Inflammopharmacology 16 (2008) 1–6

DOI 10.1007/s10787-008-8012-0

Inflammopharmacology

Research Article

Modulation of inflammatory mediators by coumarinolignoids from *Cleome viscosa* in female swiss albino mice

D. U. Bawankule[1*], S. K. Chattopadhyay[2], A. Pal[1], K. Saxena[1], S. Yadav[1], U. Faridi[1], M. P. Darokar[1], A. K. Gupta[1] and S. Preet S. Khanuja[1]

[1] Genetic resources and Biotechnology Division, Genetic Resources and Biotechnology Division, Central Institute of Medicinal and Aromatic Plants (CIMAP), Kukrail Picnic Spot Road, Lucknow-226015, India, e-mail: du.bawankule@cimap.res.in
[2] Products and Process Development Division, Central Institute of Medicinal and Aromatic Plants (CIMAP)

Received 4 March 2008; accepted 17 July 2008

Abstract. The effect of coumarinolignoid cleomiscosins A, B and C isolated from the plant *Cleome viscosa* on inflammatory mediators were studied in female swiss albino mice. A mixture of coumarinolignoid A, B, and C at 10, 30 and 100 mg/kg body weight once a day for 14 consecutive days were administered orally to the mice. Pro-inflammatory mediators such as IL-6, TNF-α and Nitric oxide were estimated from culture supernatant obtained from peritoneal macrophages stimulated by LPS and anti-inflammatory mediator IL-4 was estimated from culture supernatant obtained from spleenocytes stimulated by Con-A. For further confirmation, expressions of inflammatory mediators from serum and mortality rate were studied in LPS-induced toxicity model in mice. Pro-inflammatory mediators expression were significantly ($P < 0.05$) decreased in coumarinolignoids treatment group in dose dependent manner, whereas the anti-inflammatory mediator expression was significantly increased in coumarinolignoids at 10 mg/kg treatment. Mortality rate was also significantly reduced in treatment group in LPS-induced toxicity model. The result of this study concluded that the oral administration of coumarinolignoids inhibited the pro-inflammatory mediators and enhances the production of anti-inflammatory mediator in dose dependent manner.

Key words: Coumarinolignoids – Inflammation – Macrophages – Spleenocytes – Mice

* *Corresponding author*

Introduction

Cleome viscosa (Capparidaceae) is an annual herb with yellow flowers and strong penetrating odour, which occurs as a weed in rain fed soils from north east to northern parts of India. In Indian System of Medicine, this plant is widely used as an anti-helminthes (Asolkar et al., 1992), in fever, inflammations, liver diseases, bronchitis and diarrhea (Chatterjee and Pakrashi,1991). The rural people use the fresh juice of the crushed seed for infantile convulsions and in mental disorders (Nadkararni et al., 1992). Systematic investigation on the seeds of *cleome viscosa* has resulted in the isolation coumarinolignoid cleomiscosins A, B, and C. It is a novel class of natural products in which a lignan (C6C3 unit) is linked with a coumarin moiety through a dioxane bridge having hepatoprotective activity (Chattopadhyay et al., 1999).

Pro-inflammatory cytokines and nitric oxide are mediators of various disease conditions (Pinsky et al., 1993 and Corbett *et al.,* 1993). Clinical studies have shown that a sustained or increased release of pro-inflammatory cytokines, such as interleukin-1 (IL-1), interleukin-6 (IL-6), or tumor necrosis factor-α (TNF-α) can contribute to a variety of immunemediated inflammatory conditions such as endotoxin shock (Livingston et al., 1995 and (Jeschke *et al.,* 1999), rheumatoid arthritis (Selzman *et al.,* 1998 and De Maio *et al.,* 1998). Pleural inflammation (Pruitte et al., 1995),nephritis (Williams and Giroir,1995), uveitis (De Waal Malefyt *et al.,*1991) and liver diseases (Kimura *et al.,* 2006). Nitric oxide which is generated from inducible NO synthase (iNOS) participates in immune and inflammatory responses in many tissues (Ialenti *et al.,* 1992 and Kajekar *et al.,* 1995).Thus, the uncontrolled and prolonged action of pro-inflammatory cytokines is potentially dangerous. The physiological

