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Evaluation of adaptogenic activity profile of herbal preparation

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The herbal formulation, AVM is a proprietary formula that consists of extracts of herbs that have been used in Indian traditional medicine to promote physical and mental health, improve defense mechanisms of the body and enhance longevity. AVM (500 and 1000 mg/kg) was tested for its adaptogenic activity by determining antistress, anabolic and immunomodulatory effects. In antistress activity, pretreatment with AVM significantly attenuated the changes in ascorbic acid (from blood and adrenal), cortisol (from plasma and adrenal) and adrenal gland weights induced due to restrain stress (physical immobilization). Its antistress effect at 1000 mg/kg was comparable to that of diazepam (5 mg/kg) treated group. Leucopenia, and anemia induced by cyclophosphamide (CYP) was shown to reduce significantly by AVM. Treatment of AVM + CYP had increased spleen and thymus weights significantly as compared to CYP alone treated group. The anabolic activity was evaluated by weight gain of the levator ani muscle, ventral prostrate gland and seminal vesicles in rats as compared to untreated control.

Keywords*:* Adaptogenic, Antistress, Anabolic, Immunomodulatory, Haematological parameters

Adaptability is probably the most distinct characteristic of life. Dr. Hans Seyle defined stress as the sum of all non-specific responses of the body to any external stimuli acting up on it. Fundamentally, it is a physiological response towards external stimuli and the primary objective of such a response is to restore the normal process of life. Perhaps the single most important property of an adaptogen is its proven ability to combat stress in all forms[1].

Some medicinal plants have been demonstrated to induce a state of non- specific increase of resistance in experimental animals as well as human beings[2]. Studies indicate that adaptogens not only help the body to cope up with stress, but can enhance general health and performance[3]. Stress is the driving force that is intrinsic in the very existence of a human being. Antistress and adaptogenic agents have opened a new vista in the modern therapeutics in the management of the stress related clinical disorders.

AVM is a herbal formulation consisting of herbs that have been used in traditional medicine for centuries to promote and stimulate male health and sexual enjoyment. The formula is based on protecting men's health, promoting recovery and stimulating sexual functioning. The herbs included are *Withania somnifera* (L) Dual., *Emblica officinalis* G., *Asparagus racemosus* wild., Trikatu, *Ocimum sanctum* L., shilajit, *Tribulus terrestris* L. *and Piper longum* L. These herbs are claimed to have immunomodulatory, adaptogenic, aphrodisiac, anabolic effects and the ability to improve vital energy[4,5]. Keeping in view of the specific pharmacological activities of various constituents of these herbs, the adaptogenic activity of this formulation has been evaluated by determining antistress, anabolic and immunomodulatory activities in animal models.

Materials and Methods

Animals — Albino mice of Haffkine strain (18-23 g) and albino Wistar rats (100-120 g) of either sex, obtained from Bharat Serum and Vaccines, Thane, India were housed under standard conditions of temperature (22°±2°C), RH (60%), 12:12 hr L:D cycle and fed with standard pellet diet (Chakan Mill Ltd, Pune, India) and water *ad libitum.*

Drugs, chemicals and reagents — AVM, a standardized herbal formulation duly certified from quality control Department was obtained as a gift

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sample form Amsar Ltd, Indore, India. Cyclophosphamide (CYP; Khandelwal Laboratories, Mumbai, India) cortisol (Samarth Pharma Ltd, Mumbai, India) and diazepam (Lupin Laboratories, India) were also obtained.

All other chemical used were of analytical reagent grade and procured locally.

Treatment — The various constituents of AVM are outlined in Table 1.

Suspension of finely powdered drugs CYP, diazepam and AVM were prepared in 1% (w/v) sodium carboxymethyl cellulose (CMC) solution and administered orally. AVM I and AVM II i.e. AVM in the dose levels 500 and 1000mg/kg were used. The control animals were received vehicle.

