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Effect of Biphenyl Dimethyl Dicarboxylate on the Humoral Immunosuppression by Ketoconazole in Mice

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The present study was undertaken to investigate the effect of biphenyl dimethyl dicarboxylate (PMC) on the humoral immunosuppression by ketoconazole (KCZ) in ICR mice. PMC at a dose of 6 mg/kg was administered orally to mice daily for 14 consecutive days. KCZ was suspended in RPMI 1640 medium and orally administered at 160 mg/kg/day 2 hrs after the administration of PMC. Mice were immunized and challenged with sheep red blood cells (SRBC). The results of the present study are summarized as follows; a gain of body weight and relative weights of spleen and liver were significantly increased by combination of PMC and KCZ, as compared with those in mice treated with KCZ alone. Splenic plaque forming cells (PFC) and hemagglutination (HA) titers to SRBC were greatly enhanced by the combination of PMC and KCZ, compared with treatment of KCZ alone. The elevation of serum glutamic-pyruvic transaminase (S-GPT) and total protein levels caused by KCZ were reduced to normal level by the combination of PMC and KCZ. In addition, lower serum albumin and A/G ratio were also increased to normal level. These findings indicate that PMC has a protective effect against KCZ-induced humoral immunosuppression.

Key words : Biphenyl dimethyl dicarboxylate, Ketoconazole, Humoral immune response

INTRODUCTION

Ketoconazole (KCZ) is an imidazole antifungal agent which inhibits the biosynthesis of fungal cell-membrane ergosterol (Borgers, 1980; Van den Bossche *et al.*, 1980). It is an orally active agent with a broad spectrum antifungal activity (Levine and Cobb, 1978; Bisschop *et al.*, 1979; Dixon *et al.*, 1980; Hay *et al.*, 1980). Nevertheless, it has been shown to interfere with lymphocytic functions (Corbeel *et al.*, 1984; Gergely *et al.*, 1984; Vuddhakul *et al.*, 1988). Buttke and Chapman (1983) reported that KCZ also reduced B-lymphocyte proliferation in response to lipopolysaccharide in BALB/c mice. It has been further found that the systemic antifungal agents available for clinical use exert humoral immunotoxicity (Thong, 1986; Terrell and Hermans, 1987). Thus, it would be important to abrogate the deleterious effects of these antifungal agents using agents with a more immunobiological action as well as little adverse side-effects.

Biphenyl dimethyl dicarboxylate (PMC) is a substance derived from the synthesis of *Schizandra sp.* constituents (Xie, 1982) which have been utilized for tonic and sedative purposes in traditional Chinese medicine for over 2,000 years, and recently for antioxidative action (Toda *et al.*, 1988) and for liver-protective purposes (Maeda *et al.*, 1981, 1982; Hikino *et al.*, 1984; Kiso *et al.*, 1985; Takeda *et al.*, 1985). PMC has been shown to be effective in treating or preventing chronic hepatitis due to drug poisoning (Yu *et al.*, 1987). PMC was also found to protect mice against CCl_4-induced liver damage, and to increase hepatic microsomal cytochrome P-450 in rats (Liu *et al.*, 1981; Xie *et al.*, 1981). It was previously reported that oral administration of PMC to ICR mice can restore the suppression of humoral immunity produced by CCl_4 (Ahn and Kim, 1993). It was found that PMC was also capable of stimulating serum antibody production after immunization with ovalbumin (OVA) in complete Freund's adjuvant in BALB/c mice (Kim *et al.*, 1995). Thus, it can be considered that PMC modulates the humoral immunosuppressive effects of KCZ without adverse side-effects, suggesting that PMC enhances total serum immunoglobulin levels in experimental animals.

The present study was undertaken, therefore, to investigate the effects of PMC on the humoral immunosuppression by oral KCZ in ICR mice.

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MATERIALS AND METHODS

Animals

Male ICR mice of 6 weeks old were purchased from Sam Yuk Laboratory Animal, Korea. Animals were housed individually in each cage and acclimatized for at least 7 days prior to use. The cages were maintained at 23±2°C and 50±5% of relative humidity throughout the whole experimental period. All experimental mice were fed with animal chows (Jeil Ind. Ltd, Korea) and tap water *ad libitum* but deprived of the animal chows for 16 hrs prior to sacrifice.