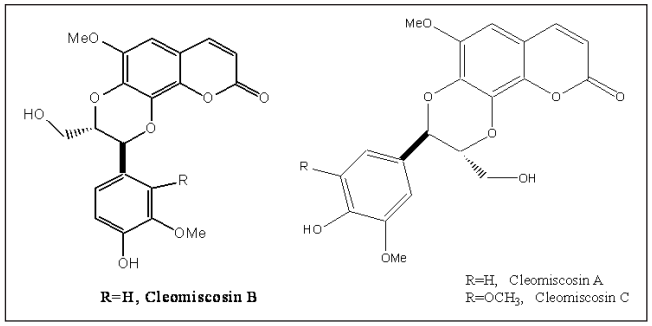

Fig. 1. The molecular structure of the purified of coumarinolignoids cleomiscosins A, B, and C.

counters of pro-inflammatory cytokines are anti-inflammatory cytokines, such as IL-2, IL-4, IL-10, and IFN-γ (Kusske *et al.,* 1996).

The idea behind the present investigation was to study the effect of coumarinolignoids, a hepatoprotective drugs on modulation of inflammatory mediators was based on our previous study, where the coumarinolignoids inhibited the TNF-α production in chronic ethanol induced liver toxicity in mice(data not shown). In the present study, we have studied whether oral administration of a combination of coumarinolignoids A, B, and C modifies the inflammatory mediators in females swiss albino mice. The result shows that coumarinolignoids possesses significant inhibition of the pro-inflammatory mediators and increased in the expression of anti-inflammatory mediator in dose dependent manner.

Materials and Methods

Isolation of coumarinolignoid cleomiscosins A, B, and C from Cleome viscosa *seeds*

Air dried pulverized seeds of *Cleome viscosa* (1 kg) were defatted with petroleum ether(1 liter × 3) for 72 hours. The defatted material was then exhaustively extracted with methanol (1 liter × 3) and concentrated to a small volume (50 ml). It was adsorbed with celite and dried at room temperature for 24 hours which was then packed in a cheese cloth and extracted with toluene followed by ethyl acetate and methanol. The toluene and ethyl acetate fractions were mixed together and concentrated (60–120 mesh) and chromatographed over silica gel (60–120 mesh) in petroleum ether. The column was eluted with mixtures of pet ether– ethyl acetate in the ratio of (1:1) and (1:3) successively. The above two eluants on concentration crystallized out and filtered with pet ether- ethyl acetate (1:1) to give a mixture of coumarinolignoids cleomiscosins 1, 2 and 3. (Fig. 1). The ratio of the three coumarinolignoids, cleomiscosins A, B and C were found to be 42:50:8 by HPLC analysis (Figure No. 2).

Experimental Animals

Inbred female swiss albino mice, weighing 16–21 g, obtained from Jeevanika, CIMAP, and Lucknow were used for this experiment. They were fed with pelleted balanced diet (Dayal feed agency, Lucknow, India) and water *ad libitum*, in an experimental animal room under a 12 h light/dark cycle at a temperature of 22 ± 1°C and a humidity of 60 ± 5%. After 1 week acclimatization, they were used for the experiment. The experimental protocol was approved by the Institutional Animal Ethics Committee for Animal Studies.

Treatment with Coumarinolignoids cleomiscosins A, B, and C

A mixture of coumarinolignoids A, B, and C was suspended in 0.5% carboxymethyl cellulose (Sigma Chemical Co. USA) and administered orally to mice @10, 30 and 100 mg/kg/day for 14 consecutive days. Vehicle control mice were given 0.5% carboxymethyl cellulose (CMC) as the vehicle (0.1 ml/10 g body wt). The animals were euthanised by cervical dislocation 24 hours after the last administration under ether anesthesia for isolation of peritoneal macrophages and spleenocytes. The experiments were conducted as per the method described by Tanaka et al., 1999 and Matsumori et al., 1997 respectively.

Isolation of Peritoneal Macrophages

All procedures were conducted under aseptic conditions. Each group consisted of four mice. Mice were euthanized by cervical dislocation under ether anesthesia and peritoneal exudate cells (PEC) were obtained by intra-peritoneal injection of Phosphate Buffer Saline, pH-7.4 (Sigma Chemicals Co. USA). Macrophages in the PEC suspension were isolated by the cell adhesion method. The PEC were suspended in RPMI 1640 medium (Sigma Chemicals Co. USA) containing 10% fetal calf serum (Gibco, USA) and incubated in a culture plate (Nunc, Germany) for 4 hours at 37 ^{0}C with 5% CO_2 in an incubator. After removing non-adherent cells by removing the medium solution, the adherent cells were resuspended in RPMI 1640 medium. The cells were used for experiments as resident peritoneal macrophages at a concentration of 2×10^6 viable cells/ml.

