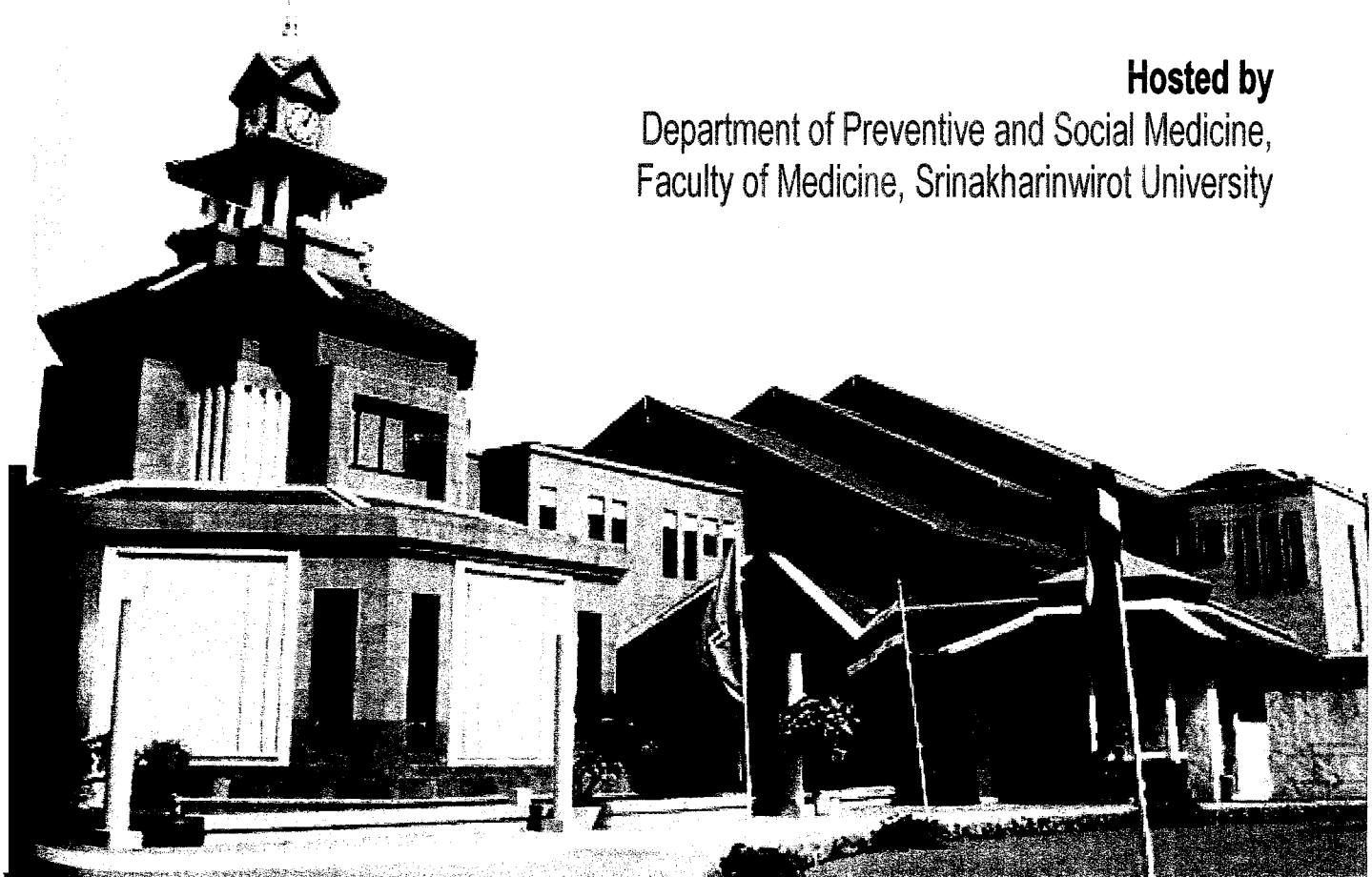
Proceedings
International Conference on Environmental Health
December 26, 2007
Srinakharinwirot University, Bangkok, Thailand

