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A study on humoral immune response, cellular immune response and delayed type of hypersensitivity reaction, on oral administration of organic selenium in Wistar Albino Rats (*Rattus norvegicus*)

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Abstract
An experiment was undertaken to examine the humoral immune response, cellular immune response and delayed type of hypersensitivity reaction, on oral administration of organic selenium in Wistar Albino Rats. The experimental rats were included in four groups of six each. Group I rats receiving distilled water, served as control and Group II got organic selenium, Group III received an antigen, served as antigen control and Group IV given an antigen with organic selenium administered orally. Group III administered antigen on day 14 and 20. The blood samples were collected on day 0, 14, 20, 28, 35 and 42. Leukocyte count (TLC), lymphocyte count (ALC) and Phagocytic indices were increased significantly ($p<0.05$) in both groups compared with their respective control groups. In Group II, TLC were increased on days 35 and 42 and in Group IV on days 20, 28, 35, and 42, when compared to the groups I and II. In both the group II and IV, increase in ALC and PI values was seen on day 28, 35 and 42, compared with group I and III. In contrast to control groups I and III, no significant ($p>0.05$) increase in skin thickness in groups II and IV, was observed showing that organic selenium might promote both humoral immune response and cellular immune response without affecting delayed type of hypersensitive reaction.

Keywords: Organic selenium antigen humoral cellular immune response hypersensitivity

Introduction
The effectiveness of an animal's defence against microbial incursions determines its ability to survive. Living things must have a functioning immune system to ward off invaders including viruses, bacteria, fungi, parasites, and environmental contaminants otherwise that can lead to sickness, mortality, and poor performance. Therefore, a strong immune system is essential for supporting host defence systems and maintaining homeostasis.

The use of herbs and plants being traditionally utilized in oriental cultures to treat a variety of ailments, seems to have given rise to modulate immunity in its most basic form. Ginseng tea was utilized in traditional medicine and has been demonstrated to have immune stimulatory properties due to its concentration of the trace mineral germanium (Goodman, 1988) [5].

A large number of vitamins and trace minerals such as selenium, have been under testing for their potential to modulate immunity, and other pharmacological interventions are currently undergoing comprehensive clinical studies as a therapy for improving the immune system.

Among many vitamins and trace minerals investigated, organic selenium has been proven to have positive impacts on enhancing immune responses and resistance to numerous types of illnesses.

Combination of selenium with Vitamin E, is used in therapeutic practice to treat exudative diathesis, sterility, abortion, and muscular dystrophy. Selenium is an essential component of the biological antioxidant system and stabilizes cell membranes. Although the immunomodulatory effects of organic selenium are somewhat understood, more research is necessary to learn more about the humoral immune response, cellular immune response, and delayed type of hypersensitivity reaction following oral organic selenium administration in Wistar Albino rats (*Rattus norvegicus*).

Keeping these facts in mind, the current experiment was carried out to examine the effects of oral administration of organic selenium in Wistar Albino Rats (*Rattus norvegicus*) on cellular immune response, delayed type of hypersensitive reaction, and humoral immune response.

Materials and Methods

The objective of the study was to evaluate humoral, cellular immune response, and delayed type of hypersensitivity reaction in Wistar Albino Rats (*Rattus norvegicus*) following oral administration of organic selenium.

Animals

24 Wistar Albino rats, each with six rats, were randomly divided into four experimental groups and procured from the Laboratory animal unit, University of Agricultural Sciences, Bengaluru. The rats were between the ages of five to six months, with body weights in the range between 100 to 200 g. They were kept clean and supplied pelleted feed and water as needed in typical laboratory settings (Alastrain, 1989) [1]. The experimental animals were acclimatized for laboratory environment, a week before the start of the experiment.

Administration of selenium

Organic selenium (selenium powder water soluble each gram containing selenium 450 mg) procured from M/s. Vetcare, Tetragon Chemie Ltd., Bengaluru was used in the experiment.

Antigen (sheep RBC suspension)

Sheep red blood corpuscles (SRBC) antigen was used the study. Blood was collected from Sheeps in Farm at University of Agricultural Sciences, Bengaluru. Alsever's solution was used to collect sheep blood, which was kept at 4 ºC for a week. Sheep RBC were washed in higher volume of pyrogen free normal saline and 2% SRBC suspension was prepared for administration in experimental groups (Shah and Gupta, 1998) [9].