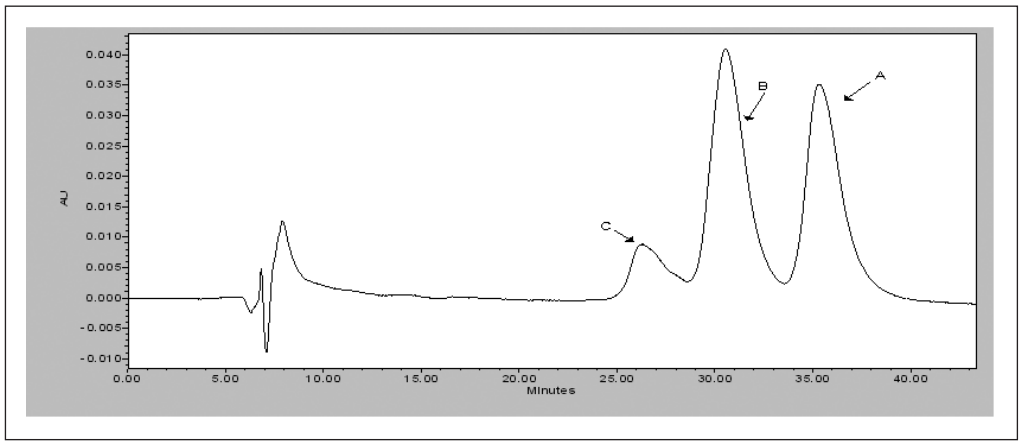

Fig. 2. HPLC chromatogram of the mixtures of cleomisconsins A, B and C (Waters RP 18 column 4,6 × 250, 5µ; solvent system: (AcCN:MeOH) (1:2): (0.5% AcOH un H_2O) in the ratio of 40:60 at a flow rate of 0.4 ml/min.).

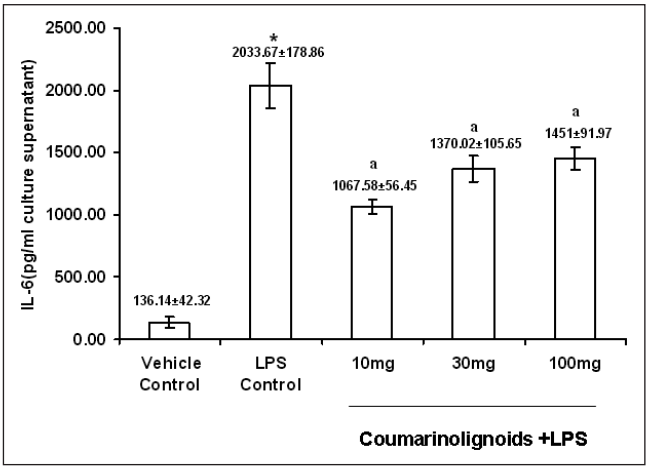

Fig. 3. Effect of coumarinolignoids on IL-6 production from peritoneal macrophages stimulated with LPS.
n = 4, P <0.05,* Vehicle control *vs* LPS, a LPS *vs* Coumarinolignoids + LPS.

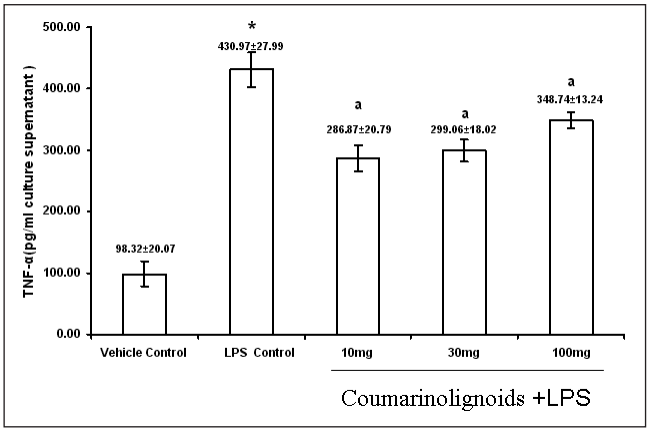

Fig. 4. Effect of coumarinolignoids on TNF-α production from peritoneal macrophages stimulated with LPS.
n = 4, P <0.05,* Vehicle control *vs* LPS, a LPS *vs* Coumarinolignoids + LPS.