Organized by

1. Korean Society of Environmental Health
2. Faculty of Medicine, Srinakharinwirot University
3. The Association of Occupational and Environmental Diseases of Thailand
4. Thai Society of Toxicology

Hosted by

Department of Preventive and Social Medicine,
Faculty of Medicine, Srinakharinwirot University



International Conference on Environmental Health (ICEH 2007)
December 26, 2007
Research building. Srinakharinwirot University,
Sukhumvit 23, Bangkok, Thailand

8.00 – 8.45 am — Travel to Meeting venue
Registration

8.45 – 9.00 am — ***Opening ceremony***
Prof. Dr. Somkiat Wattanasirichaigoon, MD
Dean, Faculty of Medicine, SWU

Dr. Rhim, Kook-Hwan, DVM, MOH, PhD
President, Korean Society of Environmental Health

9.00 – 9.20 am — ***Thai Keynote speech***
From environmental epidemiology to policy – case of lead in gasoline in Thailand
Asso. Prof. Suwanna Ruangkanjanasetr, MD
Faculty of Medicine, Ramathibodi Hospital,
Mahidol University

9.20 – 9.40 am — ***Introduction to Korean Society of Environmental Health***,
Dr. Rhim, Kook-Hwan, DVM, MOH, PhD
President, Korean Society of Environmental Health

9.40 – 10.40 am — **Session I: Current issues & Future Trend in Environmental Health**
Chairman :
(Thai) Asso.Prof.Dr.Yothin Benjawung, MD.
Faculty of Medicine, Srinakharinwirot University
(Korean)Dr. Rhim, Kook-Hwan, DVM, MOH, PhD
President, .
Korean Society of Environmental Health

- ***Lead poisoning in Thailand***
  Prof.Dr. Pornchai Sithisarankul, MD, DrPH
  Faculty of Medicine, Chulalongkorn University
- ***Air pollution in Thailand***
  Asso. Prof. Dr. Phongtape Wiwatanadate, MD, PhD
  Faculty of Medicine, ChiangMai University
- ***Risk perception and management of asbestos industry; occupational versus environmental paradigm***
  Prof. Paek, Domyung, PhD
  School of Public Health, Seoul National University

10.40 – 11.00 am *Coffee Break*

11.00 am – 12.00 pm **Session II: Research & Development in Environmental Health**

Chairman :

(Thai) Asso.Prof.Dr.Suppachai Ratanamaneechat, MD
President, The Association of Occupational and Environmental Diseases of Thailand

(Korean) Prof. Moon, Chan Seok
Dept. of Industrial Health, Catholic University of Pusan

- ***Genetic epidemiology in environmental health***
  Dr. Suleeporn Sangrajrang, PhD
  Research unit, National Cancer Institute, Thailand
- ***Environmental measurement for environmental health study : Thai experience***
  Ass.Prof.Dr. Kraichart Tantrakarnapa, Ph.D
  Head, Department of Environmental Health Sciences
  Faculty of Public Health, Mahidol University
- ***Does drinking water contribute to antibiotic levels in human?***
  Prof. Dr. Choi, Kyungho, DVM, PhD
  School of Public Health, Seoul National University

12.00 – 1.00 pm *Lunch Break*

1.00 – 2.00 pm **Session III: Poster Presentation**

2.00 -3.00 pm. **Session IV: Basic Science in Environmental Health**

Chairman :

(Thai) Dr.Sumol Pavittranon, PhD
President of Thai Society of Toxicology

(Korean) Prof. Ahn, Ryoungme
Dept. of Health Science, Dongduk Women's University

- ***Immuno-toxicology : Thai experience***
  Dr. Benjamart Chitsomboon, PhD
  Institute of Science, Suranaree University of Technology, Nakorn Ratchasima, Thailand
- ***Cellular and molecular mechanism of environmental toxicant lead-mediated immune alteration***
  Prof. Heo,Yong
  Dept. of Industrial Health, Catholic University of Daegu
- ***Molecular diagnosis in infectious disease***
  Asso.Prof. Dr. Kosum Chansiri, PhD
  Dept. of Biochemistry, Faculty of Medicine, SWU

