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Original Article
In vivo studies

Immunomodulatory Activity of *Swarna Prashana* in Charle's Foster Albino Rats

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ABSTRACT

Introduction: *Swarna Prashana* a classical incinerated gold preparation used as a *Rasayana* agent in indigenous system of medicine Ayurveda. *Swarna Prashana*, *Vacha*, cow ghee and honey are used for *Swarna Prashana* preparation. Nowadays physicians are claiming that it is a immune-modulator and prescribing it to neonates and children in India. So here it was decided to assess Immuno-modulatory action of Swarna Prashana. The aim of study was to evaluate immuno-modualtory activity of *Swarna Prashana* for humoral antibody formation and cell mediated immunity in albino rats. **Methods:** Sheep red blood corpuscles were assessed for humoral antibody formation. The test drug and vehicle were administered for 10 days. Animals were sacrificed on 11th day. Parameters like haemoagglutination titre, haematological, serum biochemical and histo-pathological study of spleen, thymus and lymph nodes were studied to assess the effect on humoral immunity. For cell mediated immunity, Immunological paw oedema was assessed. **Results:** Platelets count was significantly increased in drug treated group compared with Sheep red blood corpuscles group. Test drug observed moderate but non-significant increase in paw oedema after 24 hours and after 48 hours compared to control group. Histopathological studies show that *Swarna Prashana* increased the cellularity in spleen and lymph node. **Conclusion:** The present study demonstrates that *Swarna Prashana* produces humoral immune response.

KEYWORDS

Cell mediated immunity, haemagglutination, incinerated gold, *Swarna Bhasma*, *Swarna Prashana*

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From centuries *Swarna Bhasma* (incinerated gold) is used as a medicine in the Indian traditional system of medicine. Numbers of its properties were mentioned in the classical texts of Ayurveda like rejunavator, aphrodisiac, memory booster etc.[1] It is used in numerous disorders like *Grahani* (sprue), *Pandu* (anemia), *Asthikshaya* (osteoporosis), *Jirna Vyadhi* (chronic disorders) etc.[2] It is chief ingredient in therapeutically potent medicines like *Makaradhwaja*, *Swarna Malini Vasanata, Vasantakusumakara Rasa*. Its few therapeutic properties like analgesic[3] anticataleptic, anti anxiety and antidepressant[4], activity antioxidant[5] and augmenting effect[6] were screened earlier. Its role on a normozoospermia,[7] male infertility[8] and oligoazospermia[9] was found to have significant results also specific quantity of gold was observed in semen of healthy fertile men.[10-11] Recent studies of gold nanoparticles were found effective for antiangiogenic and anti cancer property.[12] *Swarna Bhasma* is used as a chief ingredient in *Swarna Prashana* for neonates and children. It was advocated in the classical texts of Ayurveda that *Swarna Prashana* acts as *Rasayana* (immunomodulation) and increases *Dhi* (memory) in neonates and children.[13-14] Nowadays, Ayurveda physicians are prescribing Swarna Prashana to children and neonates with claim of immunity booster in modified drop format. Many manufacturers have also manufactures Swarna Prashana drops and selling them on medical counters. Though classical texts of Ayurveda mentioned Swarna Prashana for Rasayana activity but its procedure is different from this modified drop format. So to analyze its efficacy as an immunity booster here in present it was planned to prepare Swarna Prashana Drops and evaluate its immune-modulatory activity.

MATERIALS AND METHODS

Preparation of *Swarna Bhasma*

Swarna Bhasma was prepared by following classical reference and standard guidelines in the department of *Rasashastra* and *Bhaishjya Kalpana*, Institute of Post Graduate Teaching and Research in Ayurveda (IPGT&RA), Gujarat Ayurved University (GAU), Jamnagar.[15-17] 24 caret hallmark certified gold was purchased from local gold market of Jamnagar. Thin flakes of gold were prepared by roller press. These flakes were equally weighed and used for the *Shodhana* procedure (purification and potentiating procedure).[18] After *Shodhana*, gold flakes were converted into small pieces. These small pieces of purified gold were amalgamated with purified mercury (*Shuddha Parada*)[19] followed by triturating with purified sulfur (*Shuddha Gandhaka*)[20] up to preparation of black colored homogeneous mass. The material was milled with decoction of bark of *Bahuana Verigate*

