

## Immunomodulatory effect of *Ocimum sanctum* against endosulfan induced immunotoxicity

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### Abstract

The present experiment was designed to make a systematic study of experimentally induced immunotoxicity of endosulfan and its amelioration with *Ocimum sanctum* in male Wistar rats at 6, 3 and 1.5 mg / Kg b.wt to groups II, III and IV by mixing in ground nut oil for 6 weeks. To the groups V, VI and VII in addition to endosulfan as above mentioned dose, *Ocimum sanctum* was given at 200 mg / kg b.wt daily per orally for the same duration to study immunomodulatory effect. Group I served as oil control and Group VIII as *Ocimum sanctum* control. Significant reduction in the both HA titer and DNCB contact sensitivity score was observed in the endosulfan treated groups indicates endosulfan has immunotoxic effect. But significant improvement in the immunity was observed in the *Ocimum sanctum* treated groups indicates the immunomodulatory property.

Keywords: Immunodulator, Toxicity, Herbal Drug,

### Introduction

Endosulfan is a pesticide belonging to the organochlorine group of pesticides, under the cyclodiene subgroup is used now a days on a large scale after the withdrawls of the DDT and BHC for pest control in agriculture and public health. Both humoral and cellular immune response are depressed by the endosulfan at doses that do not show overt signs of toxicity. *Ocimum sanctum* (OS) commonly known as "Tulsi" in Hindi is a medicinal plant commonly grown in India having immunomodulatory property. Hence a study was undertaken with reference to know immunotoxic effect of endosulfan and immunoprotective activity of *Ocimum sanctum* against endosulfan in rats.

### Material and methods

In the present experiment technical grade endosulfan (procured from the Hyderabad Chemicals Pvt. Ltd. Hyderabad) was fed to male wistar rats (obtained from Bros Scientific Co. Tirupati) at 6, 3 and 1.5 mg / kg b.wt. to groups II, III and IV for 45days by mixing in ground nut oil by oral intubation. To the groups V, VI and VII in addition to endosulfan as above mentioned dose, *Ocimum sanctum* (OS) (procured from Natural remedies Pvt Ltd. Bangalore) was given at 200 mg / kg b.wt. daily per orally for the same duration. Group I served as oil control and Group VIII as OS control. Six rats from each group were

randomly sacrificed at every fortnight intervals after collecting the blood from orbital sinuses. Serum was separated and used to measure the titers of the agglutination antibody by haemagglutination test using SRBCs as antigen (Swetha, 2005). Chemical contact sensitization of skin is regarded as a form of CMI. 2, 4-Dinitro Chloro benzene (DNCB) was used for contact sensitization. This test was performed as described by Thompson et al. (1975). The results were analysed statistically (Snedecor and Cochran, 1994).

### Results

#### DNCB contact sensitivity test

The mean values of DNCB contact sensitivity test scores in group I to VIII were 0.33, 0.16, 0.16, 0.19, 0.22, 0.23, 0.29 and 0.35. Significant ( $P < 0.05$ ) decrease in the skin thickness was observed in groups II, III and IV when compared to control groups. Significant improvement in the skin thickness was noticed in group V, VI and VII when compare to the group II, III and IV (Table.1).

#### Haemagglutination titers (HA)

Significant decrease was noticed in the HA titer (log) in groups II, III and V when compared control groups and these values are in between the above groups in IV, VI and VII groups. The detail of the data presented in the Table.2 and The mean values of HA titer of various groups were 2.29, 1.97, 2.09, 2.11, 2.14, 2.19, 2.24 and 2.34 in Group I to VIII respectively.