3.00 -3.15 pm.	***Coffee Break***
3.15-4.00 pm.	**Session V: Panel discussion in Environmental Health Problem(s)** • ***Thai experience*** • ***Korean experience***
4.00-4.30 pm.	***Closing Ceremony*** Best Poster Award
4.30– 5.30 pm.	***Campus visit*** Hosted by Dean, Faculty of Medicine, Srinakharinwirot University
6.00 – 9.00 pm	***Dinner with Thai traditional puppet show at Ramayana restaurant*** Hosted by The Association of Occupational & Environmental Diseases of Thailand Attended by Thai Society of Toxicology

Immuno-toxicology : Thai Experience

Dr. Benjamart Chitsomboon, PhD
Institute of Science,
Suranaree University of Technology
Nakorn Ratchasima, Thailand

Toxicity and Immunomodulatory Properties of Crude Extracts from *Aeginetia indica* Roxb. and Seed Coat of *Tamarindus indica* Linn.

B. Chitsomboon[1], W. Auttachoat[2], J. Wibuloutai[3], T. Komutarin[4], B. Meade[5], K. White[2] and M. Suttajit[6]

[1]School of Biology, Suranaree University of Technology, Nakhon Ratchasima, Thailand.[2]Department of Pharmacology and Toxicology, Virginia Commonwealth University, VA, USA,[3] Faculty of Public Health, Mahasarakham University, Mahasarakham, Thailand,[4]Faculty of Science, Mahasarakham University, Mahasarakham, Thailand,[5]National Institute Occupational Health and Safety, Morgantown, USA,[6]School of Science and Technology, Phayao Campus, Naresuan University, Thailand.

Abstract

Whole plant ethanol extract (DDDP) and water extract (WDDDP) of *Aeginetia indica* Roxb. and the seed coat extract *of Tamarindus indica* Linn induced no mortality or overt sign of toxicity in exposed mice. *In vivo* and *in vitro* studies suggested the immunomodulatory properties of both plants. *In vitro* exposure to DDDP enhance the lymphoproliferative responses to Con A and anti-CD3 antibody. Enhancement of T cell functions including the mixed function response and cytotoxic T cell activity were also observed in B6C3F1 mice treated with *A. indica* daily for 28 days. However, no effects on the antibody forming cell response and NK activity were observed. In contrast, the seed coat extract of *Tamarindus indica* Linn. enhanced the B cell function by increasing antibody forming cell response to sheep red blood cells but did not affect the mixed lymphocyte response and mitogenic response to Con A. Moreover, the seed coat extract of *T. indica* induced *in vitro* and *in vivo* suppression of NO production by LPS and IFN-□ activated RAW264.7 cells and peritoneal macrophages, respectively. The inhibitory mechanism of NO production was mediated through the suppression of iNOS expression. Overall, the whole plant extracts of *A. indica* and the seed coat extract of *T. indica* possess immunomodulatory properties in mice.

Introduction

At present, the strategy of stimulation of human's own immune system as an alternate remedy has drawn a considerable interest due to the problems of high cost, availability, limitation of efficacy, resistance and severe side effects of various drugs used in the treatment of a wide variety of diseases. People have long been used plants as traditional medicines for treatment of diseases and several plant-dervied immunomodulatory drugs have been identified and widely used. Examples include *Echinacea purpurea*, *Echinacea angustifolia*, *Panax ginseng*, *Aloe vera* and etc. Among these plants, *Aeginetia indica* Roxb. and *Tamarindus indica* Linn. are two plants that our research has been focused on.