(*Kanchanaara Twaka*) and semisolid mass was prepared. Small pellets were prepared from this semisolid mass and dried. These dried pellets were kept inside earthen pot and burned within heat of 8 cow dung cakes. The procedure was repeated for thirty subsequent times up to achievement of *Rekhapurnatva* and *Varitaratva* (characteristic features) of *Swarna Bhasma*. 52.65 % elemental gold was observed in the finished product on the inductively coupled Plasma Atomic Emission Spectrometry analysis[21]. The *Bhasma* was stored and used for the experiment. Cow ghee, Honey and *Vacha Churna* (*Acorus calamus* Linn.) were procured from the Pharmacy of GAU, Jamnagar.

Swarna Prashana was prepared by adding *Swarna Bhasma* 30 mg and *Vacha Churna* (*Acorus calamus* Linn.) 30 mg in agate mortar and pestle and milled with gentle pressure till homogenous preparation. The mixture was again triturated with 70 ml honey and cow ghee 70 ml up to homogeneous emulsion formation. The prepared emulsion was designated as SBP and kept in air tight glass container for further experimental use.[13-14]

Experimental Animals

Charle's Foster albino rats (180 to 220 g) of either sex were procured from the Animal house of Pharmacology laboratory of IPGT & RA, GAU, Jamnagar. After obtaining permission from animal ethical committee (IAEC 12/2012/02) experiments were carried out. Amrut brand rat pellet feed supplied by Pranav Agro Ltd. was fed to animals during the study period. The drinking water was given *ad libitum* in polypropylene bottles with stainless steel sipper tube.

Groups: Experimental study was carried out on 6 animals in each group; SRBC (Sheep red blood corpuscles control group) and SBP (Swarna Prashana group).

Dose fixation

The dose of the drug was calculated by extrapolating the therapeutic dose to rat on the basis of body surface area ratio by referring to the table of Paget and Barnes (1964).[22] Human dose of Swarna Bhasma 15 mg was considered as a therapeutic dose.[15] It was converted to rat dose by applying conversion formula of Paget and Barnes. SBP emulsion was administered orally at dose 1 ml/ 200 g body weight, to test drug group SBP animals.

Humoral Immune response

Sheep blood was collected aseptically from the city slaughter house in a sterilized bottle containing Elsevier's solution (2% Dextrose, 0.8% sodium citrate, 0.05% citric acid and 0.42% sodium chloride). Blood was centrifuged in cooling centrifuge at 3000 RPM (rate per minute). Sheep red blood corpuscles (SRBC) were collected and thoroughly washed with sterile normal saline and stored in a refrigerator till experimentation. SRBC from the same animal was used both for sensitizing and to determine antibody titre.

Humoral immune response activity was carried as per method described by Stone and Paget (1971) with slight modification as per experiment.[23] Test drugs and vehicle were administered for 10 consecutive days. On the third day of test drug administration, all rats were antigenically challenged by subcutaneous injection of 30% v/v SRBC in the dose of 1 ml /200g body weight in the nape of neck. On the 11th day, blood was collected by retro orbital puncturing under light anesthesia by using anesthetic ether. Blood was collected for hematological parameters and serum for evaluating haemagglutination antibody titre. All animals were sacrificed and spleen, thymus and lymph node were carefully dissected out, after noting its weight; they were transferred to 10% formalin solution for histopathological study.

Haemagglutination antibody titre

The microtitre plate was cleaned by distilled water and dried properly. 0.1 ml of sterile normal saline was added to each well of micro titre plate. Complement in the serum was inactivated by heating it at 56°C for 30 minutes in a serological water bath. Serial two fold dilutions of the serum were performed in sterile saline solution and 0.1 ml of each dilution was aliquoted into 96 well micro titre plate (16 well dilutions for each sample). 0.1 ml of sterile 3% v/v SRBC in normal saline was added and mixed. The plates were incubated for 8 hours and examined visually for agglutination. Highest serum dilution value causing haemagglutination was considered as the antibody titre and titre was converted to $\log_2$ values for easy comparison.

