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Adaptogenic activity of Siotone, a polyherbal formulation of Ayurvedic rasayanas

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Siotone (ST) is a herbal formulation comprising of *Withania somnifera, Ocimum sanctum, Asparagus racemosus, Tribulus terristris* and shilajit, all of which are classified in Ayurveda as *rasayanas* which are reputed to promote physical and mental health, improve defence mechanisms of the body and enhance longevity. These attributes are similar to the modern concept of adaptogenic agents, which are, known to afford protection of the human physiological system against diverse stressors. The present study was undertaken to investigate the adaptogenic activity of ST against chronic unpredictable, but mild, footshock stress induced perturbations in behaviour (depression), glucose metabolism, suppressed male sexual behaviour, immunosuppression and cognitive dysfunction in CF strain albino rats. Gastric ulceration, adrenal gland and spleen weights, ascorbic acid and corticosterone concentrations of adrenal cortex, and plasma corticosterone levels, were used as the stress indices. *Panax ginseng* (PG) was used as the standard adaptogenic agent for comparison. Additionally, rat brain levels of tribulin, an endogenous endocoid postulated to be involved in stress, were also assessed in terms of endogenous monoamine oxidase (MAO) A and MAOB inhibitory activity. Chronic unpredictable footshock induced marked gastric ulceration, significant increase in adrenal gland weight and plasma corticosterone levels, with concomitant decreases in spleen weight, and concentrations of adrenal gland ascorbic acid and corticosterone. These effects were attenuated by ST (50 and 100 mg/kg, p.o) and PG (100 mg/kg, p.o), administered once daily over a period of 14 days, the period of stress induction. Chronic stress also induced glucose intolerance, suppressed male sexual behaviour, induced behavioural depression (Porsolt's swim despair test and learned helplessness test) and cognitive dysfunction (attenuated retention of learning in active and passive avoidance tests), and immunosuppression (leucocyte migration inhibition and sheep RBC challenged increase in paw oedema in sensitized rats). All these chronic stress-induced perturbations were attenuated, dose-dependently by ST (50 and 100 mg/kg, p.o.) and PG (100 mg/kg, p.o.). Chronic stress-induced increase in rat brain tribulin activity was also reversed by these doses of ST and by PG. The results indicate that ST has significant adaptogenic activity, qualitatively comparable to PG, against a variety of behavioural, biochemical and physiological perturbations induced by unpredictable stress, which has been proposed to be a better indicator of clinical stress than acute stress parameters. The likely contribution of the individual constituents of ST in the observed adaptogenic action of the polyherbal formulation, have been discussed.

Slotone (ST) is a polyherbal formulation based on classical Ayurvedic literature and comprises *Withania somnifera, Ocimum sanctum, Asparagus racemosus, Tribulus terristris* and shilajit. All these herbals and shilajit, a complex entity constituted of fresh and modified remnants of humus admixed with plant and microbial metabolites[1], are classified in Ayurveda as *Dravya Rasayanas* which form an important constituent of the Ayurvedic concept of preventive medical care, aimed at improving the quality of life while promoting longevity[2]. Rasayanas are claimed to improve physical and mental health, increase the resistance of the body to infection and other external factors tending to perturb the homeostasis of the human system, promote revival of physiological functions after debilitating diseases and to augment intellect.[2] There is, thus, a remarkable similarity between the Ayurvedic concept of rasayanas and the modern concept of a new class of plant-derived drugs, the adaptogens, which appear to induce a state of non-specific increase of resistance of the organism to diverse aversive assaults which threaten internal homeostasis[3]. Adaptogenic drugs, like the rasayanas, are basically preventive rather than curative and appeared to function best when the resistance of the body is diminished, as seen after prolonged illness or in old age, or when the individual is subjected to prolonged stress[3].

There is comprehensive experimental and clinical evidence that various aspects of the immune, behavioural and endocrine systems are severely compromised following exposure to chronic stress. The stress effects appear to be determined by the duration rather than the intensity of the external or

internal stressful stimuli. Thus, acute and transient stress, of a severe nature, may be less harmful than mild stress continued over a length of time[4]. Another major factor, which initiates and aggravates stress-induced effects is the inability of the organism to cope with the stress situation[4]. The introduction of the factor of unpredictability makes it difficult for the individual to adapt, and therefore cope, to the stressor[5]. As such, the use of chronic unpredictable, though mild, stress continued over a length of time appears to be more clinically relevant than acute stress, even of a relatively severe nature[5].

