Original Article

Adaptogenic and immunomodulatory activity of Virgozest *Avaleha* – An ayurvedic proprietary formulation

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Abstract

Introduction: *Rasayana* (rejuvenator) or adaptogenic drugs have been proved to produce the complete potential to prevent diseases and degenerative changes that leads to diseases and promote longevity by providing strength and immunity. Virgozest *Avaleha* is a poly-herbal formulation claimed to serve as adaptogenic, and immunomodulatory, as a health tonic, enriched with dry fruits, and ingredients containing natural supplements of Vitamin E and proteins. **Aim:** To evaluate the adaptogenic activity and humoral immune activity of virgozest *Avaleha* in Wistar albino rats. **Materials and methods:** Virgozest *Avaleha* was evaluated for adaptogenic activity against swimming stress-induced changes and hypothermia in albino rats. The humoral immune activity of virgozest *Avaleha* was evaluated against sheep red blood cells (SRBCs)-induced response in albino rats with the inclusion of cyclophosphamide as immune suppressant agent. **Results:** In adaptogenic activity, virgozest *Avaleha* (450 and 900 mg/kg) exhibited an increase in physical activity, decrease in stress-induced hypothermia, and serum cortisol level when compared to the stress control group of albino rats. In humoral immune activity, virgozest *Avaleha* reversed the effects of cyclophosphamide-induced adverse changes on spleen and lymph node, and produced a significant increase in serum antibody titer in SRBCs-sensitized rats. **Conclusion:** The present study concluded that virgozest *Avaleha* has adaptogenic and humoral immune activity in Wistar albino rats, which may suggest the *Rasayana* like properties of Ayurvedic formulation.

Keywords: Adaptogenic, antibody titer, humoral immunity, immunomodulatory, virgozest *Avaleha*

Introduction

The autoimmune, as well as different types of cancerous ailments, involve the suppression of immunity in individuals.[1] Most surveys agree that poly-herbal and herbo-mineral remedies are the most prevalent therapies and adjuvants in many chronic disorders.[2] In *Ayurveda*, *Rasayana* is well-known therapy, by which a person gets the superiority of *Rasa* (the nourishing fluid which is produced immediately after digestion) and most operative rejuvenation therapies that keep the body young and helps to endorse health. Many *Rasayana* or adaptogenic drugs have been reported to produce the complete potential to prevent diseases and degenerative changes that promote longevity by providing strength and immunity.[3,4] Numerous Ayurvedic herbal medicines and their formulations are classified in the group of *Rasayana*.

Virgozest *Avaleha* is a poly-herbal formulation with ingredients having *Rasayana* like properties and claimed to serve as adaptogenic and immunomodulatory, health tonic, enriched with dry fruits and its constituents contains natural supplements of Vitamin E and proteins. The five constituents of virgozest *Avaleha* are reported to enhance immunity, memory, and better health to all ages from pediatric to geriatric. Various studies are reported related to *Rasayana* effects, adaptogenic and immunomodulatory activities of individual constituents such as *Badam,*[5] *Sunthi,*[6] *Safed Musali,*[7] *Ashwagandha*[8,9] and *Shatavari*[7,10] are reported. However, to date, no scientific studies are reported on the whole formulation in animal models. Therefore, virgozest *Avaleha* was assessed for its adaptogenic and humoral immune activity in Wistar albino rats.

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Materials and methods

Animals

Wistar albino rats of either sex weighing between 180 ± 20 g were used for the experiments. The animals were exposed to 12-hour light and dark cycles, relative humidity of 50%–70%, and the ambient temperature during the period of experimentation was 22°C ± 03°C. All animals were kept under the same husbandry conditions. Institutional Animal Ethics Committee (IAEC/24/2018/18) approved the experimental protocols by the guideline formulated by the Committee for the Purpose of Control and Supervision on Experiments on Animals, India.

Drug and chemicals

Virgozest *Avaleha* is polyherbal proprietary Ayurvedic formulation provided by Virgo UAP Pvt. Ltd., Ahmedabad (Gujarat) (Batch no. AVG031, Mfg. date January-2019). The ingredients with their scientific/botanical name, parts used, and quantity are given in Table 1. Cyclophosphamide was purchased from Biochem Pharmaceutical Industries Ltd., Ahmedabad (Batch no. BYU1031, Mfg. date February-2018). All other chemicals used in the present research study were of analytical grade.