Experimental Methods

The experimental rats were divided into four experimental groups. The description of the groups were as follows

Group	Treatment	Dose	Route of inoculation
Group I	Distilled water (control)	0.4 ml	orally
Group II	Organic selenium	0.4 µg/kg body weight	I/P
Group III	Antigen Plus Distilled water (antigen control)	0.4 ml each	I/P and orally
Group IV	Antigen Plus organic selenium	0.4 ml plus 0.4 µg/kg body weight	I/P and orally

I/P: intraperitoneal

Distilled water and organic selenium was given daily for 42 day. The first dose of an antigen was given on day 14 and second dose of an antigen was given on day 20 as a booster dose.

Groups I and II, non-antigen stimulated groups, along with Group I received distilled water, which served as control.

Among Groups III and IV, the antigen stimulated groups, Group III received only an antigen plus distilled water, served as an antigen control.

Collections of blood samples

For the purpose of estimation of phagocytic index and cellular parameters, respectively, blood samples were obtained in heparinised and Na2 EDTA vials. Additionally, blood was also drawn into sterile test tubes for serum separation and stored in refrigerators at 4 ºC.

Diethyl ether was used to make the rats unconscious before blood samples collection from the orbital plexus of non-antigen stimulated, and antigen stimulated groups. The blood was collected on Day zero, i.e., immediately before administering distilled water and drug and then on the 14, 20, 28, 35 and 42 days after treatment. Total leukocyte count (TLC) and absolute lymphocyte count (ALC). was done as per Jain, (1990) [7], with the blood samples collected before and after the administration on days 0, 14, 20, 28, 35 and 42. Phagocytic index (PI) as the humoral parameter was analysed with the serum samples obtained as per Vanfurth *et al.*,1979) [13] with *staphylococcus* as antigen. and Dinitro-chlorobenzene (DNCB) skin sensitivity test was done for testing delayed type of hypersensitivity reaction as per the procedure outlined by Brummerstedt and Basse, (1973) [2].

Phagocytic index (PI) was assessed as per the procedure outlined by (Vanfurth *et al.*,1979) [13] using *staphylococcus* as antigen.

To 1 ml of heparinised blood sample collected individually in sterile heparinised vials, 0.1 ml killed whole cell suspension (*staphylococcus* antigen) was added, and incubated at 37ºc for one hour. Then smears prepared were stained using Giemsa's stain. Mean number of bacteria ingested per every 100 phagocytes were calculated and recorded.

The phagocytic index was obtained after "the number of bacteria ingested by phagocytes" was divided by "the number of phagocytes involved".

Dinitro-chlorobenzene (DNCB) skin sensitivity test: The test was performed as per Brummerstedt and Basse, (1973) [2].

Statistical analysis

Student's "t" test was applied to assess the significance (Snedecor and Cocharan, 1976) [10] with mean values and standard error of mean (×±SE) as the parameter values.

Results and Discussions

The experimental rats appeared healthy during the experimental period. The group I which received distilled water, group II which received organic selenium were described as normal non-antigen stimulated groups. The group III which received an antigen plus distilled water, group IV which received an antigen plus organic selenium were described as an antigen-stimulated groups.

TLC values of normal non- antigen stimulated and anantigen stimulated groups were presented in Table 1 and Figure 1. The mean TLC valuein the Group I ranged from 5008.33±96.10 to 5308.33±39.61, in the Group II varied from 5016.66±44.09 to 5483.33±40.13. in the Group III varied from 5033.33±72.64 to 8033.33±187.82 and in the group IV ranged from 5025.00±33.54 to 8941.66±219.24. There was a significant (P>0.05) increase in the mean TLC values in normal non-antigen stimulated andan antigen stimulated groups when compared with their respective control groups. The increase in TLC was found on day 35 and 42 in non-antigen stimulated group II and in an antigen stimulated group IV on day 20, 28, 35 and 42 with their respective control group I and III. Similar results were obtained by (Bednarek *et al.*, 1996) [3] higher blood leucocyte in calves which received two intramuscular injections of (with an interval of two weeks) selenium (5.75 mg) and α tocopherol acetate (75 mg). On the contrary a dietary supplementation of Vitamin C at

200 mg/day for 90 days in young and older people had significant lower leucocyte count in older than in younger people (Jayachandran and Paneerselvam, 1998) [8].