Acute toxicity studies[6] — Different doses of AVM ranging from 500-5000 mg/kg were administered orally to the different groups of mice (10 mice/group), while one group with the same number of mice served as control. The animals were observed for various behavioral symptoms and mortality up to 7 days. Acute LD_{50} was calculated by the method of Miller and Tainter[6].

Antistress activity — The antistress activity of herbal formulation was evaluated by determining its effect on restrain stress (by immobilization of the rats) [7,8]. In this experiment rats (150-180 g) were randomly divided into 5 groups (6 rats/group). The group I and II served as control without stress and with stress respectively. Group III, IV and V mice received diazepam (5 mg/kg)[9], AVM I and AVM II respectively, orally daily for 15 days. At the end of the treatment all rats (except Group I) were immobilized[7] on a wooden plank with the help of adhesive plaster individually for 5 hr. After 5 hr of restrain stress, the rats were anaesthetized with ether and blood was collected by cardiac puncture for biochemical studies, and adrenal glands were removed and their weights were recorded. Ascorbic acid from blood and adrenal gland and cortisol from plasma and adrenal gland were assayed[10,11].

Table 1—Composition of AVM

Herbs	Quantity of extracts (mg/capsule)
Root of *Withania somnifera* (Linn) Dual.	250
Fruit of *Emblica officinalis* Gaerth.	100
Root of *Asparagus racemosus* wild	50
Tuber of *Dioscorea bulbifera* Linn.	50
Powder of Trikatu	20
Leaves of *Ocimum sanctum* Linn.	15
Powder of Shilajit	50
Areal parts of *Tribulus terrestris* Linn.	50
Fruit of *Piper longum* Linn.	5

Immunological related studies

Biological parameters[12]— In this experiment, CYP was used to suppress the immune activity of mice. Mice were divided randomly into 5 groups (6 mice/group). Group I served as control and received 1%(w/v) sodium CMC while group II, III and IV received CYP (25 mg/kg), AVM I + CYP(25mg/kg) and AVM II + CYP (25 mg/kg) respectively and group V received AVM II alone orally daily. The treatment continued for 10 days. On the 11th day mice from all the groups were sacrificed and spleen and thymus were isolated, cleaned with normal saline and their weights recorded.

Haematological parameters[13]— Four groups of mice (6 mice/group) were used for the study. The group-I received CYP (25 mg/kg) alone; the group-II and III received AVM I + CYP (25 mg/kg) and AVM II + CYP (25 mg/kg) respectively and group IV received AVM II alone orally daily. The treatment continued for 10 days and the effects on various haematological parameters were evaluated up to 28 days. Blood samples were collected from caudal vein and various haematological parameters viz: total WBC count and RBC count (haemocytometer), hemoglobin content (cyanmethaemoglobin method), and differential leukocyte counts (Fields stain) were determined initially and also on 7th, 14th, 21st and 28th day. The body weight of the animals was also recorded prior to cyclophosphamide treatment and also on every 4th day up to 28 days period.

Anabolic activity[14]— The anabolic activity was evaluated by determining the organ weights of rats viz: levator ani muscle, ventral prostate and seminal vesicles following AVM treatment. Male rats (6 rats/group: 50-65 g) were randomly divided into 4 groups. Rats were weighed and then castrated under light ether anesthesia. Group I served as control and received 1% (w/v) sodium CMC while group II and III received AVM I and AVM II, respectively orally daily for 7 days. To the fourth group testosterone (10 mg/kg) was administered, sc, daily for 7 days. On day, 8th after 24 hr of the last dose, the rats were sacrificed. The wet and dry weights of levator ani muscle, ventral prostate gland, and seminal vesicles of rats of all groups were recorded.

