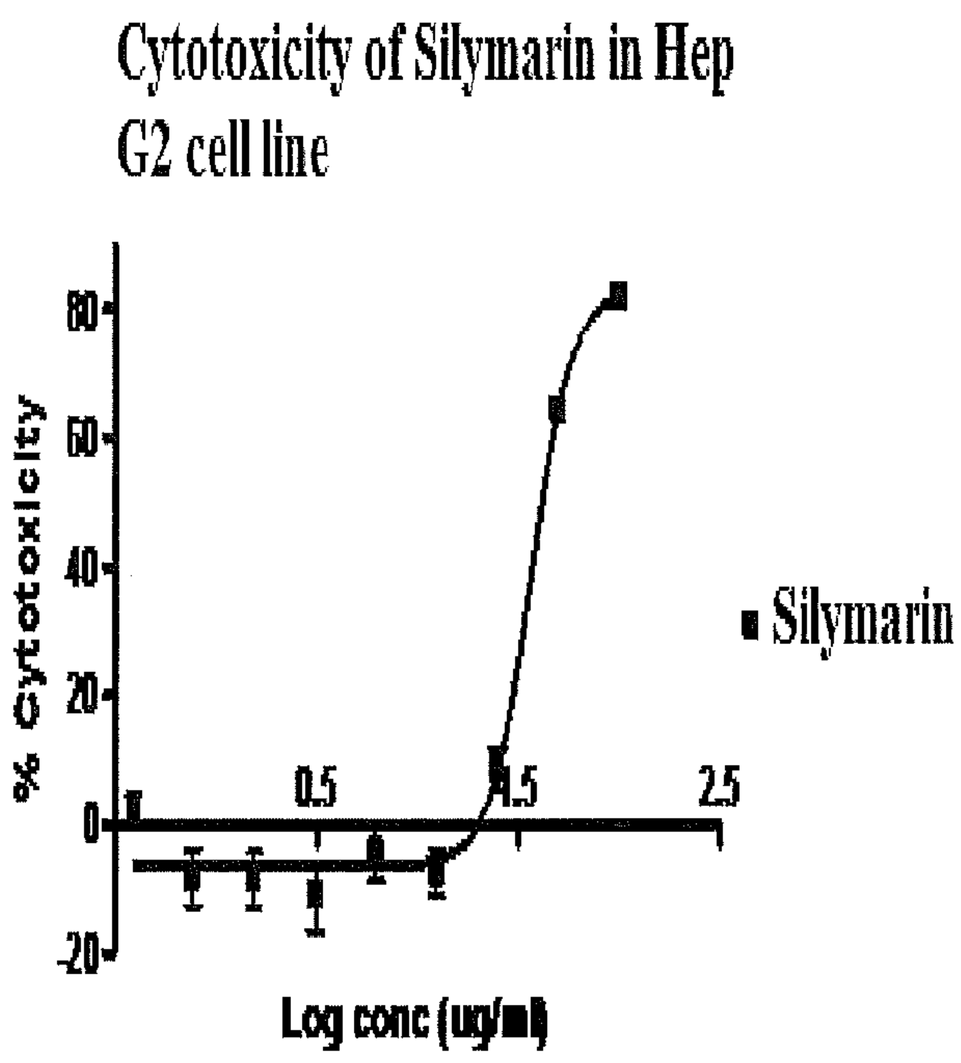

Concentration IL-12 (pg/ml) (Mean ± S.E)
80
70
60
50
40
30
20
10
0
32.9
72.9
57.4
*
51.87
**
44
**
48.8
**
47.73
Vehicle
Vehicle + Alcohol
GC +Alcohol - 1.25
GC +Alcohol - 2.5
GC +Alcohol -5
GC +Alcohol - 10
Silymarin + Alcohol
(mg / kg) p.o

FIG 12


Cytotoxicity of Silymarin in Hep G2 cell line
% Cytotoxicity
80
60
40
20
0
-20
0.5
1.5
2.5
Log conc (ug/ml)
Silymarin