Expression of IL-6, TNF-α and Nitric oxide (NO) from Lipopolysaccharide (LPS)-sstimulated Peritoneal Macrophages

Peritoneal Macrophages were cultured at 37 ^{0}C with 5 % CO_2 in humidified air for 48 h with lipopolysaccharide (E.coli 050: B5; Sigma Chemical Co. USA) @1 µg/ml. IL-6 and TNF-α activity in culture supernatants were estimated by Enzyme Immune Assay (EIA) using commercial kits for mouse IL-6 and TNF-α (Pierce Endogen, Rockford, USA) and NO production in culture supernatant was measured using griess reagent (Sigma Chemical Co. USA). The samples were frozen and stored at –80 °C until use.

Isolation of spleenocytes

Mice were euthanized by cervical dislocation under ether anesthesia and a single cell suspension was prepared by pressing the spleen between two slide glasses. The cell suspensions were passed through a 200 gauge stainless steel sieve and then left to stand to remove tissue fragments. The cell suspensions were centrifuged at 600g for 10 minutes and representative pellets were resuspended gently in RPMI 1640 medium. The cell suspensions were adjusted to 2x10^6 viable cells/ml.

Expression of IL-4 from Con-A stimulated spleenocytes

Spleenocytes cells were cultured at 37 ^{0}C with 5 % CO_2 in humidified air for 48 hour with Con-A (5 µg/ml). IL-4 expression in culture supernatants was determined using an Mouse IL-4 EIA kit (Pierce Endogen, Rockford, USA). The samples were frozen and stored at –80 ^{0}C until use.

Effect of Coumarinolignoids on LPS-induced lethal toxicity

To study the effect of coumarinolignoids on LPS-induced lethal toxicity. A mixture of coumarinolignoids were administered orally to mice for 14 consecutive days and 24 hours after the last administration, mice were injected LPS (250 µg/kg body weight) intra-peritoneal rout. Two hours after the LPS treatment blood was collected from orbital plexus of the mice using haematocrit capillaries. The blood samples was allowed to clot at room temperature for one hour and then kept at 4 ^{0}C for 30 minutes followed by centrifugation at 2500 rpm for 10 minutes. Serum sample was collected and store at –80 ^{0}C till further use. Serum sample from each group was pooled for estimation of cytokines and these mice were kept under observation for 24 hours to observe the effect of coumarinolignoids on the LPS induced mortality rate.

Statistical analysis

The data collected were compiled and analyzed using GraphPad Prism 4. Analysis of Variance (ANOVA) followed by bonferroni post-test were used to assess the statistical significance of various groups. Results are presented as the means ± SE. Differences with a P value <0.05 were considered significant.

Results

Effect of Coumarinolignoids on IL-6, TNF-α and Nitric oxide production from LPS- Stimulated Macrophages

The results on IL-6, TNF-α and Nitric oxide expression are illustrated in figure 3, 4 and 5 respectively. IL-6, TNF-α and Nitric oxide expression were significantly increased in LPS stimulated peritoneal macrophages when it compared with the normal peritoneal macrophages which was considered as a normal vehicle control. The expression of IL-6, TNF-α and NO from peritoneal macrophages in treatment group were significantly decreased in dose dependent manner when it is compared with peritoneal macrophages stimulated with LPS alone (P <0.05).

Effect of Coumarinolignoids on IL-4 expression from Concavalin-A (Con-A) stimulated Spleenocytes

The expression level of IL-4 from Spleenocytes stimulated with Con-A in mice treated with coumarinolignoids @ 10 mg/kg body weight were increased significantly when it compared with Con-A stimulated Spleenocytes (P <0.05). The representative data is depicted in figure no 6.