Materials and treatments

PMC (Bimethyl-4,4'-dimethoxy-5,6,5',6'-dimethylene-dioxybiphenyl-2,2'-dicarboxylate) was supplied by Dongkwang Pharmaceutical Co., Korea and suspended in 2% starch solution. PMC (6 mg/kg) was administered orally to mice daily for 14 consecutive days. Ketoconazole (KCZ; Dongkwang Pharmaceutical Co., Korea) was suspended in RPMI 1640 medium and orally administered at 160 mg/kg, 2 hrs after the administration of PMC, daily for 14 consecutive days. Control animals received the appropriate vehicle only and were treated at the same time as the corresponding experimental animals.

Lymphoid organ and body weights

Mice were sacrificed by cervical dislocation on the next day after the last PMC treatment. Liver and spleen were separated and weighed. The lymphoid organ weight ratio to body weight was calculated for each mouse.

Antigen preparation

Sheep red blood cells (SRBC) collected from a single female sheep were kept at 4°C in sterile Alserver's solution (pH 6.1). SRBC were washed three times with phosphate-buffered saline (PBS, pH 7.4) after centrifugation at 400×g for 10 minutes and adjusted to provide a desired concentration by hemacytometer count.

Immunization

All mice were immunized by intravenous (i.v.) injection of 0.1 ml of SRBC suspension (1×10^8 cells/ml) 4 days prior to each assay as described by Lake and Reed (1976).

Preparation and inactivation of serum

The blood sample of each mouse was obtained from the carotid artery. The blood was allowed to clot in polyethylene tubes at 4°C for 30 minutes and then centrifuged at 700×g for 20 minutes. The serum was withdrawn and heat-inactivated in polyethylene tubes at 56°C for 30 minutes.

Preparation of spleen cells

Mice were killed by cervical dislocation and their spleens were removed aseptically. Cell suspensions from spleens were prepared in complete medium (RPMI-1640 medium supplemented with 100 unit penicillin/ml, 100 μg streptomycin, and 2 mM L-glutamine) by the modified method of Mishell *et al.* (1980). The Cells were counted and the viability was determined by trypan blue exclusion test.

Hemagglutination (HA) titer

HA titer was determined in microtitration trays (Limbro Chemical Co., Inc. New Haven, Connecticut, U.S.A.) using 25 μl volume of diluent by serial dilution of inactivated pooled sera in Hank's balanced salt solution (HBSS), which was added to 50 μl volume of 0.5% packed SRBC, as described by Yoshikai *et al.* (1979). For HA titer assay, the specific plate (Flow Lab., U.S.A.) of serum and SRBC mixture was incubated for 18 hrs at 37°C. Each titration was performed in duplicate, and the mean titer was expressed as $\log_2$.

2-Mercaptoethanol resistant (2-MER) HA titer

HA titer assay in the serum treated with 2-mercaptoethanol was the same method as described previously, except that the serum was diluted with HBSS containing 0.15N 2-mercaptoethanol instead of HBSS alone. Each titration was performed in duplicate, and the mean titer was expressed as $\log_2$.

Assay of plaque forming cells (PFC)

In order to examine whether this PMC accelerates the antibody production to heterologous antigen or not, the slide technique of Cunnigham and Szenberg (1968) was utilized. The number of direct PFC was counted 4 days after the i.v. immunization by with 10^7 SRBC. The plaques were expressed either as those per 10^6 viable spleen cells or per spleen.

Serum chemistry

Glutamic-pyruvic transaminase (SGPT) levels were determined according to the method described by Reitman and Frankel (1957). Briefly, mice were anesthetized using ether. Blood was subsequently collected by cardiac puncture and plasma was immediately separated by centrifuge. The plasma was used for the estimation of SGPT using Sigma Kit 505-OP. Protein was determined by the method of Lowry *et al.* (1951) with bovine serum albumin as the standard.