Stress has been postulated to be involved in the etiopathogenesis of a variety of diseased states, ranging from psychiatric disorders like depression and anxiety, immunosuppression, endocrine disorders including diabetes mellitus, male impotency, and cognitive dysfunctions, to diseases like peptic ulcer, hypertension and ulcerative colitis[6]. The benzodiazepine anxiolytics, despite having significant anti-stress activity, have not proved effective against chronic stress induced adverse effects on immunity, behaviour, cognition and male sexual function[7]. Additionally, the problem of tolerance and physical dependence on prolonged use, limits the clinical utility of these drugs[7]. *Panax ginseng* (PG) has been used in the ancient Chinese system of medicine for a variety of clinical disorders and was the first adaptogenic agent to be used in modern medicine[3]. However, it is now known to induce several adverse effects, including the well documented 'ginseng abuse syndrome', on prolonged use[8]. There is, therefore, a need for an effective adaptogenic agent which can replace PG in the therapy of stress-induced disorders. The answer perhaps lies in the Ayurvedic rasayanas. The present investigation was conducted to evaluate the effect of a polyherbal formulation of rasayana drugs. ST, some of whose constituents like *W.somnifera*, *O.sanctum* and shilajit have earlier been reported to exhibit significant antistress activity against acute stress effects in experimental situations[9-11].

Materials and Methods

The study was conducted on adult CF strain albino rats (140-180 g), of either sex obtained from the Central Animal House of this Institute. The rats were acclimatized for at least one week in the Department animal house before use. The animals were kept in colony cages (4-5 rats/cage) at ambient temperature of 25°±2°C and 45-55% relative humidity, with a 12 hr light/12 hr dark cycle. Experiments were conducted between 0900 and 1400 hrs.

Drugs and vehicles—ST was suspended in 0.3% carboxymethylcellulose in distilled water and was administered orally (p.o) in the doses of 50 and 100 mg/kg. PG (Biological Evans) was suspended in the same vehicle and administered p.o. in the dose of 100 mg/kg. Control animals received only the vehicle (2.5 ml/kg, p.o.). The test drugs and vehicle were administered for 14 days, once daily, 1 hr before induction of stress. Experiments were conducted on day 14, one hr after the last stress procedure and 2 hr after drug or vehicle administration.

Induction of chronic stress—The method of Armario *et al.*[5] was used. The rats were randomly assigned to the unstressed control, stress and drug-treated stress groups. Those assigned to the vehicle or drug treated groups were subjected daily (including Sundays) to 1 hr of footshock through a grid floor in a standard conditioning chamber with the escape route closed. The duration of each shock (2 mA) and the intervals between the shocks were randomly programmed between 3 and 5 sec and 10 and 110 sec, respectively, in order to make them unpredictable. Animals were sacrificed on day 14, 1 hr after the last shock procedure on completion of the test procedure involved. Blood was collected and the different tissues required (adrenal glands, spleen and brain) were removed.

Techniques used for assessment of stress intensity—The following parameters were used to assess the intensity of stress-induced effects:

(a) Gastric ulceration: The stomach was removed and split open along the greater curvature. The number of discrete ulcers were noted by the help of a magnifying glass. The severity of the ulcers was scored after histological confirmation as, 0=no ulcers, 1=changes limited to superficial layers of the mucosa with no congestion, 2=half the mucosal thickness showing necrotic changes and congestion, 3=more than two-thirds of mucosal thickness showing necrotic changes and congestion, and 4=complete destruction of the mucosa with marked haemmorhage. Thereafter, the pooled ulcer severity score was calculated[12].

(b) Adrenal cortex and spleen weights.

(c) Adrenal cortex corticosterone[13] and ascorbic acid[14] concentrations.

(d) Plasma corticosterone levels[13].

Method used to assess stress-induced perturbations

(a) Stress-induced hyperglycaemia and glucose intolerance: Blood sugar estimations were done at the end of the 14-day period in vehicle—treated unstressed and stressed rats, and in drug-treated stressed animals. Glucose tolerance test was done in these groups after giving glucose load (1 g/100 g, p.o.). Blood sugar estimation was done by an enzymatic technique [15].

(a) Stress-induced behavioural depression: The following methods were to assess behavioural depression

(1) Swim stress-induced 'behavioural despair' test[16]: Rats were forced to swim individually in a polypropylene vessel (45×40×30 cm) with a water level of 20 cm, which ensured that the rat's feet did not touch the floor of the vessel and that it could not climb out of it. The rat was allowed to swim for 10 min. Thereafter, during the next 5 min, the total period of immobility, characterized by complete cessation of swimming with the head floating above water level, was noted. This immobility period, after initial frenzied attempts to escape, is postulated to represent 'behavioural despair' as an experimental model of endogenous depression[16].