Statistical analysis — Results were expressed as mean ± SE. The data obtained were subjected to

Table 2 —Effect of AVM on restrain stress (Immobilization stress) induced biochemical parameters in rats
[Values are mean ± SE from 6 animals in each group]

Group Treatment (dose/kg, po)	Adrenal weight (mg/100g of body wt.)	Blood ascorbic acid (µg %)	Plasma cortisol (µg %)	Ascorbic acid (mg/100g of adrenal wt.)	Cortisol (mg/100g of adrenal wt.)
I. Control (without stress)	9.76 ± 0.30	700.6 ± 5.88	18.25 ± 0.63	349.9 ± 7.82	3.40 ± 0.12
II. Control (with stress)	17.74 ± 0.32*	890 ± 4.77*	39.24 ± 1.07*	236.4 ± 5.4*	1.32 ± 0.0*
III. Diazepam (5mg/kg) (with stress)	10.59 ± 0.22*	729.5 ± 3.88*	25.86 ± 0.51*	319 ± 4.16*	3.028 ± 0.3*
IV.AVM (500mg/kg) (with stress)	13.71 ± 0.28*	801.16 ± 3.40*	34.00 ± 0.62 *	293 ± 5.3 *	2.92 ± 0.0*
V.AVM (1000mg/kg) (with stress)	11.63 ± 0.26*	778.5 ± 4.56*	28.47 ± 0.38*	302 ± 5.24*	2.74 ± 0.04*

One-way ANOVA followed by Dunnett's multiple comparisons test against the respective control. *P<0.01

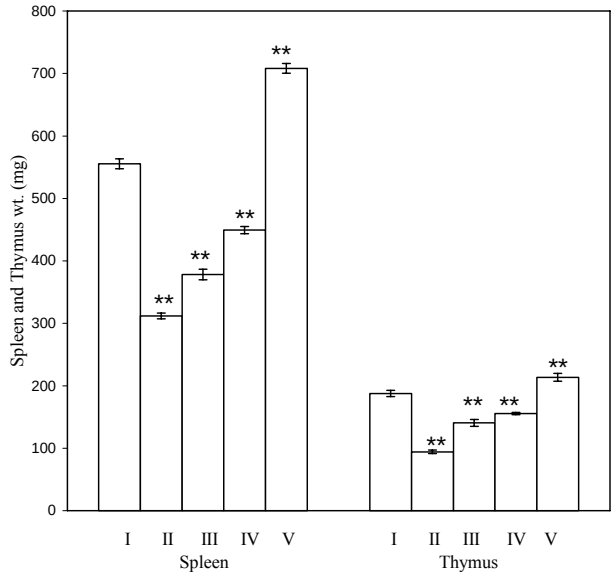

Fig.1— Effect of AVM on spleen (wt. to body wt. ratio) and thymus (wt. to body wt ratio) changes induced by cyclophosphamide in mice. I - Control, II - CYP, III - AVM I + CYP, IV - AVM II + CYP, V - AVM II; One-way ANOVA followed Dunnett's multiple comparisons test against the respective control (group I *vs* group II and V and group II *vs* group III and IV) *P* values: **<0.01

statistical analysis using one-way ANOVA followed by Dunnett's multiple comparisons test for control and multiple test groups. The *P* values <0.05 were taken as the criteria of significance.

Results

Acute toxicity studies — All AVM treated mice showed no discernible behavioral changes up to 5g/kg by oral route. No mortality was observed during 7 days observation period.

Antistress activity — Exposure to restrain stress causes hypertrophy of adrenal glands which is associated with significant depletion of adrenal contents viz. ascorbic acid and cortisol (P <0.01) and elevated blood ascorbic acid and plasma cortisol levels (P<0.01) (Table 2).

Pretreatment with diazepam (5 mg/kg), AVM I and AVM II prevented the increase in adrenal gland weight significantly (P<0.01). Depletion of adrenal ascorbic acid and adrenal cortisol contents were attenuated significantly by diazepam (P<0.01), AVM I and AVM II treatment (P<0.01) (Table 2). The antistress activity of diazepam (5mg/kg) was comparable to that of AVM II treated group.

Biological parameters — CYP (25 mg/kg) treated mice showed significant decrease in spleen and thymus weights (P<0.01) (Fig.1). Mice treated with AVM I + CYP and AVM II + CYP showed significant increase in the spleen and thymus weights as compared to CYP alone treated group (P<0.01) (Fig.1).