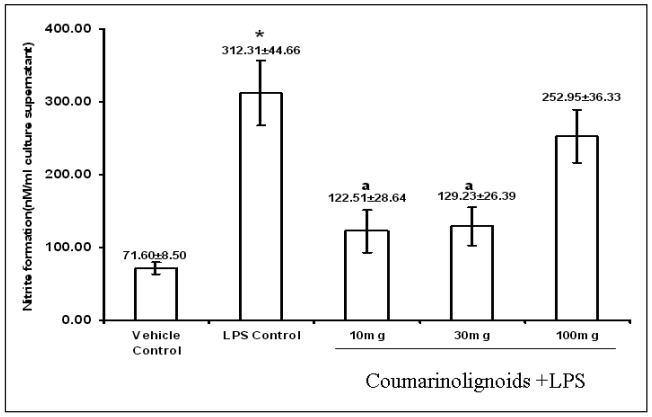

Fig. 5. Effect of coumarinolignoids on Nitride production from peritoneal macrophages stimulated with LPS.
n = 4, P <0.05,* Vehicle control *vs* LPS, a LPS *vs* Coumarinolignoids + LPS.

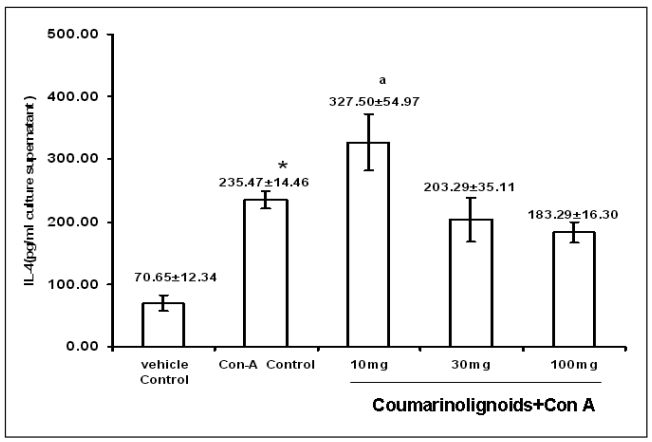

Fig. 6. Effect of coumarinolignoids on IL-4 production from spleenic lymphocytes stimulated with Con-A.
n = 4, P <0.05,* Vehicle control *vs* Con-A, a Con-A *vs* Coumarinolignoids + Con-A.

Table 1. Effect of Coumarinolignoids on LPS-induced lethal toxicity n = 06.

Treatment	% Mortality
Vehicle Control	0.00 (0/6)
LPS Control	100 (6/6)
Coumarinolignoids (10 mg/kg) + LPS	16.66 (1/6)
Coumarinolignoids (30 mg/kg) + LPS	66.66 (4/6)
Coumarinolignoids (30 mg/kg) + LPS	50 (3/6)

Effect of Coumarinolignoids on inflammatory mediators and mortality rate in LPS-induced lethal toxicity model in mice.

The expression of pro-inflammatory cytokines (TNF-α and IL-6) estimated from serum were significantly decreased in coumarinolignoids treated mice in dose dependent manner and the mortality rate was also reduced in coumarinolignoids treated mice. The results are depicted in figure no 7, 8 and Table no.1 respectively.

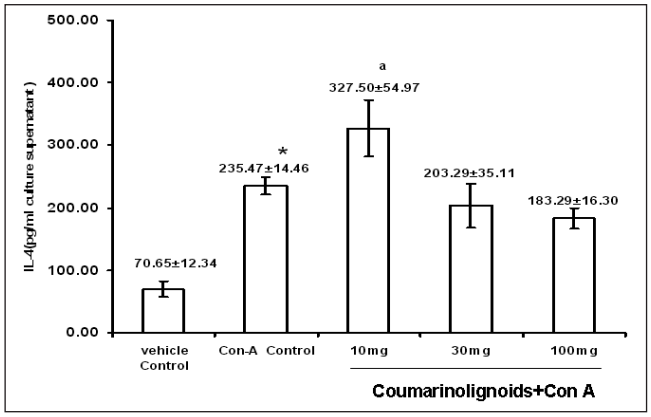

Fig. 7. Effect of coumarinolignoids on TNF-α production from serum isolated from LPS-induced endotoxemia.
n = 4 (Pooled Sample).