Aeginetia indica Roxbert

A. indica Linn., a parasitic plant grows on roots of Japanese pampa grasses or sugar canes, has been used as a tonic and anti-inflammatory medicinal herb in China and Japan (Muller-Oerlinghausen *et al.*, 1971, Bando *et al.*, 1988). The butanolic seed extract of *A. indica* Linn. has been shown to possess strong anti-tumor activity *in vivo* as evidenced by curing most ddY mice bearing allo-transplantable sarcoma (S-180) and the cured mice were resistant to subsequent rechallenge with the tumor cells. Moreover, the extract also prevented or reduced the growth of syngeneic Meth A tumor cells in ascites of the tumor bearing Balb/c mice. *In vitro* studies showed the ability of the seed extract to induce various cytokines including IL-2, IL-6, IL-10, IL-12, IL-18, IFN-□, TNF-□ and GM-CSF (Chai *et al.*, 1990, Chai *et al.*, 1994, Okamoto *et al.*, 2000 and Ohe *et al.*, 2001). In Thailand, the same species of plant with *A. indica* Linn is *Aeginetia indica* Roxbert. *A. indica* Roxb. has been called by

different local names depending on locations such as Dok Din Daeng (DDD, Trat province), So-Suai (Mae Hong Son province), Sop Laeng (Song Khla province), Pak Cha Khe (Northeastern), and Yaa Dok Khol (Loei province) (Smitinand, 2001). Similar to *A. indica* Linn., *A. indica* Roxb. (DDD) in Thailand is also a parasitic plant without any chlorophyll and hence grows primary on the roots of other plants. In Thailand, *A. indica* Roxb. often found growing under the shady areas of bamboo. Though *A. indica* Roxb. has long been used as a folk remedy to treat diabetes and dermal swelling in Thailand but there are still no published studies on the toxicity, pharmacological activity or immunological effect of this species of plant when we first conducted the studies.

Methods

Fresh plants of DDD from Wang Num Keaw, Nakhon Ratchasima province, Thailand were collected. Whole plants of DDD were dried in an electric hot-air oven at 60°C for about 3 days or until dried. To obtain the crude ethanol extract (DDDP), 80 grams of dried whole plants were ground and extracted with 95% ethanol using soxhlet apparatus and the ethanol was dried using a rotary evaporator. The powder was dissolved in 0.9% sodium chloride and filtered through 0.45 □m. The hot water crude extract (WDDDP) was also prepared to mimic the decoction procedure which is the way of Thai's traditional preparation of DDD. In this method, the 80 gm of dried homogenized powder was macerated in a pot containing 800 ml of sterile water with lid for 30 minutes at room temperature. The pot was heated at 70-80 °C for 20 minutes, subsequently cooled down to room temperature prior filtration. The filtrate were used as a 100% stock extract in the studies.

Female B6C3F1 mice (Taconic Farms, USA) were daily intraperitoneally injected with DDDP (0.25-250 mg/kg) or daily gavaged with WDDDP (10-100%) for 28 days.The effects of DDDP and WDDDP on T cell function were evaluated by three T Cell functional assays namely proliferative response to anti-CD3 antibody, mixed lymphocyte response and the cytotoxic T lymphocyte response. The effects on B cells were investigated using plaque forming cell response to sheep red blood cell and the natural killer cell activity was used to evaluate innate immunity. The *in vitro* proliferative responses to Con A, LPS and anti-CD3 were also evaluated.