Statistical analysis

The values were expressed as means±standard error (S.E.). All data were examined for their statistical significance of difference with Student's *t*-test.

RESULTS

The effect of PMC on body and selected organ weights in KCZ-treated mice is summarized in Table I. A gain in body weight was significantly decreased in KCZ-treated mice, but the decrease in body weight was significantly prevented by the combined administration of KCZ and PMC. The relative spleen weight was significantly decreased in KCZ-treated mice, compared with that in control, but the decrease was significantly prevented by the combined administration of KCZ and PMC (Table I). The relative liver weight of KCZ-treated mice was significantly decreased by about 23%, as compared with that in controls ($P<0.05$). Meanwhile, the relative liver weights of mice treated with PMC together with KCZ was elevated by 51%, as compared with that of KCZ-treated mice ($P<0.01$) (Table I).

The effect of PMC on HA titer and 2-MER HA titer in KCZ-treated mice are summarized in Fig. 1. HA titer of serum to SRBC was significantly decreased in KCZ-treated mice, compared with that in control mice. But HA titer in mice treated with PMC together with KCZ was markedly increased. In the 2-MER HA titer, 2-MER HA titers to SRBC of KCZ-treated mice was also significantly decreased by about 44% as compared with those in control mice ($P<0.01$), but the decrease was significantly prevented by the combined administration of KCZ and PMC ($P<0.01$). This result indicates that PMC can restore antibody responses suppressed by KCZ. As shown in Fig. 2, plaque forming cells (PFC) per 10^6 spleen cells of KCZ-treated mice were significantly decreased by about 30% as compared with those in control ($P<0.01$), but the decrease in PFC per 10^6 spleen cells was significantly restored by the combined

Table I. The effect of biphenyl dimethyl dicarboxylate on body and organ weights in ketoconazole-treated mice

Group	Percentage of body weight		
	Body wt. gain (%)	spleen	Liver
Control	13.78±0.47	0.61±0.06	5.82±0.55
Ketoconazole	4.40±0.28**	0.41±0.04*	4.47±0.19*
Ketoconazole+PMC	8.95±0.12§§	0.74±0.05§§	6.75±0.34§§
PMC	12.90±0.78	0.58±0.03	6.55±0.21

Biphenyl dimethyl dicarboxylate (PMC; 6 mg/kg) was administered orally to ICR mice daily for 14 consecutive days. Ketoconazole was prepared by suspension in RPMI 1640 medium and administered at 160 mg/kg orally 2 hrs after the administration of PMC daily for 14 consecutive days. Mice were immunized i.v. with 10^7 SRBC 4 days prior to each measurement. Each value represents the mean±S.E. of 6 to 7 mice. Significantly different from control at $*P<0.05$ and $**P<0.01$. Significantly different between ketoconazole and ketoconazole plus PMC groups at §§$P<0.01$

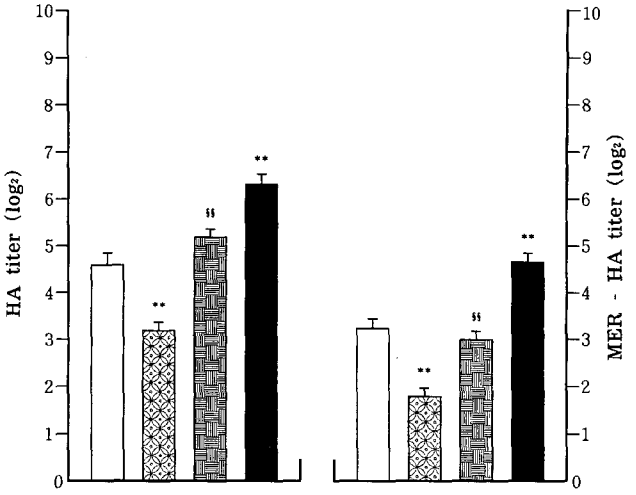

Fig. 1. The effect of biphenyl dimethyl dicarboxylate on hemagglutination titer in ketoconazole-treated mice. □ Control; ▨ Ketoconazole; ▫ Ketoconazole and biphenyl dimethyl dicarboxylate; ■ Biphenyl dimethyl dicarboxylate. Mice were immunized i.v. with 10^7 SRBC 4 days prior to each assay. Values are means±S.E. of 6 to 7 mice. Significantly different from control at $**P<0.01$. Significantly different from ketoconazole alone at §§$P<0.01$