Dose and dosage form

The dose for the experimental study was calculated by extrapolating the clinically prescribed dose of virgozest *Avaleha* to an animal dose based on body surface area ratio.[11] Thus, the calculated dose of virgozest *Avaleha* was 450 mg/kg (virgozest *Avaleha* low dose [VALD]) and 900 mg/kg (virgozest *Avaleha* high dose [VAHD]) body weight of albino rats. The suspension of virgozest *Avaleha* was prepared in fresh cow milk with adding of sugar (Madhur brand, pure and hygienic sulfur-free sugar) in the ratio of 1.68 g in 10 ml milk and administered orally with the help of oral feeding cannula in a constant volume of 10 ml/kg body weight of rat.

Experimental protocols

Adaptogenic activity

The virgozest *Avalaeha* was evaluated for adaptogenic activity against swimming stress-induced changes and hypothermia in albino rats.[12] A total of 24 animals (12M + 12F) weighing between 180 ± 20 g were taken for experimental protocol and were divided into four groups each consisting of six rats. Group (I) was kept as stress control group (SC), received distilled water (10 ml/kg, po); group (II) was kept as vehicle control (VC) group, received sweet milk (10 ml/kg, po); group (III) and (IV) were kept as drug-treated groups, received virgozest *Avaleha*, 450 mg/kg, po (VALD) and 900 mg/kg, po (VAHD), respectively.

The virgozest *Avaleha* was administered orally for 15 consecutive days, twice a day in full dose at both times. Initial body weight was noted and thereafter on the 7th and 15th days during the experimental period. On the 7th day 1 h after oral drug administration, the initial rectal temperature of the animal was taken and then exposed to the forced swimming stress for 20 min in the jiggler swimming apparatus. After 20 min., the fall in rectal temperature that is hypothermia was noted for each animal. On the 15th day again, the same protocol was followed for noting hypothermia and then, immediately blood was collected by supraorbital puncture under light ether anesthesia. The serum was used for estimation of cortisol,[13] and antioxidant parameters such as superoxide dismutase (SOD),[14] catalase,[15] total glutathione[16] and glutathione peroxidase (GPx).[17]

Immunomodulatory activity

The immunomodulatory activity of virgozest *Avaleha* was evaluated against sheep red blood cells (SRBCs)-induced humoral immune response in Wistar albino rats.[18] A total 36 animals (18M + 18F) weighing between 180 ± 20 g were taken for experimental protocol and were divided into six groups

Table 1: The ingredients of Virgozest *Avaleha* (each 500 g) contain

Ingredients	Latin name	Family	Part used	Quality (%)
Badam	*Prunusa mygdalus* Baill	Rosaceae	Seed powder	14
Khajura	*Phoenix sylvestris* Roxb.	Arecaceae	Fruit pulp	20
Draksha	*Vitis vinifera* Linn.	Vitaceae	Fruit pulp	15
Seb	*Pyrus malus* Linn.	Rosaceae	Fruit pulp	12
Anjeer	*Ficus carica* Linn.	Moraceae	Fruit pulp	3
Pista	*Pistacia vera* Linn.	Anacardiaceae	Fruit powder	1.2
Sunthi	*Zingiber officinale* Roxb.	Zingiberaceae	Rhizome powder	0.25
Elaychi	*Elletaria cardamomum* (Linn.) Maton	Zingiberaceae	Fruit powder	0.01
Chironji	*Buchanania latifolia* Roxb.	Anacadriaceae	Seed powder	1.4
Safed Musali	*Asparagus adscendus* Buch.-Ham. Ex Roxb.	Asparagaceae	Rhizome powder	0.25
Ashwagandha	*Withania somnifera* (L.) Dunal.	Solanaceae	Root powder	0.25
Shatavari	*Asparagus racemosus* Willd.	Liliaceae	Root powder	0.25
Kesar	*Crocus sativus* Linn.	Iridaceae	Style and stigma powder	0.02
Ghee	-	-	Liquid	1
Excipients	-	-	-	QS

QS: Qunatity sufficient

each consisting of six rats. The first three groups (I to III) were kept without cyclophosphamide treatment, and groups (IV to VI) were further immunosuppressed with cyclophosphamide treatment as details mentioned below.