Cell mediated immunity

The test drug was evaluated to assess its effect on cell-mediated immunity against triple antigen mediated immunological oedema. The suspension of triple antigen with alum precipitate prepared in the proportion (Triple Antigen: 1 ml + Normal saline (0.9%) 4 ml+Potash Alum (10%) 1 ml). pH of the above solution was adjusted between 5.6 to 6.8 by using 10% sodium carbonate. Initially the rats were antigenically challenged by injecting the suspension of triple antigen with alum precipitates subcutaneously in the nape of the neck in a dose of 1 ml/200g body weight.[24] Administration of test drugs and vehicle were started on the day of sensitization and continued for the next five days. On 5th day, 1 hour after administration the test drug, the rats were injected with 0.1 ml suspension of triple antigen with alum precipitates beneath plantar aponeurosis in the left hind paw. The paw volume was measured initially and 24, 48 hours after injecting this alum adjuvant. The paw volume was measured with the help of a digital plethysmograph. Percentage increase in paw volume after alum adjuvant injection in comparison to initial volume was noted. Values from control group were compared to the values from test drug administered groups. One way ANOVA followed by Dunnet's multiple 't' test for un paired data to determine significant difference between groups at $p<0.05$.

RESULTS AND DISCUSSION

The result showed that almost similar pattern of body weight gain were observed in treated group compared to control group whereas moderate insignificant decrease in spleen and thymus weight were observed in drug treated group (Table 1 and 2).

Table 1. Effect of test drugs on body weight in SRBC sensitized Albino rats

Groups	Dosage(ml/g)	Initial bodyweight (g)	Final body weight (g)	% change
SRBC control	0.5 ml/100 g	94.00 ± 7.55	124.33 ± 6.85	32.27
SBP	0.5 ml/100 g	100.66 ± 7.28	130.33 ± 9.51	29.47

Data: Mean ± SEM; *P<0.05, comparison to Control (ANOVA –Dunnett's multiple 't' test)

Table 2. Effect of test drugs on weight of spleen and thymus in SRBC sensitized albino rats

Groups	Spleen (g)	% change	Thymus (g)	% change
SRBC control	0.29 ± 0.065	-	0.2 ± 0.02	-
SBP	0.24 ± 0.02	17.24	0.16 ± 0.004	19.90

Data: Mean ± SEM; *P<0.05, comparison to Control (ANOVA –Dunnett's multiple 't' test)

Treatment with SBP insignificantly increased White Blood corpuscles (WBC), Lymphocytes, haemoglobin, Packed Cell Volume (PCV), Red blood corpuscles (RBC) count in comparison with SRBC group. Platelets count was significantly increased in drug treated group compared with SRBC group. Non significant decrease was observed in Neutrophils, Eosiophil, Monocyte, MCV (Mean Corpuscular Volume), MCH (Mean Corpuscular Hemoglobin) was observed in drug treated group in comparison with SRBC group (Table 3). Significant increase in antibody titre in SBP treated group was observed compared to control group (Table 4). The result showed moderate insignificant increase in paw oedema after 24 hours and after 48 hours in treated group compared to SRBC group (Table 5).

Table 3. Effect of test drug on Haematological parameters in albino rats

Parameter	SRBC control	SBP	% change
WBC($10e^3$)	8933.33 ± 573.68	12133.33 ± 1428.68	35.82
Neutrophils (%)	16.33 ± 1.89	11.5 ± 1.73	29.58
Lymphocytes (%)	80.67 ± 2.51	85.33 ± 1.93	5.78
Eosinophil (%)	2.33 ± 0.21	1.83 ± 0.17	21.46
Monocytes (%)	1.83 ± 0.31	1.33 ± 0.21	27.32
Hemoglobin(g/dl)	13.62 ± 0.44	15.03 ± 0.2	10.40
PCV (%)	43.33 ± 1.80	47.27 ± 0.75	9.09
RBC($10e^6$/µl)	6.97 ± 0.28	7.89 ± 0.19	13.29
Platelets(10^3/ul)	934.00 ± 61.26	1179.17 ± 83.48 *	26.25
MCV(fl)	62.233 ± 0.94	59.967 ± 0.80	3.63
MCH(Pg)	19.58 ± 0.27	19.08 ± 0.28	2.55
MCHC(g/dl)	31.5 ± 0.29	31.8 ± 0.21	0.95

Data: Mean ± SEM; *P<0.05, comparison to Control (ANOVA –Dunnett's multiple 't' test)