Haematological parameters — Mice treated with CYP alone showed significant suppression of haematological parameters viz. total WBC count and haemoglobin content. Both CYP + AVM I and CYP + AVM II treated mice showed decrease on day 7 but thereafter increase in the levels of these parameters was observed after stoppage of treatment (Table 3). Total WBC count, RBC count and haemoglobin levels in AVM + CYP treated animals were normal by 28th day while only CYP treated mice did not regain normal levels up to 28 day (Table 3). There were no significant changes in RBC count thus data not presented. Mice treated with CYP (25 mg/kg) showed decrease in body weight whereas mice treated with AVM I + CYP and AVM II + CYP showed increase in body weight (Fig. 2).

Table 3—Effect of AVM on cyclophosphamide (CYP) induced changes in hemoglobin (Hb) and WBC count
[Values are mean ± SE from 6 animals in each group]

Treatment Group	Hb (g%) on day					WBC count (x 10^3)/cmm on day				
	0	7	14	21	28	0	7	14	21	28
CYP	13.1 ± 0.52	9.8 ± 0.62**	10.15 ± 0.44^{NS}	10.4 ± 0.36^{NS}	10.8 ± 0.56^{NS}	8.15 ± 0.38	4.06 ± 0.44**	4.66 ± 0.6^{NS}	4.91 ± 0.52^{NS}	5.14 ± 0.48^{NS}
AVM I + CYP	12.8 ± 0.4	9.63 ± 0.34**	10.5 ± 0.52^{NS}	10.95 ± 0.46^{NS}	11.5 ± 0.32*	7.88 ± 0.54	5.25 ± 0.39**	5.96 ± 0.4^{NS}	6.51 ± 0.58^{NS}	6.98 ± 0.62^{NS}
AVM II + CYP	12.9 ± 0.24	9.6 ± 0.4**	10.8 ± 0.36^{NS}	11.25 ± 0.42*	11.9 ± 0.28**	8.08 ± 0.45	5.12 ± 0.35**	5.61 ± 0.45^{NS}	6.56 ± 0.34*	6.89 ± 0.38*
AVM II	13.1 ± 0.46	13.4 ± 0.36^{NS}	13.7 ± 0.5^{NS}	13.6 ± 0.42^{NS}	13.4 ± 0.32^{NS}	8.12 ± 0.38	8.42 ± 0.52^{NS}	8.06 ± 0.44^{NS}	7.95 ± 0.34^{NS}	7.82 ± 0.28^{NS}

One-way ANOVA followed Dunnett's multiple comparisons test against the respective control (day 7 *Vs* day 0 readings and day 14, 21 and 28 *Vs* day 7 readings). *P* values: *<0.05, **<0.01, ^{NS}Not-significant

Table 4—Effect of AVM on Levator ani muscle, ventral prostrate, seminal vesicles weight in rats after 7 days treatment
[Values are mean ± SE from 6 animals in each group]

Treatment (dose and route)	Wet Levator ani muscle wt (mg)	Wet ventral prostate wt (mg)	Wet seminal vesicles wt (mg)	Dry Levator ani muscle wt (mg)	Dry ventral prostate wt (mg)	Dry seminal vesicles wt (mg)
I. Control						
(1% Na CMC, po)	8.52 ± 0.44	5.38 ± 0.20	9.58 ± 0.16	1.40 ± 0.07	0.80 ± 0.11	1.1 ± 0.06
II. Testosterone (10mg/kg, sc)	95.28 ± 2.66**	163.46± 3.20 **	121.66 ± 3.84**	13.30 ± 0.69 **	21.92 ± 1.37 **	17.02 ± 0.38 **
III. AVM (500mg/kg, po)	21.36 ± 0.82 **	24.72 ± 0.74 **	19.34 ± 0.64 *	3.98 ± 0.62 **	5.42 ± 0.46 **	2.72 ± 0.41*
IV. AVM (1000mg/kg, po)	39.55±1.04 **	45.35±0.88 **	33.64±1.56 **	6.18±0.48 **	6.96±0.82 **	4.66±0.52 **