. ALC values of experimental groups were shown in Table 2 and Figure 2. The mean ALC values in the group I ranged from 75.33±1.80 to 81.50±1.52, in the group II varied from 74.50±2.44 to 86.00±0.96, in the Group III varied from 75.66±2.31 to 85.66±0.80 and in the group IV ranged from 75.50±2.02 to 88.00±0.51. There was a significant (p<0.05) increase of ALC values in both group II and IV compared to their respective control groups (Group I and III), the increase was seen on day 28, 35and 42. Similar results were observed in calves supplemented with Vitamin E at the dose rate of 1 g orally it showed increase in the mean lymphocyte stimulation indices (Cipriano, *et al*., 1982) [14]. (Tamara *et al.*, 1999) [12] noted that increased in lymphocyte count in patients supplemented with Vitamin $B_{12.}$ However, there was significantly low lymphocyte numbers on supplementation with Vitamin C 200 mg/day for 90 days (Jayachandran and Pannerselvam, 1998) [8].

Neutrophil, the phagocytic cell. is involved in phagocytosis. PI values of normal non- antigen stimulated and an antigen stimulated groups were presented in Table 3 and Figure 3. The mean PI values in the Group I ranged from 1.72±0.04 to 1.88±0.02, Group II varied from 1.70±0.04 to 1.99±0.04, in the Group III varied from 1.71±0.03 to 1.94±0.05 and in the group IV ranged from 1.71±0.02 to 2.11±0.02. There was a significant (p>0.05) increase in PI values in group II and IV compared to their respective control group I and III. on day 28, 35 and 42 in both the group II and IV compared with their respective control group I and III. Similar results were reported by (Bednrek *et al*., 1996) [3] found greater phagocytic index in calves treated with selenium (5.75 mg) and α-tocopherol acetate (75 mg). In contrary (Sommer, *et al*., 1975) [11] reported in calves which were fed daily doses of 1000 or 1500 mg of zinc oxide did not show any change in the phagocytic activity.

One of the in-vivo techniques for evaluating a person's cell-mediated immune state is the cutaneous sensitization test with DNCB, presented in Table 4 and Figure 4, the mean skin thickness of the normal, non-antigen-stimulated and an antigen-stimulated groups were displayed. The mean skin thickness varied from 1.25 0.15 to 2.33 0.08 in Group I, 1.29 0.10 to 2.08 0.01 in Group II, 1.29 0.13 to 2.25 0.09 in Group III, and 1.12 0.05 to 2.37 0.15 in Group IV. When group II and group IV were compared to their control group I and group III, there was no appreciable (P>0.05) increase in skin thickness. The indication of delayed hypersensitive reaction is unchanged. Similar findings were made by (Girodonet al., 1999) [6] in institutionalized elderly people who received long-term daily supplements of trace minerals (Zinc sulphate and Selenium sulphide) or vitamins (ascorbic acid and Vitamin E) without any effect on delayed type hypersensitivity reaction.

Table 1: Total leucocyte count (per cu.mm) in normal non-antigen stimulated and antigen stimulated rats

Day	Group I (Distilled water Control)	Group II (Organic selenium)	Group III (Antigen and distilled water antigen control)	Group IV Antigen and organic selenium
0	5008.33±96.10	5016.66±44.09	5033.33±72.64	5025.00±33.54
14	5066.66±57.24	5075.00±69.21	5075.00±54.38	5133.33±54.26
20	5041.66±47.28	5133.33±33.33	7900.00±263.31	8775.00±250.58*
28	5141.66±61.12	5241.66±20.66	8033.33±187.82	8941.66±219.24*
35	5308.33±39.61	5483.3±40.13*	7983.33±190.02	8908.33±239.58*
42	5158.33±63.79	5416.6±40.13*	7933.33±200.27	8841.66±214.24*