Table 4. Effect of test drug on *Antibody formation* against SRBC in rats after 7 days

Treatment	Dosage (ml/100g)	Antibody titer (Log_2 values)	Percentage change
SRBC control	0.5	4.16 ± 0.18	--
SBP	0.5	5.08 ± 0.23*	22.25

Data: Mean ± SEM; *P<0.05, comparison to Control (ANOVA –Dunnett's multiple 't' test)

Table 5. Effect of test drug on triple antigen induced immunological paw oedema in pre-sensitized Albino rats

Groups	Dosage (g/kg)	% Increase in paw volume at different time intervals after the injection of the Triple antigen			
		After 24 hours	% change	After 48 hours	% change
SRBC Control	0.5	25.57 ± 3.94	-	19.79 ± 3.46	-
SBP	0.5	31.75 ± 3.73	24.17	28.84 ± 3.03	45.73

Data: Mean ± SEM; *P<0.05, comparison to Control (ANOVA –Dunnett's multiple 't' test)

Examination of sections of spleen, thymus and lymph node from control group exhibited normal cytoarchitecture (Figure 1-3). The sections of SBP showed increase in the white pulp and cellularity in spleen along with mild increase in the cellularity of lymph node (Figure 1-2). Normal cytoarchitecture of thymus was observed in SBP treated group (Figure 3).

Figure 1. Photomicrographs of representative section of spleen of albino rats

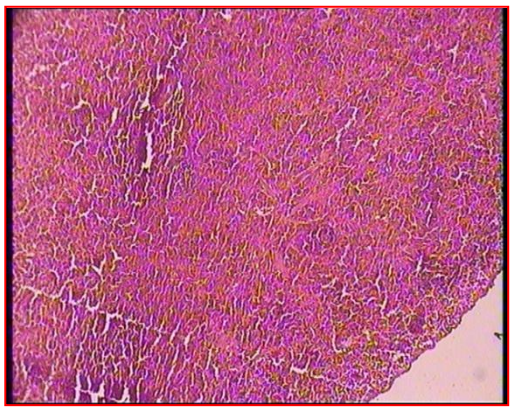

Fig 1a. Photomicrographs of representative section of spleen of albino rats from SRBC control group (1×102 magnification). **Note:** Normal cytoarchitecture

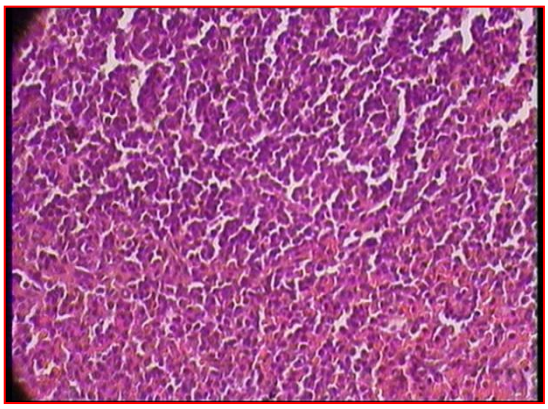

Fig 1b. Photomicrographs of representative section of spleen of albino rats from SRBC control group (1×400 magnification). **Note:** Normal cytoarchitecture

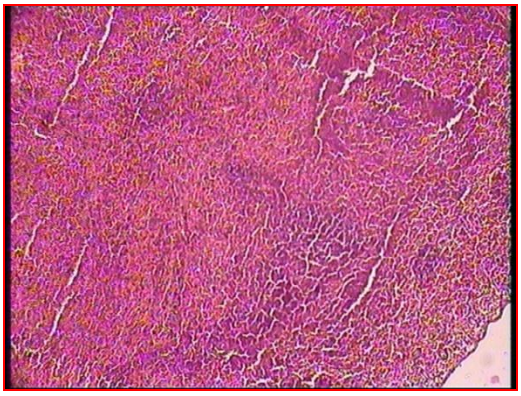

Fig 1c. Photomicrographs of representative section of spleen of albino rats from SBP group (1×100 magnification). **Note:** Normal cytoarchitecture