(2) 'Learned helplessness' test [17]: On day 12 of the investigation, rats were subjected to footshock (60 scrambled shocks, 15 sec duration, 0.8 mA, every min) in a two compartment jumping box (Techno) with the escape door to the adjoining unelectrified compartment closed. The exercise continued for 1 hr. On day 14, 48 hr later, the rats were subjected to avoidance training, using the same apparatus but keeping the escape route to the unelectrified chamber open. During this avoidance training the rats were placed in the electrified chamber and allowed to acclimatize for 5 min before being subjected to 30 avoidance trials, with an inter-trial interval of 30 sec. During the first 3 sec of the trial, a buzzer stimulus (conditioned stimulus, CS) was presented followed by electroshock (unconditioned stimulus, UCS) (0.8 mA) delivered via the grid floor for the next 3 sec. The avoidance response was characterized by escape to the adjoining 'safe' chamber during CS. Failure to escape during UCS within 15 sec was assessed as 'escape failure' which is postulated to indicate despair or depression[17].

(c) Stress-induced inhibition of male sexual behaviour: A male rat was placed in a cage in a dimly-lit room for 10 min with 2 oestrinized (sequentially treated with oestradiol valerate 5 µg/rat, followed 48 hr later by hydroxyprogesterone 1.5 mg/rat, sc) female rats. The parameters observed were the latency (in min) to initiate licking of female genitalia, first mount and first intromission, and the total number of mounts and intromissions[18].

(d) Stress-induced cognitive dysfunction: The following parameters were used to assess the effect of stress on retention of a learned task as memory

(1) Active avoidance test[19]: Rats were trained for an active-avoidance task before subjecting them to stress. During training, the rat was placed in the right electrified compartment of a shuttle box (Techno) and allowed to acclimatize for 5 min. Thereafter, the animal was subjected to 15 sec of a buzzer stimulus (CS) which was followed by electric shock (1. mA, 50 Hz) given through the grid floor (UCS). The rats were given at least 10 trials, with an inter-trial interval of 60 min, until they reached the criterion of 100% avoidance response of jumping to the unelectrified left chamber of the shuttle box during CS. The test was repeated on day 14 in order to assess the retention of the active-avoidance learning.

(2) Passive avoidance test[20]: The test apparatus was a rectangular box (45×30×40 cm) with an electrified grid floor. An 8 cm high platform (17×12 cm) was fixed to the centre of the floor. A rat was placed on the platform and allowed to step down. 24 hr later, on day 1 of the experiment, the rat was again placed on the platform and on stepping down, received footshock (0.75 mA, 2 sec) through the grid floor. The rat was given 3 more trials until the latency of step down had stabilized. The test was repeated on day 14. Retention of learning, as memory, for each animal was determined by calculating the 'inflexion ration' in sec (cut off point 300 sec) by the formula, Inflexion ratio $=L_1-L_{14}/L_{14}$ (L_1 and L_{14} represent step down latencies, in sec, on days 1 and 14).

(d) Stress-induced suppression of immune function: Stress-induced inhibition of both humoral and cell mediated immune responses were assessed—

(1) Cell—mediated immune response: Rats were immunized with sheep RBC (SRBC, 1×10^8 cell, sc) injected into the dorsum on day 1. Rats were thereafter, challenged with SRBC, (1×10^8) injected into the left hind paw on day 14 one hr after the last stress procedure, whereas saline was injected into the right paw. The difference in the footpad thickness, left-right, was assessed 24 hr later by mercury displacement technique[21].

(2) Leucocyte migration test[22]: Rats were sensitized with 0.5 ml egg albumin (25 mg/ml) admixed with 0.5 ml complete Freund's adjuvant, and administered s. c. on day 1 before induction of stress. On day 14 one hr after the last stress procedure, the rats were sacrificed and blood was collected. The leucocyte rich plasma was separated and the final cell suspension was adjusted to contain 15×10^6 cells/ml. Leucocyte migration was assessed in the absence (control) and presence of antigen (egg albumin 1μg/ml). Percentage migration inhibition was calculated by the formula:

$$100-\frac{\text{Area of migration in antigen chamber}}{\text{Area of migration in control chamber}}\times100$$

(e) Rat brain tribulin activity : Tribulin activity was assessed in terms of endogenous monoamine oxidase (MAO) A and B inhibiting activity. Extraction and the assay procedure for tribulin activity were similar to earlier described procedures[23]. The enzyme sources and substrates for estimating MAO A and MAO B inhibiting activity were human placental homogenate and human platelets, and (^{14}C) 5-hydroxytryptamine and (^{14}C) phenylethylamine, respectively.