Table-1: Mean values of DNCB contact sensitivity score (mm) in animals of different experimental groups

Groups	Age in weeks			Mean±SE
	2	4	6	
I	0.33	0.31	0.36	0.33±1.45c
II	0.23	0.17	0.08	0.16±4.35a
III	0.27	0.13	0.09	0.16±5.45a
IV	0.24	0.19	0.16	0.2±2.33ab
V	0.26	0.21	0.19	0.22±2.08ab
VI	0.25	0.22	0.22	0.23±1.0ab
VII	0.28	0.31	0.28	0.29±1.0bc
VIII	0.32	0.39	0.36	0.35±2.03c

Mean values with different subscripts differ significantly (P<0.05)  
One way ANOVA, SE –Standard Error

Discussion

DNCB contact sensitivity test

Significant decrease in the skin thickness was observed in groups II, III and IV when compared to control groups. Significant improvement in the skin thickness was noticed in group V, VI and VII when compared to the group II, III and IV. These results are in accordance with the findings of Kurkure et al. (1993), Garg et al. (2004) in birds. Banerjee and Hussain (1986 and 1987) observed the decreased MMI and LMI in rats these are another forms of test to know CMI. Contact hypersensitivity of skin is regarded as a form of CMI response, the T lymphocytes count mirrored the total leukocyte count. The number of circulating lymphocytes in peripheral blood is an index of functional ability of lymphoid organs and lymphocytes are the main agents of immunogenic response in mammals and birds. Thus reduction in the number of total lymphocytes may be an indication of the lower immunocompetence. (Garg et al., 2004) The reduction in the dermal reaction in this study might be due to the lowered peripheral lymphocytes, or might be due to depletion of lymphoid organs like spleen, thymus and lymph nodes.

Haemagglutination titers (HA)

Significant decrease was noticed in the HA titer (log) in groups II, III and V when compared control groups. These results were in accordance with the findings of Banerjee and Hussain, (1986 and 1987) and Turan Akay et al. (1999) in rats where they observed decreased Ab titer against Tetanus toxoid by Radio Immune Diffusion Test. Varshaneya et al. (1988b) observed decreased Ab titer against Bovine serum albumin in birds and Kurkure et al. (1993) and Saxena et al. (2006) observed decrease HI titer against NDV. The decreased HA titer might be due to reduced circulating B lymphocyte population (Garg et al., 2004), or due to pathological changes in the thymus and spleen (Kurkure et al., 1993).

Further more Ach E activity in RBCs and serum was significantly reduced in rats after exposure to the endosulfan, indicating possible role of AchE, Gamma Glutamyl transpeptidase, and glutathione in producing immunosuppression. (Banerjee et al., 1999 and Felten and Felten, 1994). Hence immunosuppression by endosulfan might be a consequence of toxic chemical stress induced cholinergic stimulation and its effects on immune cells (lymphocytes) function. (Seth et al., 2001) or endosulfan might be affecting the rough

Table 2: Mean values of HA titer in animals of different experimental groups

Groups	Age in weeks			Mean±SE
	2	4	6	
I	2.32	2.32	2.23	2.29±3c
II	2.17	2.02	1.72	1.97±0.133 a
III	2.23	2.02	2.02	2.09±7 a
IV	2.23	2.1	2.02	2.11±6.1 bc
V	2.17	2.23	2.02	2.14±6.24 a
VI	2.23	2.17	2.17	2.19±2 bc
VII	2.32	2.23	2.17	2.24±4.3 bc
VIII	2.4	2.4	2.32	2.34±5.6 c

Mean values with different subscripts differ significantly (P<0.05)  
One way ANOVA, SE –Standard Error

endoplasmic reticulum of IgM producing cells and there by decreasing the synthesis of antibodies. In our study decreased titer might be due to pathological changes in the lymphoid organs.

In OS ameliorated groups the HA titer and CMI response were improved significantly. This might be due to anti apoptotic activity of OS (Mohanty et al., 2006) or OS appears to modulate both HI and CMI, GABAergic pathways may mediate these immuno modulatory effects. (Surender Singh et al., 2007).

#### Acknowledgement

The authors are grateful to the ICAR and SVVU for the facilities provided.

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