Results

General toxicity

Exposure to DDDP and WDDDP extracts for 28 days had no effect on body weight and selected organ weights and no signs of overt toxicity were observed. Only mice exposed to the highest treatment group (250 mg/kg) of DDDP showed an increase in absolute spleen and liver weights. But the increase was not statistically significant when the data were expressed as relative weight (organ to body weight ratio). Neither DDDP nor WDDDP altered most of the hematological parameters evaluated, except the MCV level which was slightly increase (2%) in all the treatment groups. However, the increase might not be biological relevant as no alterations of other hematological parameters suggesting haematological abnormality were observed. (Auttachoat *et al.*, 2004a,b)

Immunological studies

DDDP as well as WDDDP stimulated the T cell functions in the treated mice as assessed by the lymphoproliferative responses to anti-CD3 antibody, mixed lymphocyte response, and cytotoxic T lymphocyte activity. Exposure to DDDP significantly enhanced the responses in MLR (Fig.1) and CTL assays (Fig. 2) whereas WDDDP enhanced the lymphoproliferative responses to allogeneic antigen in mixed lymphocyte reaction (Fig. 3) and to anti-CD3 antibody (Fig. 4) (Auttachoat *et al.*, 2004a) Both DDDP and WDDDP produced minimal changes in humoral and innate immunities. No alterations in the antibody-forming cell response to the T-dependent antigen, sheep red blood cells, were observed in

both DDDP and WDDDP treatments. The natural killer cell activity against Yac-1 tumor target cells was also not affected by DDDP or WDDDP treatment (Auttachoat *et al.*, 2004b).

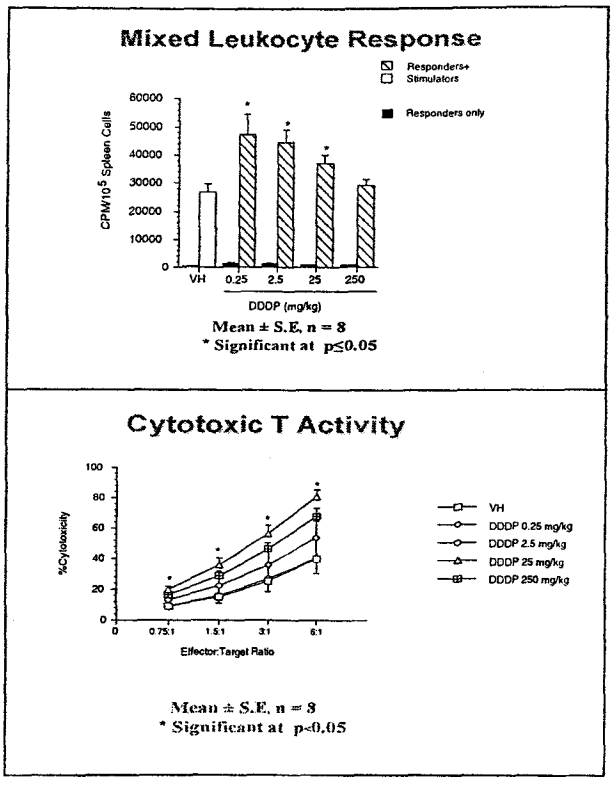


Fig 1-2 The enhanced mixed lymphocyte and cytotoxic T cell responses by DDDP treatment.

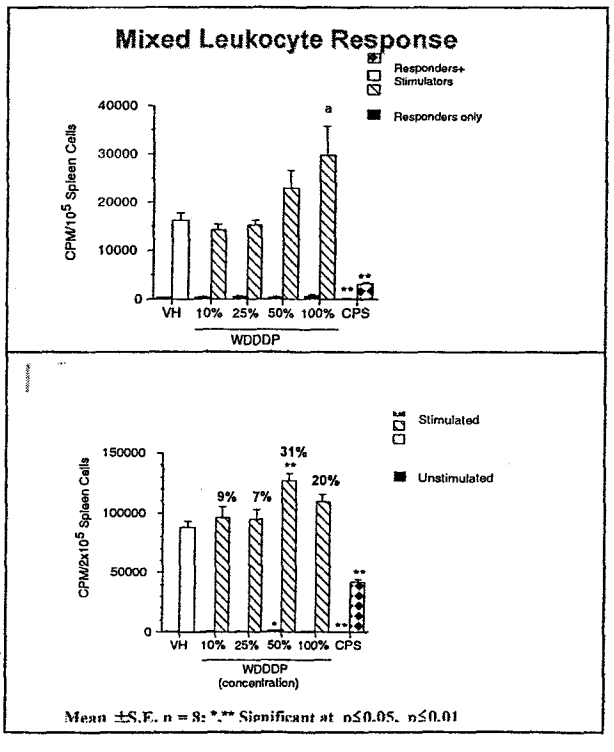


Fig. 3-4 The enhanced lymphoproliferative responses to allogeneic cells and anti-CD3 by WDDDP treatment.