Statistical analysis

The data were analysed by the Dunnett's t- test. P values lower than 0.05 were considered as statistically significant. The Chi-square test was used for analysing quantal data (ulcer incidence; leucocyte migration).

Results

Chronic stress markedly increased the incidence, number and severity of gastric ulcers. ST (50 and 100 mg/kg, p.o.) and PG (100 mg/kg, p.o.) significantly reduced these stress-induced gastric indices (Table 1).

Chronic stress significantly increased adrenal gland weight and reduced that of the spleen. These stress-induced changes were attenuated by ST (50 and 100 mg/kg, p.o) and PG (100 mg/kg, p.o.) (Table 2).

Chronic stress markedly reduced adrenal gland ascorbic acid and corticosterone concentration while increasing the levels of plasma corticosterone. Both ST (50 and 100 mg/kg, p.o.) and PG (100 mg/kg, p.o.) reversed these stress- induced effects (Table 3).

Blood sugar levels were significantly increased by chronic stress. This stress-induced hyperglycaemia was significantly decreased by ST (50 and 100 mg/kg, p.o.) but not by PG (100 mg/kg, p.o.). However, both ST and PG, in the doses used tended to normalise stress- induced glucose intolerance (Table 4).

Chronic stress increased the duration of immobility in the swim-stress immobility test, while increasing escape failures with concomitant decrease in avoidance response in the learned helplessness test, features indicative of depression. ST (50 and 100 mg/kg, p.o.) and PG (100 mg/kg, p.o.) tended to reverse the stress- induced behavioural changes (Tables 5 and 6).

Chronic stress significantly decreased the sexual behaviour of male rats, as indicated by the increase in the latencies of licking of female genitalia, mounting and intromission, with concomitant decrease in the number of mountings and intromissions. These stress effects were reversed by ST (50 and 100 mg/kg, p.o.) and PG (100 mg/kg, p.o.) (Table 7).

Chronic stress produced significant decrease in the retention of acquired active and passive learning. These stress- induced memory deficits were reduced by ST (50 and 100 mg/kg, p.o.) and by PG (100 mg/kg, p.o.). (Tables 8 and 9).

Table 1—Effects of Siotone (ST) and *Panax ginseng* (PG) on chronic-stress induced gastric ulcerations in rats

[Data are mean±SE]

Treatment groups (mg/kg, p.o.)	n	Ulcer incidence (%)	Number of ulcers	Severity of ulcers
Vehicle+stress (VS)	16	100	18.8±2.4	30.9±5.6
ST (50)+VS	8	50*	10.6±2.0[a]	15.4±2.3[a]
ST (100)+VS	10	20*	6.2±1.1[a]	8.9±1.8[a]
PG (100)+VS	10	40*	9.2±1.6[a]	12.8±1.9[a]

* $P < 0.05$ (Chi-square test); [a] < 0.05 (Dunnett's t-test)

Table 2—Effects of Siotone and *Panax ginseng* (PG) on chronic stress-induced changes in adrenal gland and spleen weights in rats

[Data are mean±SE]

Treatment groups (mg/kg, p.o.)	n	Adrenal gland wt (mg/100 g)	Spleen wt (mg/100 g)
Vehicle	8	23.4±3.6	194.9±12.4
Vehicle+Stress (VS)	16	39.8±4.9*	123.4±9.8*
ST (50)+VS	8	29.8±3.2[a]	159.4±6.8[a]
ST (100)+VS	10	24.4±2.9[a]	179.6±5.8[a]
PG (100)+VS	10	26.8±3.9[a]	168.2±8.3[a]

* $P < 0.05$ different from control vehicle group;
[a] $P < 0.05$ different from group VS (Dunnett's t-test)

Chronic stress produced significant suppression of the immune responses used in this study. Thus, there was significant decrease in leucocyte migration and SRBC induced inflammation in sensitized rats. These stress- induced effects tended to be reversed by both ST (50 and 100 mg/kg, p.o.) and PG (100 mg/kg, p.o.) (Tables 10 and 11).

Rat brain concentration of tribulin was markedly increased following chronic stress, as assessed in terms of endogenous MAO A and MAO B inhibitory activity. The MAO B inhibitor component of tribulin was 53.8% higher than the MAO inhibitor component. Chronic stress induced 117.9% and 53.2% increase in the MAO A and MAO B inhibitor activities, respectively. ST (50 and 100 mg/kg, p.o.) induced 38.5% and 17.2% and 47.6% and 23.2% decrease in the MAO A inhibitor and MAO B inhibitor components of tribulin raised by chronic stress. PG (100 mg/kg, p.o.) induced a decrease of 43.3% and 25.4%, respectively, in stress-induced increases in the MAO A and MAO B inhibitor components of rat brain tribulin (Table 12).