One-way ANOVA followed Dunnett's multiple comparisons test against the respective control. *P* values: *<0.05, **<0.01

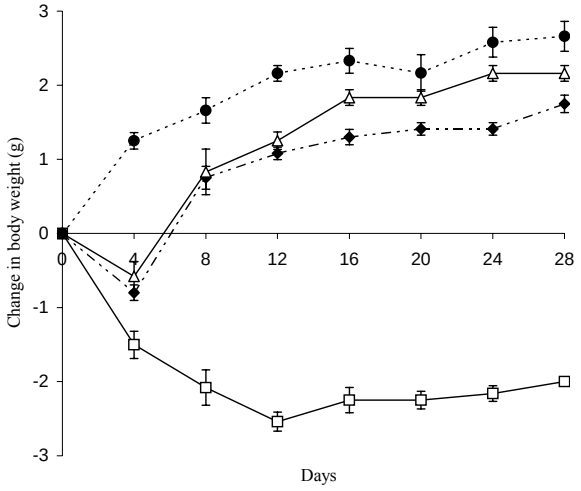

Fig. 2— Effect of AVM on cyclophosphamide induced changes in body weight in mice. -□- CYP -♦- AVMI+ CYP -Δ- AVM II+CYP -●- AVM II

Anabolic activity – AVM I, AVM II and testosterone (10 mg/kg, sc) treated rats showed a significant increase in the wet and dry levator ani muscle, ventral prostrate and seminal vesicles weights as compared to that of untreated control group. (P<0.01) (Table 4). The anabolic activity of testosterone was higher as compared to AVM treated groups.

Discussion

The adrenal glands contain relatively large amounts of ascorbic acid and cortisol, which are markedly decreased and cause hypertrophy when they are stimulated by stress or injection of adrenocorticotropin hormone[15,16]. Pretreatment with AVM at both the doses significantly prevents the depletion of ascorbic acid and cortisol and hypertrophy of adrenal glands. Further, AVM treatment also prevents the rise in blood ascorbic acid and plasma cortisol, indicating that constituents of AVM appear to have a corticosteroid sparing effect as observed in the present study and which is in agreement with reported studies[15,17]. The present findings have demonstrated that AVM changes the course of primary physiological indicators of stress possibly via reducing the activation of adrenal cortex and sparing the corticosteroid effect.

The AVM contains herbs (Table 1), which are known to potentiate cellular immunity[18]. The results demonstrated that AVM treatment was able to reverse the immune suppression significantly, reflected in partial restoration of thymus and spleen weight loss due to CYP (25 mg/kg) (Fig. 1). Further, administration of AVM significantly reduces the leucopenia and anemia induced by CYP in mice. Analysis of the present findings in different experimental conditions suggests that AVM has the ability to stimulate haemopoietic system and to reverse the CYP induced haematological changes.

Major side effects of chemotherapy observed in clinics are impairment and suppression of the immune system. In such clinical situation, the pharmacological effects (immunomodulatory activity) of AVM may be of therapeutic importance in the treatment of patients with severely impaired or suppressed immune system. The exact mechanism(s) of action of constituents of AVM in the amelioration of CYP induced immunological as well as haematological changes is not yet clear. However, effects of the some of the constituents of AVM on enhancing production of growth factors such as granulocyte colony-stimulating factors (GS-CSF) cannot be ruled out.

The anabolic activity evaluation study revealed that the AVM II treated rats showed greater gain in wet and dry Levator ani muscle, ventral prostate gland, and seminal vesicles weights. AVM has also restored the changes in body weight induced CYP. The anabolic activity observed with AVM can be attributed to the presence of steroidal and other active components of AVM. These findings suggest that AVM can be useful as a growth promoter and can be beneficial in the treatment of variety of geriatric problems.

In conclusion it can be stated that AVM exhibited significant antistress, immunomodulatory and anabolic activities in different animal models thereby proving a promising adaptogen.

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