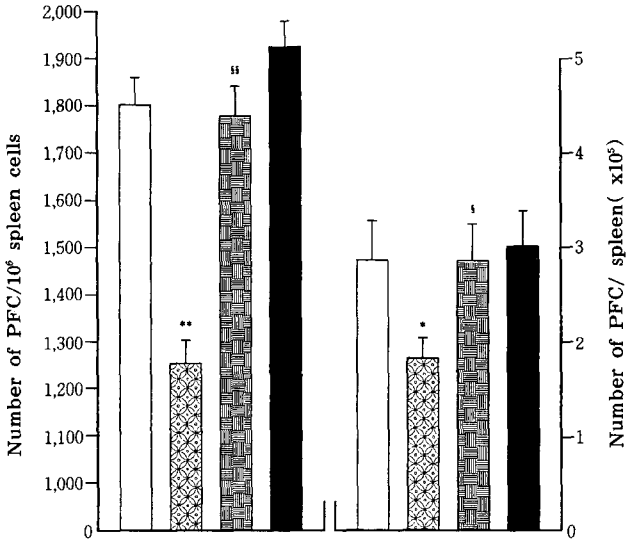

Fig. 2. The effect of biphenyl dimethyl dicarboxylate on splenic plaque forming cell in ketoconazole-treated mice. □ Control; ▨ Ketoconazole; ▫ Ketoconazole and biphenyl dimethyl dicarboxylate; ■ Biphenyl dimethyl dicarboxylate. Mice were immunized i.v. with 10^7 SRBC 4 days prior to each assay. Values are means±S.E. of 6 to 7 mice. Significantly different from control at $*P<0.05$ and $**P<0.01$. Significantly different from ketoconazole alone at §$P<0.05$ and §§$P<0.01$

Table II. The effect of biphenyl dimethyl dicarboxylate on serum chemistry in ketoconazole-treated mice

Group	GPT (IU/ml)	Albumin (g/dl)	Protein (g/dl)	A/G ratio
Control	38.20±3.32	4.18±0.33	6.98±0.13	1.49±0.08
Ketoconazole	64.80±5.88**	3.38±0.16*	8.04±0.36*	0.73±0.14**
Ketoconazole+PMC	38.00±5.68§§	4.02±0.17§	6.82±0.18§§	1.44±0.11§§

Abbreviations: Biphenyl dimethyl dicarboxylate, PMC. Each value represents the mean±S.E. of 6 to 7 mice. Significantly different from control at *P<0.05 and **P<0.01. Significantly different between ketoconazole and ketoconazole plus PMC groups at $^{§}P$ <0.05 and $^{§§}P$<0.01

administration of KCZ and PMC (P<0.01). The PFC per spleen also showed similar alteration (Fig. 2).

The effects of PMC on serum chemisty is shown in Table II. The elevated SGPT and total protein levels were significantly reduced to normal value by the combined administration of KCZ and PMC (*i.e.*, approximately 41% and 15%, P<0.01 as compared with KCZ-treated mice, respectively). In addition, lower serum albumin level and A/G ratio were also restored to normal value.

Thus, PMC is thought to inhibit KCZ-induced hepatic damage such as the degenerations of endoplasmic reticulum of liver cells associated with immune function.

DISCUSSION

The objective of this study was to investigate the protective effects of PMC against the humoral immunosuppression of KCZ. We selected the toxic dose of 160 mg/kg from the toxicological studies of KCZ, which had been previously reported by Lavrijsen *et al.* (1986). On the other hand, PMC at 6 mg/kg is known to be nontoxic and quite effective in enhancing antibody production, without causing any adverse side-effects (Kim *et al.*, 1995).