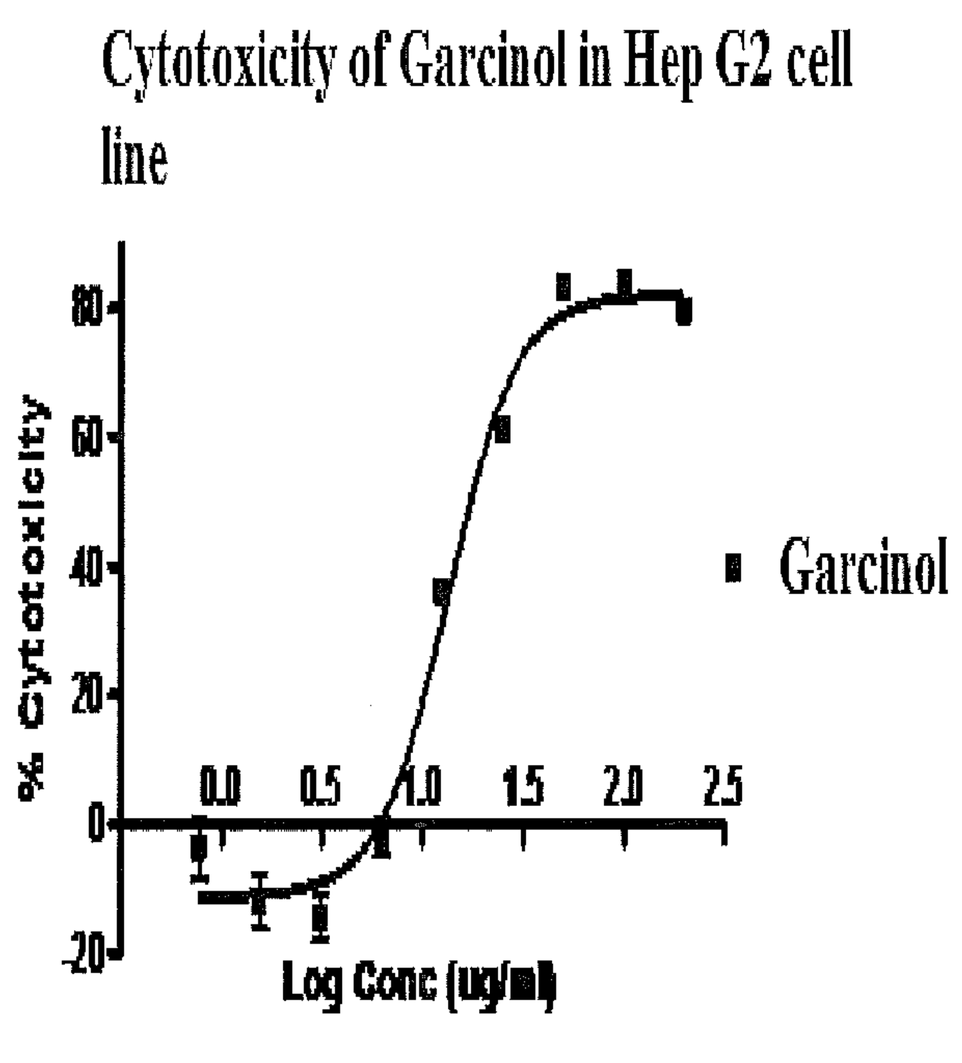
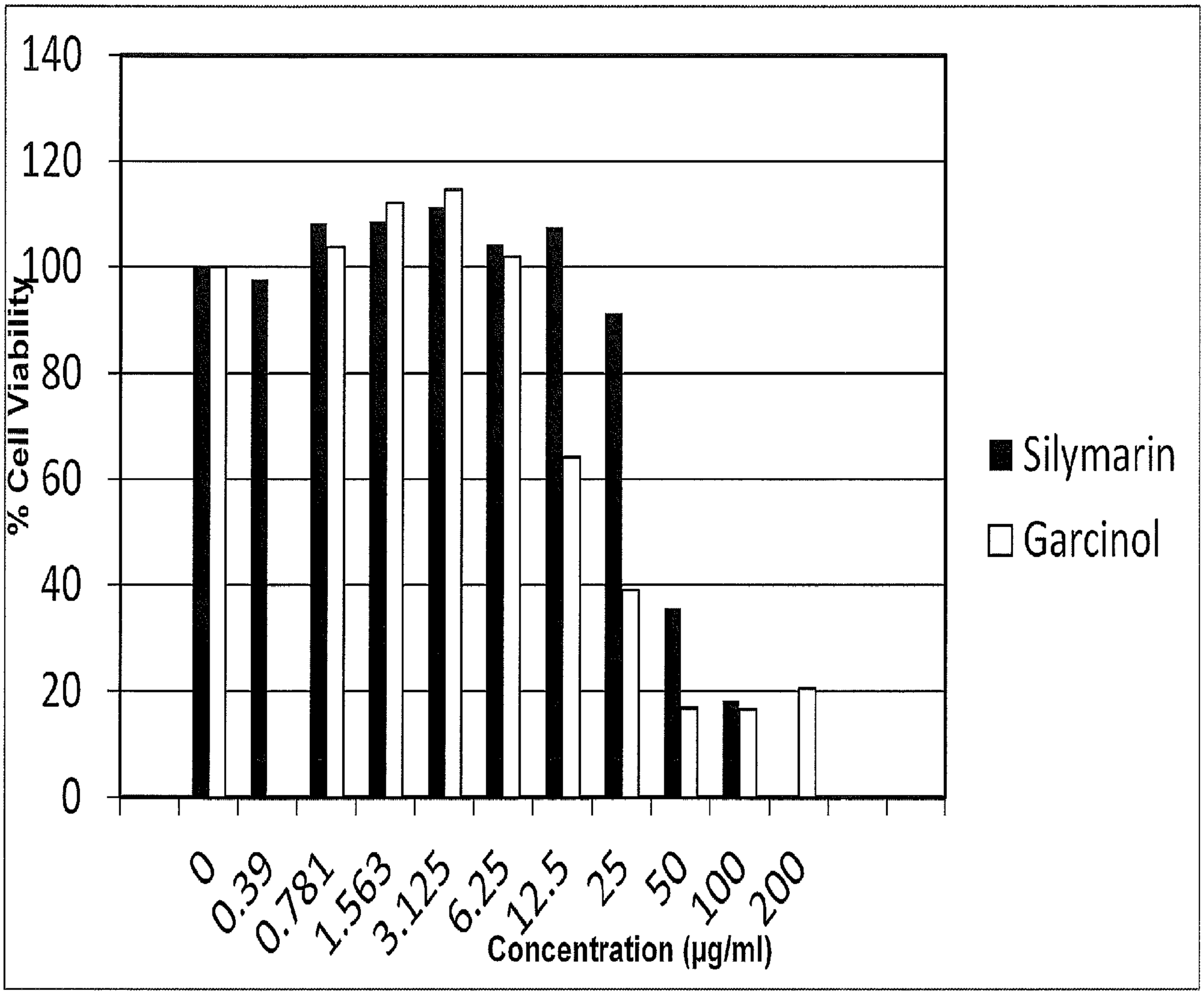

Cytotoxicity of Garcinol in Hep G2 cell line
% Cytotoxicity
80
60
40
20
0
-20
0.0
0.5
1.0
1.5
2.0
2.5
Log Conc (ug/ml)
Garcinol

FIG.13


140
120
100
80
60
40
20
0
% Cell Viability
Silymarin
Garcinol
0
0.39
0.781
1.563
3.125
6.25
12.5
25
50
100
200
Concentration (µg/ml)