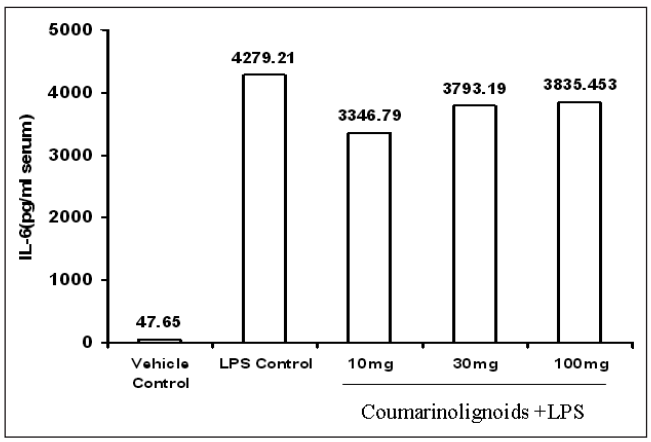

Fig. 8. Effect of coumarinolignoids on IL-6 production from serum isolated from LPS-induced endotoxemia.
n = 4 (Pooled Sample).

Discussion

Many medicinal plants in traditional medicine are believed to be useful in strengthening the immune system of an individual. It may also play an important role in modern health care, particularly where the effective as well safe treatment is not available (Patwardhan et al., 1990). Several Indian Ayurvedic products can reduce chemically induced mammary tumors in rats without any toxicity (Sharma et al., 1990). Lignoid from *Curcuma. longa* exhibits anti-inflammatory, antioxidant, and chemopreventive activities (Yadav et al., 2005) and anti-proliferating and anti-carcinogenic properties (Cui et al., 2006). Several recent studies also reported that plant-derived flavonoids shows in-vitro and in-vivo anti-inflammatory activity by inhibiting the experssion of inflammatory cellular targets at mRNA and protein level (Kim et al., 2004; Wai et al., 2008; Zheng et al., 2008; Sang et al., 2008). In present study we have studied the modulation of inflammatory mediators by coumarinolignoids from *Cleome viscose* in female swiss albino mice.

The result of the study suggested that the oral administration of coumarinolignoid significantly decreased the expression of pro-inflammatory mediators (IL-6, TNF-α and Nitric oxide) and increased the anti-inflammatory (IL-4) cytokines production estimated from macrophages and spleenocytes supernatant in a dose dependent. In LPS-induced lethal toxicity model, pro-inflammatory mediators (TNF-α and IL-6) expression estimated from the serum and mortality rate were also significantly reduced ($P < 0.05$) in dose dependent manner in mice. Our finding is correlated with the previous study that *Allum sativum* (garlic) decreases pro-inflammatory cytokines and increased the anti-inflammatory cytokines (Hodge et al., 2002). Lipopolysaccharide (LPS) is a major constituent of the outer membrane of the Gram-negative microbe. LPS serves as a potent immune system stimulus known to induce various inflammatory mediators. Also, it is well accepted that LPS is responsible for triggering the development of hypotension and ultimate organ failure often associated with severe Gram-negative infections in humans (Mayeux, 1997). The pro-inflammatory cytokines are upregulated in various pathophysiology of diseases and disorders include heart failure (Kurrer et al., 2002), Cerebral malaria (Grau et al., 1989, Miller et al., 1989, Taverne et.al., 1994 and Desouza et al., 2002), hepatitis-B (Chee-Kin and George,2005) and auto-immune diseases (Goldring,1999). Cytokines triggers signal transduction and gene activation cascades that regulate cellular activation, proliferation, differentiation and survival. Thus the functioning of the immune system is finely balanced by the activities of the pro-inflammatory cytokines such as IL-1, IL-6, TNF-α and anti-inflammatory cytokines such as IL-4, IL-10, TGF-β (Jeschke et al., 2002). Among them IL-6, TNF-α and IL-4 are representative cytokines secreted, when the macrophages and Spleenocytes are stimulated with LPS and Con-A respectively (Inaba et al., 1996). Nitric oxide (NO) which is generated from inducible nitric oxide synthase (iNOS) participates in immune and inflammatory responses in many tissues. iNOS, is found in tissues after its induction, such as in response to the inflammatory stimuli (Ialenti et al., 1992). In conclusion, the results suggested that coumarinolignoid cleomiscosins A, B, and C isolated from seeds of *Cleome viscosa* can be used as a supportive therapy to overcome the undesired effect of chemotherapeutic agents used to treat the diseases or disorders and to restore the normal health where the pro-inflammatory cytokines are released.

Acknowledgments. We are thankful to DBT, CSIR New Delhi, National Gene Bank of Medicinal and Aromatic Plants, Faculty, Technical staff and Students of the GRB Division, CIMAP Lucknow India for providing funding, seed material, and technical support respectively during this work.