Antigen was administered on days 14 and 20; values in Mean±S.EM.; n = 6; p>0.05

Table 2: Absolute lymphocyte count (%) in experimental rat groups

Day	Group I (Distilled water Control)	Group II (Organic selenium)	Group III (Antigen and distilled water antigen control)	Group IV Antigen and organic selenium
0	75.33±1.80	74.50±2.44	75.66±2.31	75.50±2.02
14	78.33±2.13	79.16±0.60	79.33±2.15	80.83±1.70
20	80.16±2.02	82.83±0.65	84.33±1.30	87.00±0.68
28	81.50±1.52	86.00±0.96*	85.66±0.80	88.00±0.51*
35	81.00±0.57	85.33±1.30*	84.83±0.87	87.66±0.71*
42	80.83±1.13	85.33±0.91*	84.00±0.85	87.16±0.54*

values in Mean ±S.EM.; n = 6; p>0.05

Table 3: Phagocytic index values in various groups

Day	Group I (Distilled water Control)	Group II (Organic selenium)	Group III (Antigen and distilled water antigen control)	Group IV Antigen and organic selenium
0	1.72±0.04	1.70±0.04	1.71±0.03	1.71±0.02
14	1.82±0.02	1.85±0.03	1.83±0.02	1.85±0.02
20	1.83±0.01	1.86±0.01	1.87±0.12	1.90±0.02
28	1.85±0.01	1.93±0.02*	1.92±0.03	2.04±0.02*
35	1.88±0.02	1.99±0.04*	1.94±0.05	2.11±0.03*
42	1.87±0.02	1.99±0.04*	1.93±0.04	2.08±0.04*

Values are in Mean± S.E; N = 6; p>0.05

Table 4: Dinitrochlorobenzene skin sensitivity test (mm) in normal non-antigen stimulated and antigen stimulated rats

Day	Group I (Distilled water Control)	Group II (Organic selenium)	Group III (Antigen and distilled water antigen control)	Group IV Antigen and organic selenium
0	1.25±0.15	1.29±0.10	1.29±0.13	1.12±0.05
24	2.33±0.08	2.08±0.01	2.25±0.09	2.37±0.15
48	1.66±0.13	1.79±0.10	1.75±0.01	1.79±0.10

Values are in Mean± S.EM; n= 6; $p>0.05$

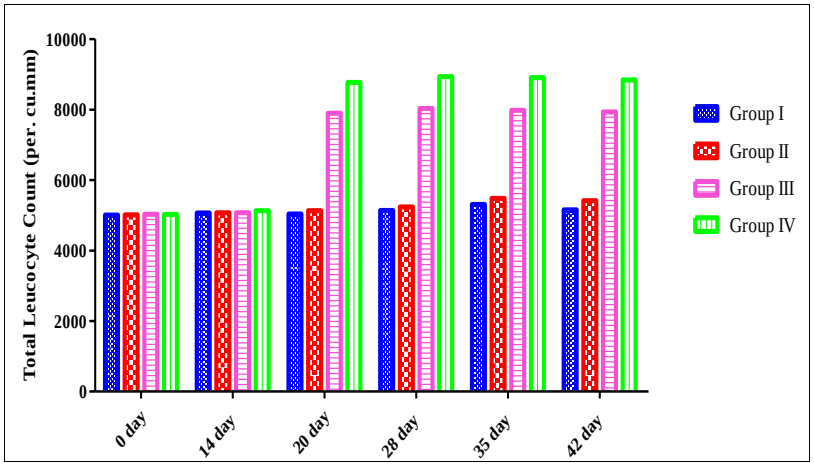

Fig 1: Total leucocyte count (Per common) in normal non-antigen stimulated and antigen stimulated rats