The humoral immune functions of KCZ or/and PMC-treated mice were evaluated by the T-dependent antigen, SRBC. The *in vivo* T-dependent antibody response to SRBC, which requires functionally competent B cells, T cells, and macrophages (Mosier and Coppelson, 1968; Claman and Mosier, 1976), was most affected by KCZ treatment. Previous studies of KCZ had indicated that KCZ did not affect serum immunoglobulin levels, serum C_3 and C_4 complement levels and total haemolytic complement at therapeutic concentrations for a short period in humans (Van Rensburg *et al.*, 1983) but dose-dependently reduced *in vitro* B-lymphocyte proliferation in response to lipopolysaccharide in BALB/c mice (Buttke and Chapman, 1983). Marshall *et al.* (1981) further reported that KCZ markedly suppressed lymphocyte transformation induced by all three mitogens (namely phytohaemagglutinin A, concanavalin A and pokeweed mitogen) in a dose-dependent manner. It was shown, however, that PMC significantly recovered humoral immune response to SRBC in CCl_4-immunosuppressed mice (Ahn and Kim, 1993), and that it significantly enhanced antibody forming response to ovalbumin in BALB/c and C3H/HeN mice (Kim *et al.*, 1995). The present study suggests that KCZ suppresses antibody production as demonstrated in the decrease of both splenic PFC and HA titer (Figs 1 and 2). In contrast, PMC combined with oral KCZ significantly enhanced splenic PFC and HA titer as well as spleen weight as compared with those in immunosuppressed mice by KCZ alone (Table I, Figs 1 and 2). This indicates that PMC has a restorative effect against oral antifungal agent-induced suppression of humoral immune response.

In vivo studies were performed to determine whether PMC also would result in a significant recovery of the hepatotoxicity produced by KCZ. The basis of selecting PMC for these studies was previously published in a report by Xie *et al.* (1981), in which they demonstrated that pretreatment of mice with PMC resulted in the abrogation of CCl_4-induced liver damage. The present study has further shown that PMC combined with KCZ significantly decreased serum GPT levels and total serum protein level elevated by KCZ alone. Serum albumin levels and A/G ratio as well as liver weight were also found to be significantly increased (Tables I and II). Then, it is thought that PMC may prevent hepatic damage such as the degenerations of endoplasmic reticulum of liver cells by KCZ, as discussed in the enhancement of reticuloendothelial system associated with immune function through the inhibition of liver damage induced by $CC1_4$ in mice (Ahn and Kim, 1993).

In summary, PMC ameliorated the adverse effects of KCZ treatment. As a result of the finding that PMC reduced the humoral immunosuppression induced by KCZ, we are now performing further studies on KCZ in cell-mediated immune responses and non-specific host defenses. While further immunological studies of the effects of PMC are required, our results suggest that it may be useful in preventing or minimizing the humoral immunosuppression of KCZ.

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REFERENCES CITED

Ahn, Y. K. and Kim, J. H., Preventive effects of diphenyl dimethyl dicarboxylate on the immunotoxicity of carbon tetrachloride in ICR mice. *J. Toxicol. Sci.*, 18, 185-195 (1993).

Bisschop, M. P. J. M., Merkus, J. M. W. M., Scheijgrong, H., Van Cutsem, J. and Van de Kuy, A., Treatment of vaginal candidiasis with ketoconazole, a new, orally active, antimycotic. *Eur. J. Obs. Gynaecol. Rep. Biol.*, 9, 235-239 (1979).

Borgers, M., Mechanism of action of antifungal drugs with special reference to the imidazole derivatives. *Rev. Infect. Dis.*, 2, 520-534 (1980).

Buttke, T. M. and Chapman, S. T., Inhibition by ketoconazole of mitogen-induced DNA synthesis and cholesterol biosynthesis in lymphocytes. *Antimicrob. Agents Chemother.*, 24, 478-485 (1983).

Claman, H. N. and Mosier, D. E., Cell-cell interaction in antibody production. *Progr. Allergy*, 16, 40-80 (1976).