References

Asolkar, L. V., Kakkar, K. K., Chakre, O. J. (1992). Second supplement to Glossary of Indian medicinal plants with active principles part I (A–K), PID, Dr. K. S. Krishnan Marg, New Delhi 1965–1981, 217.

Chatterjee, A., Pakrashi, S. C. (1991). The treatise on Indian medicinal plants PID. New Delhi: CSIR.

Chattopadhyay, S. K., Thakur, R. S., Patnaik, G. K. Shrimal, R. C. (1999). Indian patent, patent no.182638.

Chee-Kin, H., George, K. K. (2005). Advances in immunomodulating therapy of HBV infection. *Int. J. Med. Sci.* **2**(1), 24–29.

Corbett, J. A., Kwon, G., Turk, J., Mcdanial, M. L. (1993).IL-1β induces the coexpression of both nitric oxide synthase and cyclooxygenase by islet of lagerhans: Activation of cyclooxygenase by nitric oxide. *Biochemistry* **32**(50),13767–13770.

Cui, S. X., Xie, Y. Y., Xie, Y. Y., Nakata, M., Makuuchi, M. and Tang, W. (2006). Curcumin inhibits telomerase activity in human cancer cell lines. *Int. J. Mol. Med.* **18**(2), 227–31.

De Maio, A., de Mooney, M. L., Matesic, L. E., Paidas, C. N., Reeves, R. H.(1998). Genetic component in the inflammatory response induced by bacterial lipopolysaccharide. *Shock* **10**, 319–323.

De Souza, J. B., Riley, E. M.(2002). Cerebral malaria: the contribution of studies in animal models to our understanding of immunopathogenesis. *Microbes Infection* **4**, 291–300.

De Waal., Malefyt, R., Abrams, J., Bennett, B. (1991). Interleukin 10 (IL-10) inhibits cytokine synthesis by human monocytes: an autoregulation role of IL-10 produced by monocytes. *J. Exp. Med.* **174**, 1209–1220.

Goldring, M. B. (1999). The role of cytokines as inflammatory mediators in osteoarthritis: lessons from animal models. *Connect Tissue Res.* **40**, 1–11.

Grau, G. E., Taylor, T. E., Molyneux, M. E.(1989). Tumor necrosis factor and disease severity in children with faciparum malaria. *New England Journal of Medicine* **320**, 1586.

Hodge, G., Hodge, S., Han, P. (2002). *Allium sativum* (garlic) suppresses leukocyte inflammatory cytokine production in vitro: potential therapeutic use in the treatment of inflammatory bowel disease. *Cytometry*; **48**, 209–215.

Ialenti, A., Ianaro, A., Moncada, S., Di Rosa, M. (1992). Modulation of acute inflammation by endogenous nitric oxide. *Eur. J. Pharmacol.,* **211**, 177–82.

Inaba, R., Sugiura, H., Iwata, H., Mori, H., Tanaka, T. (1996). Immunomodulation by Maharishi Amrit Kalash in mice. *J. Appl. Nutr*, **48**, 10–21.

Jeschke, M. G., Wolf, S. E., DebRoy, M. A., Herndon, D. N. (1999). The combination of growth hormone with hepatocyte growth factor alters the acute phase response. *Shock* **12**, 181–187.

Jeschke, M. G., Barrow, R. E., Suzuki., F., Rai, J., Benjamin, D., Herndon, D. N. (2002). IGF-I/IGFBP-3 equilibrates ratios of pro- to anti-inflammatory cytokines, which are predictors for organ function in severely burned pediatric patients. *Molecular Medicine* **8**(5), 238–246.

Kajekar, R., Moore, P. K., Brain, S. D.(1995). Essential role for nitric oxide in neurogenic inflammation in rat cutaneous microcirculatio n;Evidence for an endothelium-independent mechanism. *Circ. Res.* **76**, 441–7.

Kim, H. P., Son, K. H., Chang, H. W., Kang, S. S. (2004). Anti-inflammatory plant flavonoids and cellular action mechanisms. *J Pharmacol Sci,.***96**(3), 229–45.

Kimura, K., Moriwaki, H., Nagaki, M., Saio, M., Nakamoto, Y., Naito, M., Kuwata, K., Chisari, F. V. (2006).Pathogenic role of B cells in anti-CD40-induced necroinflammatory liver disease. Am J Pathol. **168**(3), 786–95.