Discussion

Stress research in laboratory animals has assumed an important role in understanding the biological and behavioural consequences of external or internal stressors which threaten to perturb homeostasis and may induce a number of clinical diseases when the body fails to counter the stress situation[24]. One difficulty with the concept of stress first propounded by Selye, is that it has become so broad as to include virtually every type of environmental change. It has now been proposed that stressors be classified based upon dimensions of intensity, frequency of exposure and the duration of stress exposure. A variety of

Table 5—Effects of Siotone and *Panax ginseng* (PG) on chronic stress-induced increase in swim stress immobility in rats

[Data are mean±SE]

Treatment groups (mg/kg, p.o.)	n	Duration of immobility (sec)	*P*
Vehicle	12	114.6±9.8	--
Vehicle+Stress (VS)	12	259.4±4.2	<0.05*
ST (50)+VS	10	186.2±9.4	<0.05[a]
ST (100)+VS	10	142.4±7.3	<0.05[a]
PG (100)+VS	8	149.2±8.5	<0.05[a]

* Difference with vehicle treated group;
[a]Difference with group VS (Dunnett's t-test)

Table 3—Effects of Siotone (ST) and *Panax ginseng* (PG) on chronic stress-induced changes in adrenal ascorbic acid and corticosterone concentrations, and plasma corticosterone levels in rats

[Data are mean±SE]

Treatment groups (mg/kg, p.o.)	n	Adrenal ascorbic acid (μ/100 g)	Adrenal corticosterone (μ/100 g)	Plasma cortico-sterone (μ/dl)
Vehicle	8	304.9±42.3	4.9±0.9	14.6±1.9
Vehicle+Stress (VS)	16	124.9±10.6*	1.2±0.6*	22.4±3.2*
ST (50)+VS	8	206.0±11.4[a]	2.8±0.8[a]	18.8±1.6[a]
ST (100)+VS	10	242.2±16.3[a]	3.4±1.0[a]	16.2±1.2[a]
PG (100)+VS	10	239.2±16.4[a]	3.4±0.8[a]	17.2±2.0[a]

* $P < 0.05$ different from control vehicle treated group; [a]$P < 0.05$ different from group VS (Dunnett's t-test)

Table 4—Effects of Siotone (ST) and *Panax ginseng* (PG) on chronic stress-induced hyperglycaemia and glucose intolerance in rats

[Data are mean±SE]

Treatment groups (mg/kg, p.o.)	n	Blood glucose (mg %) Day 14	Glucose tolerance (glucose load 1 g/100 g, p.o.) Blood glucose (mg %) 0 hr	0.5 hr	1 hr	3 hr
Vehicle	6	74.9±3.6	78.4±3.8	182.3±6.9	126.2±6.2	82.6±5.5
Vehicle+Stress (VS)	10	144.2±8.4*	139.4±8.6*	242.0±9.8*	196.6±8.6	134.2±7.6
ST (50)+VS	10	118.4±8.2[a]	119.0±5.4[a]	208.2±6.8[a]	164.4±7.2[a]	114.3±4.8[a]
ST (100)+VS	10	102.3±4.9[a]	98.6±4.4[a]	199.4±7.7[a]	138.7±5.4[a]	96.3±8.4[a]
PG (100)+VS	6	129.6±8.8	126.3±6.9	208.4±7.3[a]	169.4±6.9[a]	122.2±6.3

* $P < 0.05$ different from control vehicle treated group; [a]$P < 0.05$ different from group VS (Dunnett's t-test)

stressful situations have been employed and the lack of consistency of the stress protocols is astounding[24]. Likewise, there is wide variation in the physiological consequences of the stressor utilized in animal research[25]. However, it is now widely accepted that chronic intermittent stress, particularly of an unpredictable pattern, is more likely to induce neural, endocrine, biochemical and immune perturbations than either acute stress or chronic stress of a predictable character[24]. In addition, the factor of coping and control over the aversive stimulation, plays an important part in stress research since stress responses are minimal in such situations[24]. The method used in this study employs mild electroshock stress, unpredictable in nature, administered over a period of 2 weeks[5]. The validity of the method is demonstrated by the biological effects induced by it which include gastric ulcerations, decrease in adrenal ascorbic acid and corticosterone concentrations, with concomitant increase in plasma corticosterone levels, increase in adrenal gland weight and decrease in the weight of the spleen. All these parameters have been conclusively shown to be stress-induced effects[26]. In addition, the increase in rat brain tribulin activity, provides supportive evidence since the endocoid has been proposed to be an endogenous marker of stress in animals and in man[27].