The group (I) was kept as a VC group, received sweet milk (10 ml/kg, po). The group (II) and (III) were kept as test drug-treated groups, received Virgozest *Avaleha*, 450 mg/kg, po (VALD) and 900 mg/kg, po (VAHD), respectively. Group (IV) was Cyclophosphamide treated group received distilled water (10 ml/kg, po) + cyclophosphamide (80 mg/kg, po) (CP); group (V) and (VI) were kept as test drug-treated groups, received virgozest *Avaleha* 450 and 900 mg/kg, respectively + Cyclophosphamide (80 mg/kg, po).

The drugs were administered for 11 consecutive days to the respective groups. On the third day, fresh sheep red blood (SRBCs) was collected in a sterilized bottle containing Elsever's solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid, and 0.42% sodium chloride) aseptically. Finally, the SRBCs suspension (30% w/v) was made into normal saline and injected subcutaneously (0.5 ml/100 g) to each rat. SRBCs from the same animal were used for sensitizing and to determine antibody titer. In rats, group (IV) to (VI), immunosuppression was produced by giving two doses of cyclophosphamide (80 mg/kg, po) on the 4th day and 6th day of drug administration.

At the end of the experiment, the rats were overnight fasted and on the 11th day, blood was collected by retro-orbital puncturing under light anaesthesia by ether. Serum was separated for evaluating hemagglutination antibody titer values for each rat. Thereafter, animals were sacrificed and spleen and lymph nodes were carefully dissected out. The relative weights of the organs were noted and transferred in 10% buffered formalin solution for histopathological study.

Antibody values were determined by the hemagglutination technique.[19] The micro-titer plate was filled with 0.1 ml sterile normal saline and serial two-fold dilutions of 0.1 ml of the serum in sterile saline solution were made into the micro-titer plate. About 0.1 ml thrice saline washed 3% SRBCs were added to each well of the micro-titer plate. The plate was incubated overnight and examined for visual agglutination. The value of the highest serum dilution shows visible hemagglutination taken as antibody titer and converted to log2 values for comparison between the groups.

Statistical analysis

The results are expressed as mean ± standard error of the mean for six rats per experimental group. One-way analysis of variance was used to compare the mean values of quantitative variables among the groups followed by Dunnett's multiple *t*-test for unpaired data by using Sigma stat software to determine the significant difference between groups at $P < 0.05$.

Results and Discussion

Adaptogenic activity

In the present study, virgozest *Avaleha* was evaluated for adaptogenic activity against swimming stress-induced hypothermia in albino rats. Stress causes very real physical changes in the body, including harming the neurological, endocrine, and immune system.[20] Adaptogens have stimulating properties that help counteract those harmful effects during stress conditions. Many studies have shown that restraint stress suppresses body weight gain and food intake in rodents.[21] All the groups showed an increase in body weight in comparison to initial body weight, and reversed the magnitude of weight loss due to stress in adaptogenic activity except VAHD showed insignificant decrease in body weight of rats. There were no significant changes between the treated and SC group [Table 2].

Hypothermia (fall in rectal temperature) was observed in albino rats after forced swimming in SC group. Virgozest *Avaleha* at both doses reversed the magnitude of hypothermia on the 7th day when compared to SC group and VC group, while on the 15th day only a higher dose produced effects which may suggest the drug having adaptogenic activity in stress conditions in dose-dependent manner [Table 3]. It is reported that *Rasayana* or adaptogenic drugs have the potential to prevent diseases and degenerative changes. It counteracts the stress-induced changes in the body that leads to diseases and promote longevity by providing strength and immunity.[3] Virgozest *Avaleha* at both dose levels also increases the strength and physical activity as revealed by an increase in physical activity in the jiggler cage in comparison with an SC group [Table 4].

Prolonged exposure to stress causes a high level of cortisol and other hormones involved in resistance reaction cause wasting of muscle, suppression of immune system, and ulceration of gastrointestinal tract. The increased cortisol levels are reversed by anti-stress agents.[22] Virgozest *Avaleha* at both dose levels nonsignificantly reduced cortisol level [Table 5] which may suggest its anti-stress effects.

Repeated exposure to chronic stressors can stimulate numerous pathways, including an increase in free radical formation. Inactivation or reduced the protective anti-oxidant enzymes leading to increased oxidative stress.[23] Virgozest *Avaleha* at both dose levels, non-significantly increased the antioxidant parameters such as SOD, catalase, glutathione and glutathione peroxidation when compared to the stress control group [Table 5 and Figure 1]. The result of the present study suggests the antioxidant activity, which may be responsible for its adaptogenic activity. The ingredients of virgozest *Avaleha* contains glycosides, alkaloids, flavonoids, phenolic compounds, and carbohydrates, which can modify the alarm stage and increase the resistance stage of the stress response, prevent or at least delay the state of exhaustion, and hence, provide a certain level of protection against long-term stress.