Tamarindus indica **Linn.**

The whole plant of *T indica* Linn. has several ethnomedical usages such as fruits have been used as digestive, carminative, laxative, expectorant and blood tonic, seeds have been used as anthelmntic, antidiarrheal, and emetic. The seed coat is used for burn treatment, wound healing and antidysentery. Pumthong (1999) have reported the strong antioxidative activity of the seed coat. Sudajaroen (2005) reported the polyphenolic extract of the seed coat of *T. indica* Linn was composed of flavonoids, among its major constituents include tannins, anthocyanidins, oligomeric proanthocyanidins, procyanidin B2 (II), procyanidin trimer (IV), procyanidin tetramer (V), procyanidin pentamer (VI) and procyanidin hexamer (VII). At present, there is almost no information about the general toxicity and biological functions of the seed coat of *T. indica*.

Method The polyphenolic compounds were extracted from the seed coat of *T. indica* by 50% acetone using the soxhlet extraction system, then acetone was evaporated at 45°C and the extract was dried by lyophilization. The antioxidant acitivty of the crude extract was confirmed by DPPH and FRAP assays. The general toxicity of *T. indica* was assessed in female B6C3F1 mice by oral gavaged daily for 14 days. The inhibitory effect of the seed coat extract on nitric oxide production was evaluated *in vivo* and *in vitro* using LPS and IFN-□ activated peritoneal macrophages or RAW264.7 macrophage cell lines, respectively. The effect on T cell function was evaluated by lymphoproliferative responses to Con A, and to allogeneic cells in mixed lymphocyte reaction. The *in vitro* antibody forming cell response to sheep red blood cells was used to determine the modulatory effect of the seed coat extract on B cell function.

Results

General toxicity and Antioxidant studies

The seed coat extract exhibited a strong antioxidative activity compared to the positive control, vitamin C and the grape seed extract as assessed by the ability to scavenge stable free radical and the reduction ability in DPPH (Table 1) and FRAP assays, respectively. *In vivo* exposure to the seed coat extract of TAM up to 1000 mg/kg for 14 days did not induce any mortality, clinical signs of toxicity or alteration in all haematological parameters evaluated. However, a maximum of 14% decrease in body weight was observed in the highest treated group (1000 mg/kg) on day 11 but the weight loss was partly recovered by day 14 (Fig.5) (Komutarin *et al.*, 2004). *In vitro*

Inhibition of NO Production

Exposure to the seed coat extract induced a dose dependent inhibition of NO production by LPS and IFN-γ activated RAW264.7 cells (Fig.6) The suppression was not due to direct cytotoxicity as the same range of concentrations of the extract did not affect cell viability of RAW264.7 cells measured by both trypan blue and the resazurin-based assays (Fig. 7) . The inhibitory role of the extract was also confirmed by *in vivo* treatment. Peritoneal macrophages obtained from *T. indica* treated mice and subsequently activated with LPS and IFN-□ also showed lower NO production compared to the VH controls (Fig. 8) (Komutarin *et al.*, 2004). The inhibitory mechanism of NO production was mediated through the suppression of iNOS expression (Fig.9) (Wibuloutai, 2006).

Immunological Studies

In vitro investigation of the effects of *T. indica* seed coat extract on immune functions revealed no alteration on T cell function as evaluated by proliferative responses to Con A (Fig. 10) and allogeneic cells in mixed lymphocyte response. In contrast, the seed coat extract increased B cell function as suggested by increasing number of IgM forming cell response to SRBC antigen in a dose dependent manner (Fig. 11)

Table 1 The percentage of yield, amount of total phenolic content and DPPH free radical scavenging activity of plant samples and standards (vitamin C).