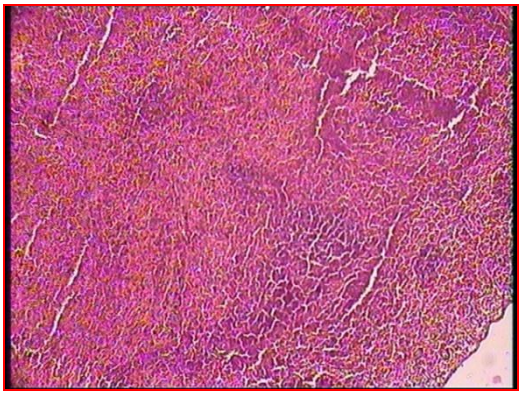

Fig 1d. Photomicrographs of representative section of spleen of albino rats from SBP control group (1×400 magnification). **Note:** Increased cellullarity

Figure 2. Photomicrographs of representative section of Lymph node of albino rats

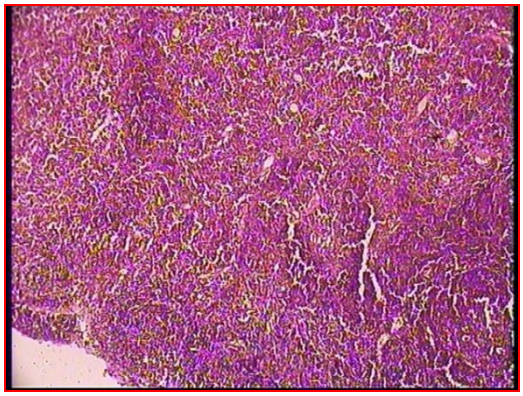

Fig 2a. Photomicrographs of representative section of Lymph node of albino rats from SRBC control group (1×102 magnification). **Note:** Normal cytoarchitecture

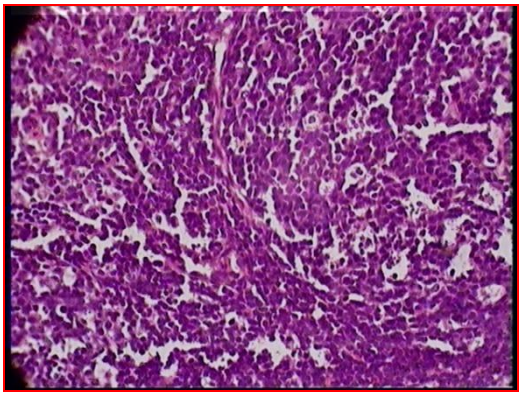

Fig 2b. Photomicrographs of representative section of Lymph node of albino rats from SRBC control group (1×400 magnification). **Note:** Normal cytoarchitecture

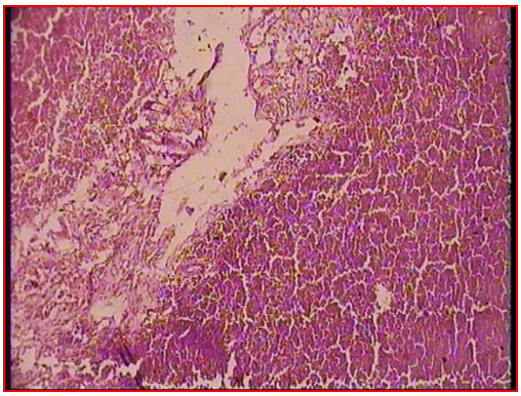

Fig 2c. Photomicrographs of representative section of Lymph node of albino rats from SBP group (1×100 magnification). **Note:** Normal cytoarchitecture

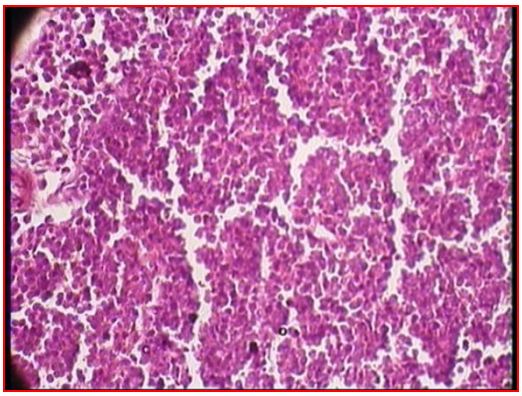

Fig 2d. Photomicrographs of representative section of Lymph node of albino rats from SBP control group (1×400 magnification). **Note:** Increased cellularity