Table 2: Effects of test drugs on body weight of albino rats in adaptogenic activity

Groups	Body weight (g)			
	Initial	7th day	15th day	Percentage
SC	175.80±15.21	185.20±12.82	194.50±12.63**	10.63↑
VC	182.20±14.76	188.20±13.44	204.60±8.89*	12.29↑
VALD	185.20±6.03	185.40±6.95	199.40±8.41	7.66↑
VAHD	190.20±2.13	187.00±1.48	182.50±4.84	4.04↓

*P<0.05, **P<0.01, when compared with the initial body weight of rats (paired t-test), Data presented as mean±SEM (n=6). SEM: Standard error of the mean, ↑: Increase, ↓: Decrease, VC: Vehicle control, SC: Stress control, VALD: Virgozest *Avaleha* low dose, VAHD: Virgozest *Avaleha* high dose

Table 3: Effects of test drugs on hypothermia in albino rats subjected to forced swimming stress on 7th and 15th days

Groups	Rectal temperature (C)					
	7th day			15th day		
	Initial	After	Percentage	Initial	After	Percentage
SC	40.17±0.74	31.18±0.42**	22.37↓	40.94±0.99	29.56±0.61**	27.79↓
VC	39.73±0.94	31.77±0.70**	20.03↓	39.94±0.38	29.96±0.63**	24.98↓
VALD	38.02±0.41	30.43±0.53**	19.96↓	39.62±0.33	28.28±0.37**	28.62↓
VAHD	37.53±0.43	30.31±1.41*	19.23↓	39.04±0.90	29.72±0.47*	23.87↓

*P<0.01, **P<0.001, when compared with initial values of respective group (paired t-test), Data presented as mean±SEM (n=6). SEM: Standard error of the mean, ↑: Increase, ↓: Decrease, VC: Vehicle control, SC: Stress control, VALD: Virgozest *Avaleha* low dose, VAHD: Virgozest *Avaleha* high dose

Table 4: Effects of test drugs on physical activity of rats in terms of Jiggeler's cage rotation on 7th and 15th days

Groups	Rotations (Nos.)			
	7th day	Percentage	15th day	Percentage
SC	29.83±5.15	-	32.33±5.69	-
VC	43.00±10.29	44.15↑	31.28±8.86	3.24↓
VALD	41.00±9.57	37.44↑	33.67±7.59	4.14↑
VAHD	38.33±5.67	13.40↑	44.00±12.19	36.09↑

Data presented as mean±SEM (n=6), Percentage compared with Stress control group. SEM: Standard error of the mean, ↑: Increase, ↓: Decrease, VC: Vehicle control, SC: Stress control, VALD: Virgozest *Avaleha* low dose, VAHD: Virgozest *Avaleha* high dose

Table 5: Effect of test drug on serum parameters in albino rats subjected to forced swimming stress

Groups	Cortisol (ng/ml)	Total protein (mg/dL)	Total glutathione (μmoles/dL)	GPx (μmoles/dL)
SC	2.02±0.23	852.80±29.89	54.51±14.58	9.61±0.17
VC	1.94±0.23	762.20±7.68†	35.15±9.40	9.53±0.14
VALD	1.84±0.17	734.72±18.85‡	42.64±10.66	10.47±0.57
VAHD	1.87±0.16	784.89±10.22	70.08±15.91	10.48±0.71

†P<0.05, ‡P<0.02, when compared to the normal control group (Annova followed by Dunnett's multiple t-test), Data presented as mean±SEM (n=6). SEM: Standard error of the mean, VC: Vehicle CONTROL, SC: Stress control, VALD: Virgozest *Avaleha* low dose, VAHD: Virgozest *Avaleha* high dose, GPx: Glutathione Peroxidase

Immunomodulatory activity

Humoral immunity involves the interaction of B-cell with the antigen and their subsequent proliferation and differentiation into antibody-secreting plasma cells. Immunomodulatory agents can enhance or inhibit the immunological responsiveness of an organism by interfering with its regulatory mechanisms.