Plant or standard	Yield (%) [a]	Phenolic content (GAE mg/g)	IC_{50} (μg/ml) [b]
TAM	45.8	178.6 ± 3.36	13.2
GSE	-	133.2 ± 2.39	20.7
Vitamin C	-	-	38

Data represent means of three determinations ± SD (standard deviation)

[a] Percentage yields from the weight of dried plant material.

[b] DPPH free radical scavenging activity of TAM, GSE and standard vitamin C.

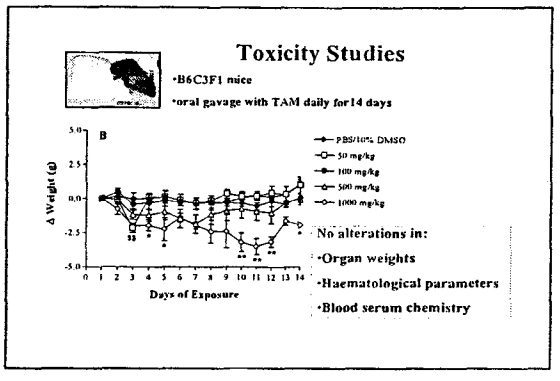


Fig. 5 Body weight of B6C3F1 mice treated with *T. indica* seed coat extract.

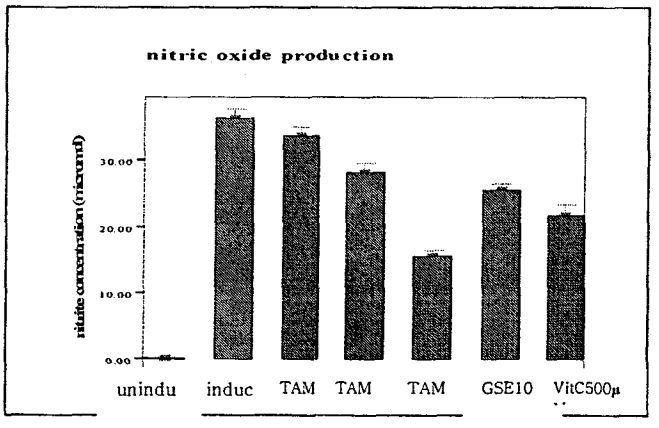


Fig. 6 Inhibitory of NO production by *In vitro* exposure to T. indica seed coat extract

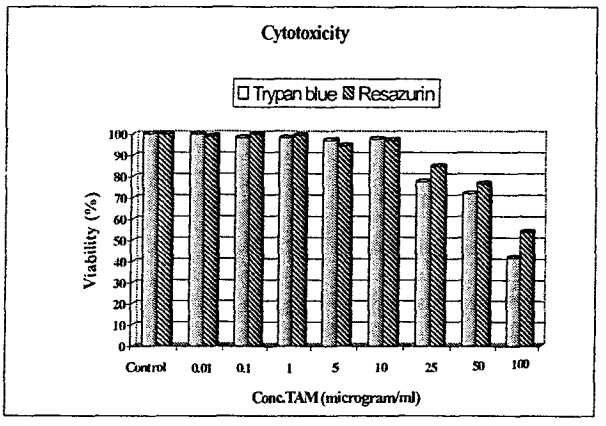


Fig. 7 *In vitro* Cytotoxicity of the seed coat extract of *T. indica* to RAW264.7 cells