Figure 3. Photomicrographs of representative section of Thymus of albino rats

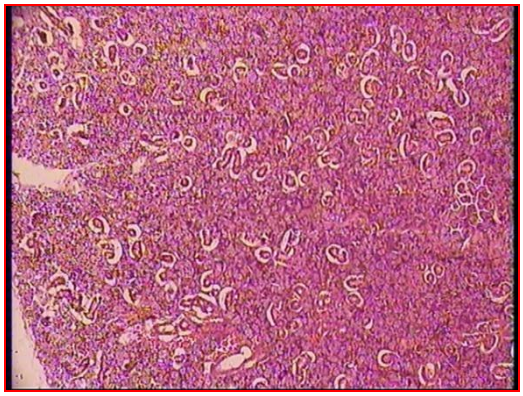

Fig 3a. Photomicrographs of representative section of Thymus of albino rats from SRBC control group (1×102 magnification). **Note:** Normal cytoarchitecture

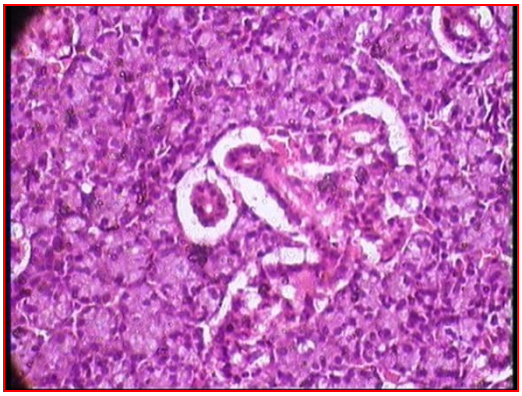

Fig 3b. Photomicrographs of representative section of Thymus of albino rats from SRBC control group (1×400 magnification). **Note:** Normal cytoarchitecture

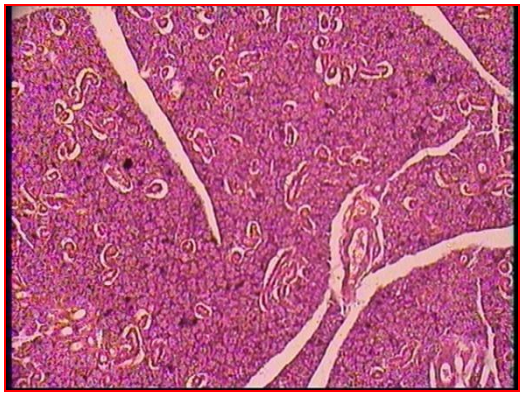

Fig 3c. Photomicrographs of representative section of Thymus of albino rats from SBP group (1×100 magnification).
Note: Normal cytoarchitecture

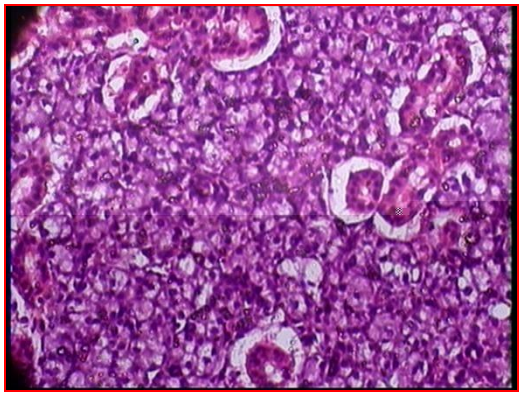

Fig 3d. Photomicrographs of representative section of Thymus of albino rats from SBP control group (1×400 magnification). **Note:** Almost Normal cytoarchitecture.

Test drug, *Swarna Prashana* studied for its effect on SRBC induced humoral immune response in Albino rats. The humoral immunity involves interaction of B-cell with the antigen, and their subsequent proliferation and differentiation into antibody secreting plasma cells. Antibody functions as the effectors of the humoral response by binding to antigen and neutralizing it to form clusters that are more readily ingested by phagocytic cell. Both drugs enhanced the agglutination titre to SRBC (antigen) but significant increase was seen in SB treated group. This indicates the enhanced responsiveness of macrophages and T, B

lymphocytes which are involved in antibody synthesis.[25] Increased level of antibodies gives higher agglutination titer against sheep red blood cells.[26]

Control group and test drug treated groups showed consistent body weight gain throughout experimental period. The moderate insignificant decrease in spleen and thymus weight was observed in test drug treated animals. Generally decrease in the weight of the organ is indicative of loss of tissue mass in that organ, exception being the secretory organs in which decrease in weight sometimes is seen along with increased activity. In present study the findings of the histopathological study the weight reduction does not seem to represent loss in the tissue mass as no pathological changes was observed in histological study (Figure 1-3).