In the first three groups, without cyclophosphamide treatment showed a significant increase in body weight in vehicle and VAHD treated groups. Cyclophosphamide arrested the magnitude of increase in body weight of rats in comparison to VC group. Cyclophosphamide, a cytotoxic bi-functional alkylating agent belongs to the class of nitrogen mustard. It is used for the treatment of various cancer as well as an immunosuppressant in organ transplantation, rheumatoid arthritis and other benign diseases.[24] In the present study, the immunosuppressant effects of cyclophosphamide were revealed by a significant decrease in the weight of the spleen and decrease in antibody titer values.

Spleen and lymph nodes are important immune organs and can relatively reflect the immune function of animals. Organ index is considered the most elementary and conventional index, which have been generally used to evaluate the whole immune state of the organism.[25] Virgozest *Avaleha* at a higher dose produced nonsignificant increase in spleen weight of SRBC-sensitized rats when compared to VC group. Virgozest *Avaleha* at both dose levels in cyclophosphamide treated rats showed increase in spleen weight when compared to the cyclophosphamide control group [Table 6]. Administration of cyclophosphamide produced a significant decrease in spleen weight when compared to VC group.

Antibodies, the product of B-lymphocytes and plasma cells, are central to the humoral immune responses. IgG and IgM are the major immunoglobulins that are involved in complement activation, opsonization, neutralization of

Table 6: Effect of test drug on body weight and relative weight of spleen of sheep red blood cells sensitized albino rats

Groups	Body weight (g)			Spleen (g/100 g BW)
	Initial	Final	Actual change	
VC	202.50±7.09	229.42±3.16*	26.92±7.69	0.198±0.015
VALD	191.00±2.57	222.83±12.63	31.83±12.47	0.170±0.007
VAHD	193.50±5.30	206.33±6.26*	12.83±2.21	0.202±0.012
VC+CP	212.67±14.31	226.25±19.08	13.58±9.94	0.139±0.008**
VALD + CP	194.67±12.07	204.00±14.56	9.33±4.26	0.151±0.017
VAHD + CP	192.75±7.07	200.50±9.93	7.75±6.97	0.143±0.013†

*P<0.02 when compared with initial body weight of rats (paired t-test), **P<0.001 when compared with vehicle control group, †P<0.05, when compared with vehicle + cyclophosphamide group (Annova followed by Dunnett's multiple t-test), Data presented as mean±SEM (n=6). SEM: Standard error of the mean, VC: Vehicle control, VALD: Virgozest *Avaleha* low dose, VAHD: Virgozest *Avaleha* high dose, CP: Cyclophosphamide, BW: Body weight

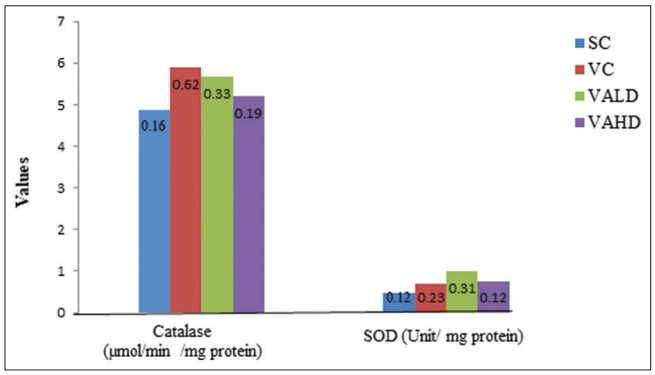

Figure 1: Effect of test drug on serum parameters in albino rats subjected to forced swimming stress

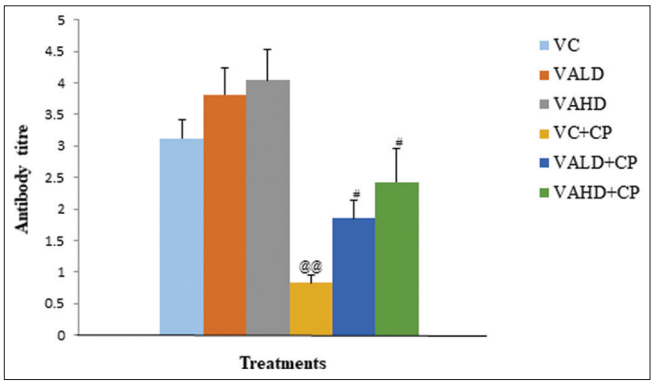

Figure 2: Effect of test drug on antibody titer in serum of SRBCs sensitized albino rats; @@$P < 0.01$, when compared with vehicle control group; #$P < 0.05$, when compared with vehicle control + cyclophosphamide control group (Annova followed by Dunnett's multiple t-test)