The prevention and management of stress disorders remains a major clinical problem. Benzodiazepines (BDZs) appear to be effective against acute stress but fail to prevent the consequences of chronic stress [24]. In addition, the problems of tolerance and physical dependence exhibited by BDZs, on prolonged use, limit their utility[24]. An answer to this vexing problem was first provided when Brekhman and Dardymov[3] reported that some plant-derived agents could induce a state of non-specific increase of resistance to diverse aversive assaults which threaten to adversely affect internal homeostasis. These agents, named adaptogens, appeared to be effective only when the physiological perturbations were discernible following prolonged illness, old age and exposure to chronic stress[3]. A number of such plants, the most important one being *P. ginseng*, were extensively used in the erstwhile USSR and the Far East, for promoting physical and mental health, while helping the body to resist internal and external stressors[3]. These adaptogens were shown to be effective in

Table 6—Effects of Siotone and *Panax ginseng* (PG) on chronic stress-induced changes in the learned helplessness test in rats

[Data are mean±SE]

Treatment groups (mg/kg, p.o.)	n	Escape failures (N)	Avoidance response (N)
Vehicle	10	13.6±1.9	3.9±0.8
Vehicle+Stress (VS)	12	24.8±1.2*	0.9±0.2*
ST (50)+VS	10	17.4±1.9[a]	1.6±0.6[a]
ST (100)+VS	10	14.9±1.2[a]	2.8±0.9[a]
PG (100)+VS	8	14.9±2.8[a]	2.8±0.8[a]

* $P < 0.05$ different from vehicle treated control group;
[a] $P < 0.05$ different from group VS (Dunnett's t-test)

Table 8—Effects of Siotone (ST) and *Panax ginseng* (PG) on chronic stress-induced memory deficit in active-avoidance response in rats

Treatment groups (mg/kg, p.o.)	n	Active avoidance response on day 14 (%)	*P*
Vehicle	20	80	--
Vehicle+Stress (VS)	12	20	< 0.01*
ST (50)+VS	10	50	N.S.
ST (100)+VS	10	70	< 0.05[a]
PG (100)+VS	10	70	< 0.05[a]

* Difference with vehicle-treated control group; [a] difference with group VS (Chi-square test)

Table 7—Effects of Siotone (ST) and *Panax ginseng* (PG) on chronic stress-induced suppression of sexual behaviour in male rats

[Data are mean±SE]

Treatment groups (mg/kg, p.o.)	n	Latency (min)			Actions (N)	
		Licking	Mounting	Intromission	Mounting	Intromission
Vehicle	12	2.9±0.9	6.9±0.9	9.0±1.8	4.6±0.6	3.9±0.8
Vehicle+Stress (VS)	10	6.8±0.9*	14.6±1.6*	18.4±.1.8*	1.2±0.8*	1.6±0.6*
ST (50)+VS	10	4.2±0.9[a]	10.9±1.6[a]	13.2±1.6[a]	2.4±0.8	2.6±0.7
ST (100)+VS	10	3.5±1.2[a]	8.2±1.2[a]	11.6±1.4[a]	3.8±1.0[a]	3.9±0.6[a]
PG (100)+VS	8	3.6±1.0[a]	9.0±0.9[a]	11.4±0.8[a]	4.0±0.9[a]	3.4±0.8[a]

* $P < 0.05$ different from vehicle treated control group; [a] $P < 0.05$ different from group VS (Dunnett's t-test)

attenuating stress induced adverse effects in astronauts, soldiers and athletes in the USSR[3].

The ancient Indian system of medicine, Ayurveda, mentions a group of plant-derived drugs, the *rasayanas*, which provide freedom from disease by increasing the defense mechanism of the body, increase lifespan, improve intellect, help recovery after prolonged illness and generally enhance the quality of life[2].The ancient Ayurvedic compendiums attributed to Charaka and Sushruta summarize the objectives and advantages of rasayanas. They mention that the aims of *rasayanas* is to retard aging (*vayasthapana),* enhance lifespan *(ayukaram),* promote intellect and physical strength (*medhabalakaram*) and increase resistance to disease (*rogapaharanasamartha*). It is, therefore apparent that there is a remarkable similarity between the Ayurvedic concept of rasayanas and the modern concept of adaptogens. Unfortunately, unlike the spate of scientific investigations on adaptogens, there has been little concerted effort to investigate Ayurvedic *rasayanas* using, modern experimental and clinical indices. Ayurveda advocates the use of polyherbal *rasayana* formulation on the premise that such formulations provide synergistic clinical effect since some *rasayanas* appear to influence the body whereas others have a beneficial effect on the mind (*medhyarasayanas*)[2]. ST is such a polyherbal formulation based on recommendations of Ayurvedic compendiums. *Panax ginseng* (PG), the first clinically used adaptogen, has been extensively investigated experimentally and clinically for its stress-attenuating activity[8].