The significant increase was observed in platelet count in SBP treated group. Test drug moderately increased the values of WBC and lymphocytes which plays important role in immune activity. Test drug also moderately increased the level of RBC and haemoglobin which indicative of its role in red blood cell formation.[27]

Histopathological studies show that SBP increased the cellularity in spleen and lymph node. Increase in cellularity in white pulp in spleen of test drug treated group may be due to the increase in lymphatic tissue and free lymphocytes in spleen. The increase in cellularity in the lymph node is indicative of increased immune activity. It may be due to increased formation of cytokines from the cells involved in immune mechanism.[28]

Cell mediated immunity is a part of the process of graft rejection, tumour immunity and many intracellular infections or to micro-organisms, which cause chronic diseases. During delayed type hypersensitivity (DTH) responses, sensitized T-lymphocytes, when challenged by the antigen, are converted to lymphoblasts and secret lymphokines, attracting more scavenger cells to the site of reaction.[29] The infiltrating cells are immobilized to promote inflammatory reaction.[30] Test drug insignificantly influenced T-cell activity by increasing vascular permeability, vasodilatation, macrophage accumulation and activation, which finally result in the increase in the paw volume that promotes phagocytic activity. This behavior itself is suggestive of activation of immune system at cellular level with lymphocytes, Cytokines, prostaglandulin E etc.are also liberating from the neighboring cells.[25]

CONCLUSION

It could be concluded that, *Swarna Prashana* exhibited dramatic immunomodulatory effects in SRBC sensitized rats. *Swarna Prashana* non-significantly influenced T–cell activity which in turn increases vascular permeability, induce vasodilatation, macrophage accumulation and activation, and which finally result in the increase in the paw volume which promotes phagocytic activity. *Swarna Prashana* produces good humoral immune response. Therefore, findings provide experimental evidence that *Swarna Prashana* can be used to improve one's health and immune function efficiently. Further studies are required to develop an effective administration of *Swarna Prashana*, and to investigate the mechanism underlying the immunomodulation before administration in humans is performed.

CONFLICTS OF INTEREST

Nil

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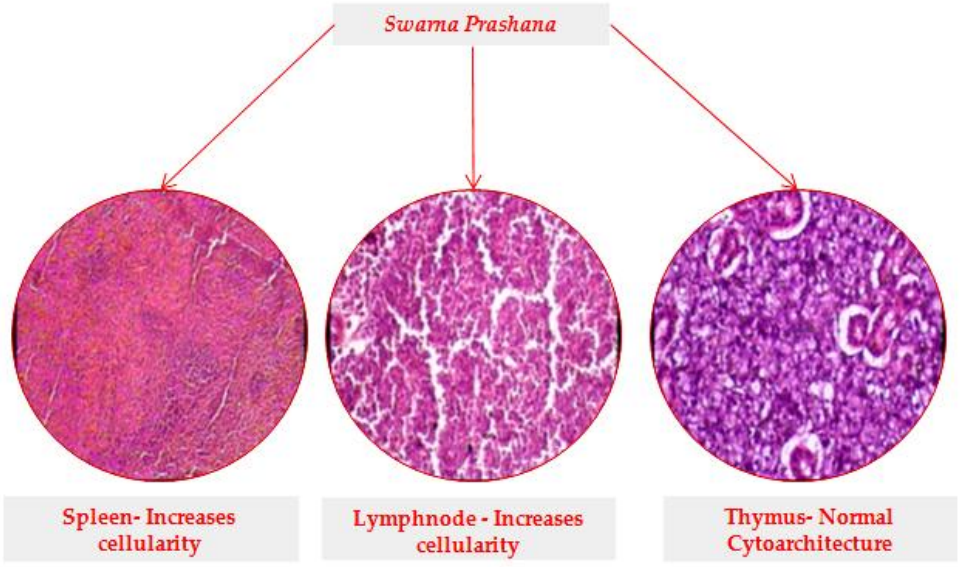
GRAPHICAL ABSTRACT



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