Corbeel, L., Ceuppens, J. L., Van den Berghe, G., Claeys, H. and Casteels-Van D., Immunological observations before and after successful treatment of chronic mucocutaneous candidiasis with ketoconazole and transfer factor. *Eur. J. Pediatr.*, 143, 45-48 (1984).

Cunnigham, A. J. and Szenberg, A., Further improvements in the plaque technique for detecting single antibody forming cells. *Immunology*, 14, 599-600 (1968).

Dixon, D., Shadomy, H. J., Espinel-Ingroff, A. and Korkering, T. M., The activity of ketoconazole in mixed cultures of leukocytes and *Candida albicans*. *Sobouraudia*, 18, 197-210 (1980).

Gergely, P., Nekman, K., Lang, I., Gonzales-Cabello, R. and Perl, A., Ketoconazole *in vitro* inhibits mitogen-induced blastogenesis, antibody dependent cellular cytotoxicity, natural killer activity and random migration of human leukocytes. *Immunopharmacology*, 7, 167-170 (1984).

Hay, R. J., Well, R. S., Clayton, Y. M. and Wingfield, H. J., The use of oral ketoconazole in chronic resistant fungal infections of the skin. *Br. J. Dermatol.*, 103, 22-23 (1980).

Hikino, H., Kiso, Y., Taguchi, H. and Ikeya, Y., Antihepatotoxic actions of lignoids from *Schizandra chinensis* Fructus. *Planta Med.*, 50, 213-218 (1984).

Kim, J. H., Ahn, Y. K. and Ohsawa, M., Enhancing effects of diphenyl dimethyl dicarboxylate on serum antibody production in BALB/c mice. *Biol. Pharm. Bull.*, 18, 24-27 (1995).

Kim, J. H. and Ohsawa, M., Oral tolerance to ovalbumin in mice as a model for detecting modulators of the immunologic tolerance to a specific antigen. *Biol. Pharm. Bull.*, 18, 854-858 (1995).

Kiso, Y., Tohkin, M., Hikino, H. and Ikeyai, Y., Mechanism of antihepatotoxic activity of wuweizisu C and gomisin A. *Planta Med.*, 51, 331-334 (1985).

Lake, J. P. and Reed, N. D., Characterization of antigen specific immunologic paralysis induce by a single low dose of polyvinylpyrrolidone. *J. Reticuloendothel. Soc.*, 20, 307-316 (1976).

Lavrijsen, K., Houdt, J. V., Thijs, D., Meuldermans, W. and Heykants, J., Induction potential of antifungals containing an imidazole of triazole moiety. *Biochem. Pharmacol.*, 35, 1867-1878 (1986).

Levine, H. B. and Cobb, J. M., Oral therapy for experimental coccidiomycosis with R41400 (ketoconazole) a new imidazole. *Am. Rev. Res. Dis.*, 118, 715-721 (1978).

Liu, K. T., Cresteil, T., Provost, E. L. and Lesca, P., Specific evidence that schizandrins induce a phenobarbital-like cytochrome P-450 form separated from rat liver. *Biochem. Biophys. Res. Comm.*, 103, 1131-1137 (1981).

Lowry, O., Rosebrough, N. J., Farr, A. L. and Randall, R. J., Protein determination with the Folin phenol reagent. *J. Biol. Chem.*, 193, 265-275 (1951).

Maeda, S., Kazuhiko, S., Aburada, M., Ikeya, Y., Taguchi, H., Yoshioka, I. and Harada, M., Pharmacological studies on Schizandra Fruit. I. General pharmacological effects of gomisin A and schizandrin. *Yakugaku Zasshi*, 101, 1030-1041 (1981).

Maeda, S., Sudo, K., Miyamoto, Y., Takeda, S., Shinbo, M., Aburada, M., Ikeya, Y., Taguchi, H. and Harada, M., Pharmacological studies on Schizandra Fruit. II. Effects of constituents of Schizandra Fruits on drugs induced hepatic damage in rats. *Yakugaku Zasshi*, 102, 579-588 (1982).