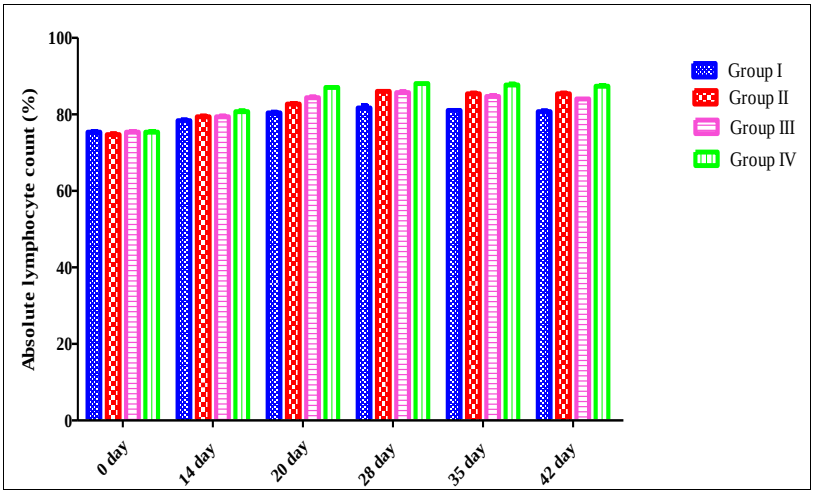

Fig 2: Absolute lymphocyte count (%) in normal non-antigen stimulated and antigen stimulated rats

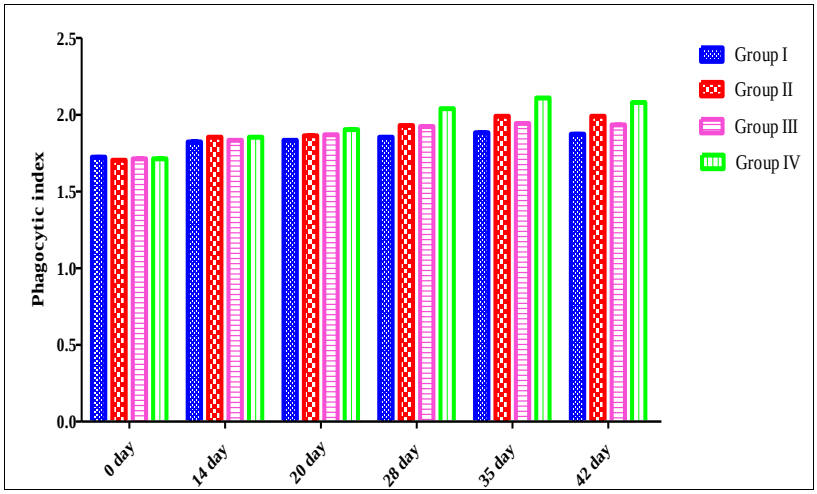

Fig 3: Phagocytic index in normal non-antigen stimulated and antigen stimulated rats

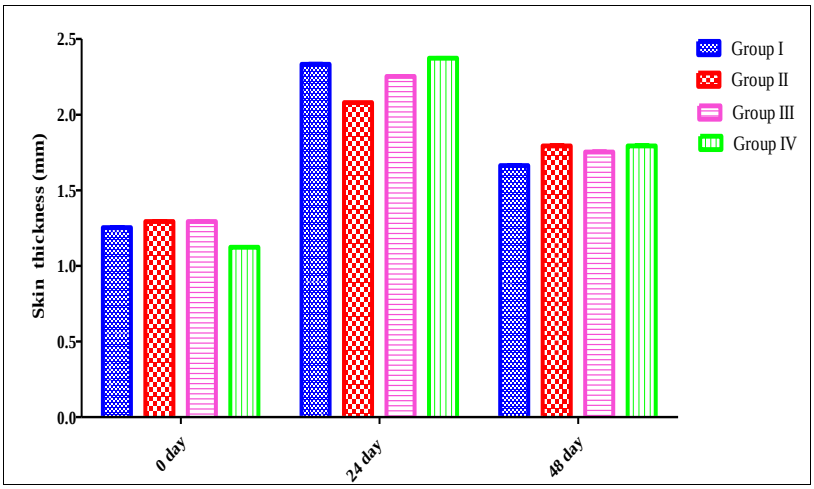

Fig 4: Dinitrochlorobenzene skin sensitivity test (mm) in normal non-antigen stimulated and antigen stimulated rats

Conclusion

The present study concluded that organic selenium could stimulate humoral immune response and cellular immune response but could not stimulate delayed type of hypersensitivity reaction, on oral administration of organic selenium in animals which were vaccinated even if they were administered before or after the vaccination which might help in boosting the immune response, necessary to protect living organisms from pathogenic external microbial invaders and certain environmental pollutants associated with ill health.

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