toxins, etc.[26] In the present study, antibody titer value has been nonsignificantly increased in SRBC-sensitized rats treated with virgozest *Avaleha* at both dose levels when compared to VC group. Administration of cyclophosphamide produced a significant decrease in antibody titer when compared to VC group. Virgozest *Avaleha* significantly and in dose-dependent manner increased the antibody titer against cyclophosphamide-induced immunosuppression in albino rats [Figure 2]. The result suggests that the increase in agglutinating antibodies in a hypersensitive condition may lead to immune complex-mediated reactions, with a significant rise in complement-fixing antibodies points out the development of protective immune responses and the counteraction of undesired immune reactions. Virgozest *Avaleha* may be useful in drug or chemical-induced immune-compromised conditions.

Cyclophosphamide significantly decrease the white pulp, peripheral lymphocytosis, lymphoid cell depletion, and fibrosis in the spleen [Figure 3], and produced peripheral lymphocytosis, lymphoid cell depletion, and congestion in the lymph node [Figure 4], same pathological changes were reversed by virgozest *Avaleha* at both dose levels in cyclophosphamide treated rats.

Immune deficiency diseases decrease the body's ability to fight invaders ending in immune deficiency disorders, which in *Ayurveda* designates as *Ojokshaya*. The process of preventing disease development and the capacity to resist disease are jointly known as *Vyadhikshamatva*.[20] Virgozest *Avaleha* is constituted by well-known *Rasayana* drugs, and individual ingredients of virgozest *Avaleha* are mostly classified as *Vrishya*, *Balya* and *Rasayana* in Ayurvedic classical books and many references have been proven that the ingredients used in this formulation have adaptogenic, antioxidant and immunomodulatory activities. Besides, *Ashwagandha* which has several properties generally associated with adaptogens,[27] including immunomodulatory[28] and antioxidant[29] properties; *Kharjur*,[30] *Draksha*,[31] *Ela*,[32] *Anjeer*[33] and *Zingiber officinale*[34] have proven antioxidant effects. *Safed Musali* has an inhibitory effect on pro-inflammatory cytokines and the production of nitric oxide reduced the level of corticosterone in the swimming stress model.[7] *Shatavari* exerts an inhibitory effect on pro-inflammatory cytokines, which may suggest beneficial effects in the management of stress and inflammatory conditions.[7] Therefore, the present research study provides evidence for its adaptogenic and immunomodulatory activities of virgozest *Avaleha*.

Conclusion

The present study concluded that virgozest *Avaleha* has adaptogenic and humoral immune activity in experimental