Marshall, G. D., Wilson, J. B., Mader, J. T. and Reinarz, J. A., Immunostimulatory effects of ketoconazole in patients with invasive fungal diseasea. *Clin. Res.*, 29, 327A (1981).

Mishell, B. B., Shiigi, S. M., Henry, C., Chan, E. L., North, J., Gallily, R., Slomich, M., Miller, K., Marbrook, J., Parks, D. and Good, A. H., Preparation of mouse cell suspensions, In Mishell, B. B. and Shiigi, S. M. (Eds.). *Selected Methods in Cellular Immunology* 3. Freeman, San Francisco, CA, pp. 27-32, 1980.

Mosier, D. E. and Coppelson, L. W., A three-cell interaction required for the induction of the primary immune response *in vitro*. *Pro. Natl. Acad. Sci.* USA, 61, 542-547 (1968).

Reitman, S. and Frankel, S., A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminase. *Am. J. Clin. Pathol.*, 28, 56-63 (1957).

Takeda, S., Funo, S., Iizuka, A., Kase, Y., Arai, I., Ohkura, Y., Sudo, K., Kiuchi, N., Yoshida, C., Maeda, S., Aburada, M. and Hosoya, E., Pharmacological studies on Schizandra Fruit. III. Effects of wuweizisu C, a lignan component of Schizandra Fruits, on experimental liver injuries in rats. *Folia*

Pharmacol. Japon., 85, 193-208 (1985).

Terrell, C. L. and Hermans, P. E., Antifungal agents used for deep-seated mycotic infections. *Mayo Clin. Proc.*, 62, 1116-1128 (1987).

Thong, Y. H., Immunomodulation by antimicrobial agents, In Jeljaszewicz, J. and Pulverer, G. (Eds.). *The Antimicrobial Agents and Immunity.* Academic Press, London, pp. 87-129, 1986.

Toda, S., Kimura, M., Ohnishi, M., Nakashima, K., Ikeya, Y., Taguchi, H. and Mitsuhashi, M., Natural antioxidants. IV. Antioxidative components isolated from schisandra fruit. *Shoyakugak Zasshi*, 42, 156-159 (1988).

Van den Bossche, H., Willemsens, G., Cools, W., Cornelissen, F., Lauwers, W. F. and Van Cutsem, J. M., *In vitro* and *in vivo* effects of the antimycotic drug ketoconazole on sterol synthesis. *Antimicrob. Agents Chemother.*, 17, 922-928 (1980).

Van Rensburg, C. E. J., Anderson, R., Joone, G., Van der Merwe, M. F. and Eftychis, H. A., The effects of ketoconazole on cellular and humoral immune functions. *J. Antimicrob. Chemother.*, 11, 49-55 (1983).

Vuddhakul, V., McCormack, J. G., Seow, W. K., Smith, S. E. and Thong, Y. H., Inhibition of adherence of *Candida albicans* by conventional and experimental antifungal drugs. *J. Antimicrob. Chemother.*, 21, 755-763 (1988).

Xie, J. X., Zhou, J., Zhang, C. Z., Yang, J. H., Chen, J. X. and Jin, H. Q., Synthesis of schizandrin C analogs. *Pharm. Factory Shanghai*, 15, 306-309 (1981).

Xie, J. X., Zhou, J., Zhang, C. Z., Yang, J. H. and Jin, H. Q., Synthesis of schizandrin C analogs. II. Synthesis of dimethyl-4,4'-dimethoxy-5,6,5',6'-dimethylenedioxylate-2,2'-dicarboxylate and its isomers. *Acta Pharmaceutica Sinica*, 17, 23-27 (1982).

Yoshikai, Y., Maike, S., Matsumoto, T., Nomoto, K. and Takeya, K., Effect of stimulation and blockade of mononuclear phagocyte system on the delayed footpad reaction to SRBC in mice. *Immunology*, 38, 577-583 (1979).

Yu, H. Q., Yang, X. Y., Zhang, Y. X. and Shi, J. Z., Diphenyl dimethyl dicarboxylate in treating and preventing hepatitis due to drug poisoning. *Chinese Medical J.*, 100, 122-123 (1987).