Both ST and PG prevented chronic stress-induced gastric ulcerations, in terms of the incidence and severity of the ulcers. Likewise, both the drugs attenuated chronic stress effects on adrenal ascorbic acid and corticosterone, and plasma corticosterone. The depletion of adrenal ascorbic acid and corticosterone, with concomitant increase in plasma corticosterone levels, represents the phase of resistance of the stress syndrome, characterized by increased adrenocortical activity. Continued exposure to the stressor would later lead to the phase of exhaustion when the capacity of the adrenals to synthesize, store and secrete glucocorticoids would exceed the demands of the body[24]. Involution of the spleen and increase in adrenal gland weight, are also consequences of chronic stress[24], both responses being reversed by ST and PG.

There is considerable experimental and clinical evidence to suggest that chronic stress induces endogenous depression[28]. A number of animal models of depression are based on the use of uncontrollable stress and the biochemical correlates of such tests are consonant with those seen in chronic stress, including monoamine deficiency and increased activity of the corticotrophin-releasing factor[28]. Both ST and PG were able to reverse chronic stress-induced indices

Table 9—Effects of Siotone (ST) and *Panax ginseng* (PG) on chronic stress-induced memory deficit in passive-avoidance response in rats

[Data are mean±SE]

Treatment groups (mg/kg, p.o.)	n	Step-down latency inflexion ratio-day 14	*P*
Vehicle	16	8.8±1.3	--
Vehicle+Stress (VS)	12	2.6±0.9	< 0.05*
ST (50)+VS	10	4.2±0.8	N.S.
ST (100)+VS	10	6.2±0.6	< 0.05[a]
PG (100)+VS	8	4.9±0.8	< 0.05[a]

* Difference with vehicle-treated control group; [a] difference with group VS (Dunnett's t-test)

Table 10—Effects of Siotone (ST) and *Panax ginseng* (PG) on chronic stress-induced leucocyte migration inhibition in rats

Treatment groups (mg/kg, p.o.)	n	% Leucocyte migration inhibition day 14	*P*
Vehicle	8	42	--
Vehicle+Stress (VS)	8	4.4	<0.001*
ST (50)+VS	8	19.2	<0.01[a]
ST (100)+VS	8	34.4	<0.001[a]
PG (100)+VS	6	26.4	<0.001[a]

* Difference with vehicle-treated control group; [a] difference with group VS (Chi-square test)

Table 11—Effects of Siotone (ST) and *Panax ginseng* (PG) on chronic stress-induced suppression of cell-mediated immunity in rats

Treatment groups (mg/kg, p.o.)	n	Increase in paw volume (units) day 14	*P*
Vehicle	16	2.16±0.12	--
Vehicle+Stress (VS)	10	0.98±0.09	< 0.05*
ST (50)+VS	10	1.28±0.08	<0.05[a]
ST (100)+VS	10	1.88±0.2	<0.05[a]
PG (100)+VS	8	1.76±0.4	<0.05[a]

* Difference with vehicle-treated control group; [a] difference from group VS (Dunnett's t-test)

Table 12—Effects of Siotone (ST) and *Panax ginseng* (PG) on chronic stress-induced increase in rat brain tribulin activity

[Data are mean±SE]

Treatment groups (mg/kg, p.o.)	n	Brain wt (g)	Percent inhibition/g wet wt	
			MAO A	MAO B
Vehicle	16	1.96±0.16	21.2±1.9	32.6±3.4
Vehicle+Stress (VS)	12	1.88±0.2	46.2±2.8*	48.4±2.9
ST (50)+VS	8	1.92±0.19	28.4±2.2[a]	40.1±2.9[a]
ST (100)+VS	8	2.02±0.06	24.2±1.8[a]	37.2±1.8[a]
PG (100)+VS	8	1.96±0.18	26.2±2.4[a]	36.0±2.2[a]

* $P < 0.05$ different from control vehicle treated group; [a] $P < 0.05$ different from group VS (Dunnett's t-test)

validated as animal models of depression. Chronic stress is known to affect other endocrine responses as well, which can induce sexual debility in males[29] and perturb glucose metabolism[30]. Maturity-onset diabetes mellitus may represent a state of stress-induced disturbance in glucose homeostasis[30]. ST and PG reversed chronic stress-induced inhibition of male sexual behaviour and glucose intolerance.