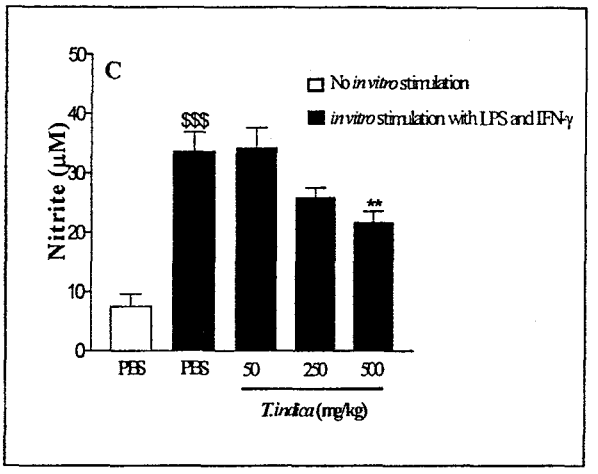


Fig. 8 Inhibitory of NO production by *in vivo* exposure to *T. indica* seed coat extract.

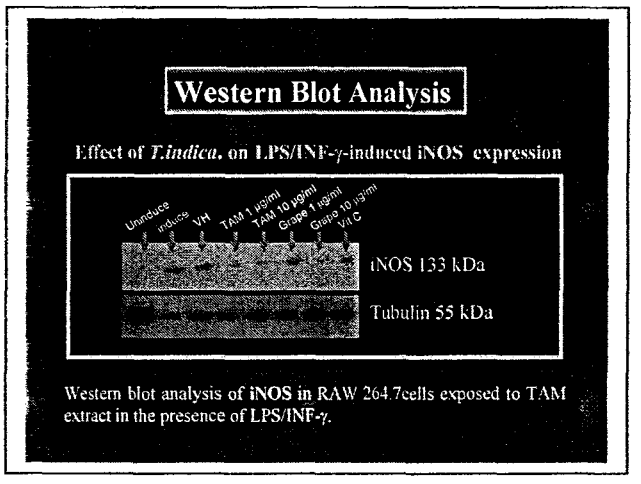


Fig. 9 The suppression of iNOS expression by *T. indica* seed coat extract.

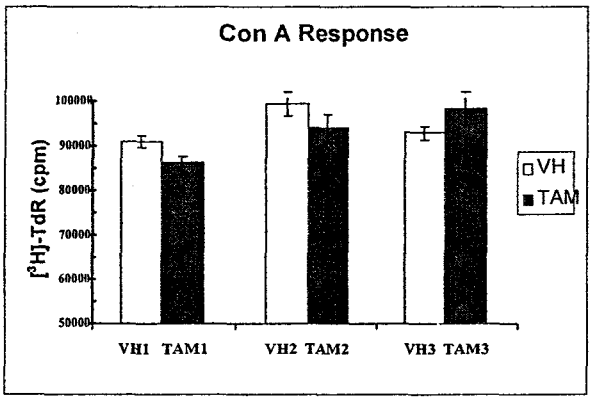


Fig.10 Seed coat extract of *T indica* had no effect on proliferative response to Con A.

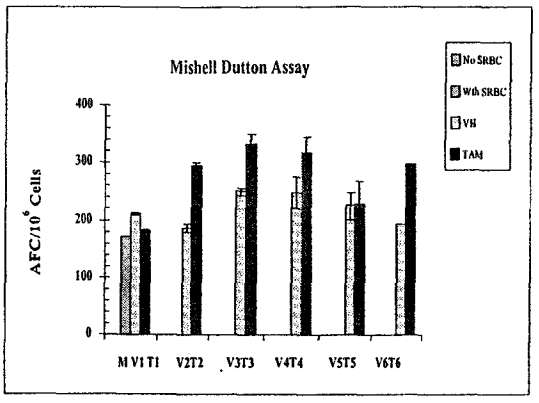


Fig. 11 The enhancement of IgM antibody-forming cell response to sheep red blood cells by *in vitro* xposure to *T. indica* seed coat extract.

Conclusion

The whole plant of ethanol or water extracts of *A. indica* Roxb. and the seed coat extract of *T. indica* Linn. induced no mortality or overt signs of toxicity in treated mice. Both plant extract *of A. indica* and the seed coat extract of *T. indica* possessed immunomodulatory properties as assessed by various *in vivo* and *in vitro* studies.

References

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