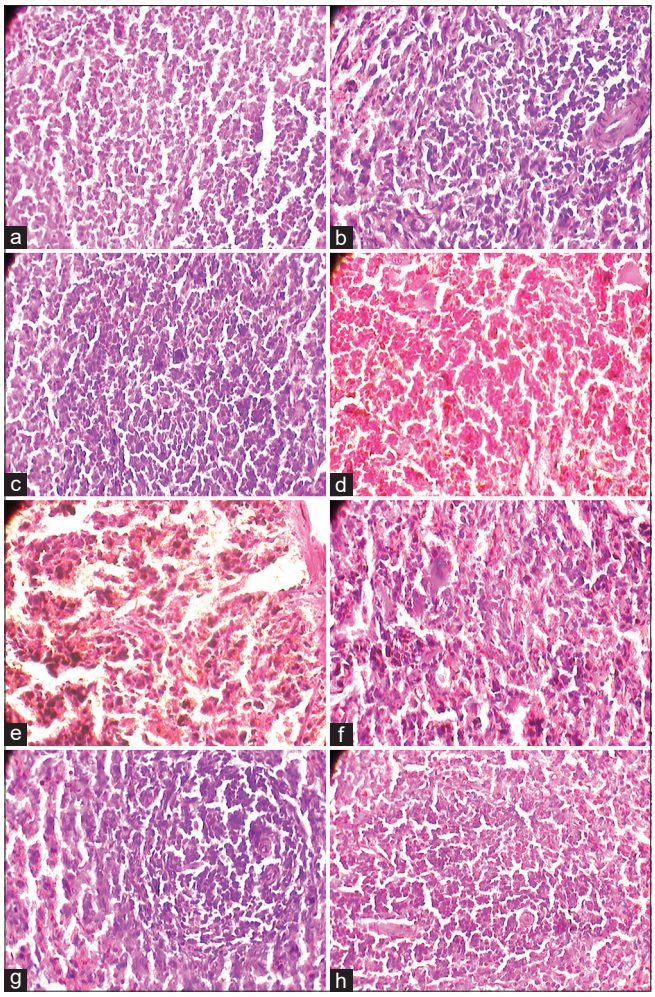

Figure 3: Photomicrograph sections of spleen of albino rats (1 × 400) (a) Normal cytoarchitecture (vehicle control) (b) Normal cytoarchitecture (virgozest *Avaleha* low dose) (c) Normal cytoarchitecture (virgozest *Avaleha* high dose) (d) Decrease in white pulp and lymphoid cell depletion (vehicle control + cyclophosphamide) (e) Lymphoid cell depletion and peripheral lymphocytolysis (vehicle control + cyclophosphamide) (f) Mild lymphoid cell depletion (virgozest *Avaleha* low dose + cyclophosphamide) (g) Normal cytoarchitecture (virgozest *Avaleha* low dose + cyclophosphamide) (h) Normal cytoarchitecture (virgozest *Avaleha* high dose + cyclophosphamide)

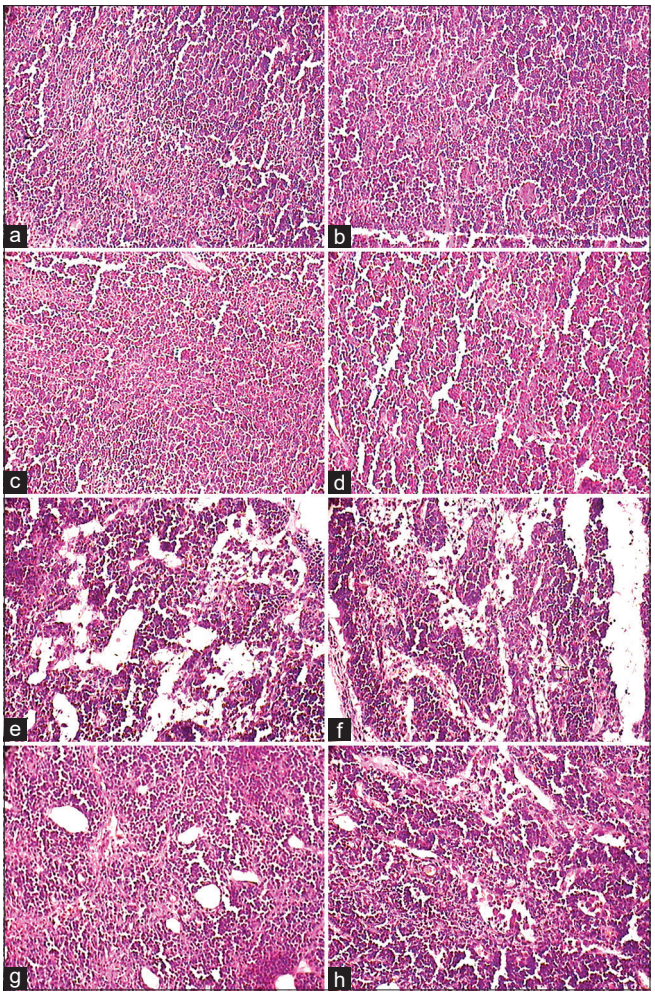

Figure 4: Photomicrograph sections of Lymph node of albino rats (1 × 400) (a and b) Normal cytoarchitecture (vehicle control) (c) Normal cytoarchitecture (virgozest *Avaleha* low dose) (d) Normal cytoarchitecture (virgozest *Avaleha* high dose) (e and f) Lymphoid cell depletion and lymph node congestion (vehicle control + cyclophosphamide) (g) Mild lymphoid cell depletion (virgozest *Avaleha* low dose + cyclophosphamide) (g) Mild lymphoid cell depletion and lymph node congestion (virgozest *Avaleha* low dose + cyclophosphamide) (h) Mild lymphoid cell depletion and lymph node congestion (virgozest *Avaleha* high dose + cyclophosphamide)

studies in Wistar albino rats. The result suggests that virgozest *Avaleha* is useful in drug or chemical-induced immune-compromised conditions.

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Conflicts of interest

There are no conflicts of interest.

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