Stress is known to interfere with cognitive functions, tending to retard the memory engram rather than the acquisition of learning[31]. The mechanisms involved in the memory-attenuating effect of stress remains conjectural but a similar neurochemical basis operating in the induction of stress-induced depression, may be responsible[31]. ST and PG attenuated the stress-induced deficit of retention of learned tasks, both in the active and passive avoidance parameters, thus facilitating memory and its recall.

There is a large and relatively consistent literature on the effects of stressful life events on predisposition to both physical illness and infections. Evidence of impaired immune function has been conclusively shown in both experimental and clinical stress situations. All components of the immune response appear to be suppressed. The cause of stress-induced immunosuppression may be related to the endocrine and neurochemical changes induced by stress[32]. It has been suggested that stress-induced activation of the corticotrophin releasing factor may be the common factor underlying stress–induced depression and immunosuppression[28]. Both ST and PG attenuated chronic stress-induced suppression of cell-mediated and humoral immunity.

Tribulin is an endogenous low molecular weight substance widely distributed in mammalian tissues and body fluids. It appear to have at least two components, one, chemically identified as isatin (2, 3-dioxoindole), which inhibits MAO B and functions as an antagonist at atrial natriuretic receptors, whereas the other, as yet chemically unidentified, selectively inhibiting MAO A and BDZ receptors[33]. Extensive experimental and clinical studies have indicate that tribulin may function as an endocoid marker of stress and anxiety, with its MAO A inhibiting component being more important than the MAO B inhibitor component[33,34]. Consonant with this postulate, the increase in the MAO A inhibitor component of tribulin, following chronic stress, was more accentuated than the increase in the MAO B inhibitor component. ST and PG were able to prevent the stress-induced increase in rat brain tribulin function.

The findings indicate that, like the standard adaptogen PG, the herbal formulation ST, can attenuate chronic stress-induced biochemical, behavioural and physiological perturbations in rats. PG has earlier been reported to reverse chronic stress-induced effects in humans[5]. Japanese traditional medicinal plant formulations, like *Kyushin, Reiousan* and *Gosya-jinki-gan*, essentially based on *Ginkgo biloba*, have been reported to reduce the adverse effects of chronic hanging stress on sexual and learning behaviours in mice[35]. The major plant constituent of ST, *Withania somnifera*, and its active principles, have been shown to inhibit acute stress effects in rats[36], stimulate immune responses and attenuate memory deficits in Alzheimer's disease models in this species, and to augment rat brain cholinergic and GABAergic activity[37-39]. Recently, a standardized extract of the plant was shown to attenuate chronic stress induced gastric ulceration, adrenocortical dysfunction, hyperglycemia and glucose intolerance, behavioural depression and sexual dysfunction, and immunosuppression, in rats[40]. It is, therefore, likely that the observed effects of ST may be due to *W. somnifera*. However, the other

constituents, *O. sanctum*[10] and shilajit[11], have also been shown to exhibit significant antistress effects as well. Shilajit, long regarded as inorganic asphalt, has now been thoroughly investigated and shown to comprise of several organic constituents, including oxygenated dibenzo-alpha-pyrones[41].

Increased generation of oxidative free radicals (OFR), or impaired antioxidant defence mechanisms, have been implicated in chronic stress induced perturbed homeostasis including immunosuppression, inflammation, diabetes mellitus, peptic ulceration and other stress-related disease[42]. Among the constituents of ST, *Withania somnifera*[43], *Ocimum sanctum*[44] and shilajit[45], have been shown to exert significant anti-oxidant activity induced by augmented activity of OFR scavenging enzymes, superoxide dismutase, catalase and glutathione peroxidase. Thus, at least part of the observed adaptogenic antistress effect of ST may be due to the anti-oxidant activity of constituents.

The present investigation indicates that the herbal formulation, ST,has significant adaptogenic activity as shown by its mitigating effects on several chronic stress induced biochemical, physiological and behaviour perturbations, comparable to that induced by the well accepted adaptogenic agent, *P. ginseng*. Acute, subacute and chronic toxicity studies,as well as investigations on teratogenicity have shown that ST in devoid of significant toxicity or teratogenicity in doses 4 to 5 times that used in the present investigation (unpublished data).

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