Kurrer, M. O., Kopf, M., Penninger, J. M., Eriksson, U. (2002). Cytokines that regulate autoimmune myocarditis. *Swiss Med Wkly* **132**, 408–13.

Kusske, A. M., Rongione, A. J., Reber, H. A.(1996). Cytokines and acute pancreatitis. *Gastroenterology,* **110**, 639–642.

Livingston, D. H., Mosenthal, A, C., Deitch, E. A. (1995).Sepsis and multiple organs dysfunction syndrome: a clinical-mechanistic overview. *New Horizons.* **3**, 276–287.

Matsumori, A., Ono, K., Nishio, R., Igata, H.,Shioi S., Furukawa Y., Iwasaki, A., Nose, Y., Sasayama, S. (1997). Modulation of Cytokine Production and Protection against lethal endotoxemia by the cardiac glycoside ouabain. *Circulation*, **96**(5), 1501–1506.

Mayeux, P. R. (1997). Pathobiology of lipopolysaccharide. *Journal Toxicology Environmental Health.* **51**, 415–435.

Miller, K. L., Silverman, P. H., Kullgren, B., Mahlmann, L. J. (1989). Tumor necrosis factor alpha and the anemia associated with murine malaria. *Infection and Immunity.* **57**, 1542–1546

Nadkararni, K. M., Nadkarani, A. K.(1992). Indian materia medica, vol 1. Bombay: *Prakashan,* **498**.

Pinsky, M. R., Vincent, J. L., Deviere, J. (1993). Serum cytokine levels in human septic shock: relation to multiple system organ failure and mortality. *Chest.* **103**, 565–575.

Pruitte, J. H., Copeland, E. M., Moldawer., L. L.(1995). Interleukin-1 and interleukin-1 antagonism in sepsis systemic inflammatory response syndrome and septic shock. *Shock* **3**, 35–251.

Sang, K. H., Pyeongjae, L., Jeong, A. P. et al. (2008). Apigenin inhibits the production of NO and PGE2 in microglia and inhibits neuronal cell death in a middle cerebral artery occlusion-induced focal ischemia mice model. *Neurochemistry International.* **52**(4–5), 878–886

Selzman, C. H., Shames, B. D., Miller, S. A.(1998). Therapeutic implications of interleukin-10 in surgical disease. *Shock* **10**, 309–318.

Sharma, H. M., Dwivedi, C., Satter, B. C., Gudenithlu, K. P., About-Issa, H., Malarkey, W., Tejwani, G. A.(1990). Antineoplastic properties of MAK-4 against DMBA-induced mammary tumors in rats. P*harmacol Biochem. Behav*. **35**, 1–7.

South, E. H., Exon, J. H., Hendrix, K. (1997). Dietary curcumin enhances antibody response in rats. *Immunopharmacol. Immunotoxicol*. **19**, 105–119.

Tanaka, T., Sugiora, H., Inaba, R., Koshimizu, K., Ohigashi, H. (1999). Immunomodulatory action of citrus acuraptene on macrophage-functions and cytokine production of lymphocytes in female BALB/c mice. *Carcinogenesis.* **20**(8), 1471–76.

Taverne, J., Sheikh, N., de Souza, J. B. (1994). Anemia and resistance to malariin transgenic mice expressing human tumor necrosis factor. *Immunology* **82**, 397.

Wai M. L., Julie M. P., Scott S., Allan, J. M., Paul, W. N., Paul, A. K., Jonathan, M. H., Kevin, D. C. (2008). Metabolic transformation has a profound effect on anti-inflammatory activity of flavonoids such as quercetin: Lack of association between antioxidant and lipoxygenase inhibitory activity. *Biochemical Pharmacology*, **75**(5), 1045–1053.

Williams, G., Giroir, B. (1995). Regulation of cytokine gene expression: tumor-necrosis factor, interleukin-1, and the emerging biology of cytokine receptors. *New Horizons* **2**, 276–287.

Yadav, V. S., Mishra, K. P., Singh, D. P., and Singh, V. K. (2005). Immunomodulatory effect of curcumin: *Immunopharmacol and Immunotoxicol.* **27** (3), 465–97.

Zheng, L. T., Jiyeon Ock J., Kwon B. M., Kyoungho, Suk. (2008). Suppressive effects of flavonoid fisetin on lipopolysaccharide-induced microglial activation and neurotoxicity. *International Immunopharmacology*. **8**(3), 484–494.